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EDITORIAL

Esophageal cancer: Risk factors, screening and endoscopic treatment in Western and Eastern countries

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Abstract

Esophageal cancer is one of the most unknown and deadliest cancers worldwide, mainly because of its extremely aggressive nature and poor survival rate. Esophageal cancer is the 6th leading cause of death from cancer and the 8th most common cancer in the world. The 5-year survival is around 15%-25%. There are clear differences between the risk factors of both histological types that affect their incidence and distribution worldwide. There are areas of high incidence of squamous cell carcinoma (some areas in China) that meet the requirements for cost-effectiveness of endoscopy for early diagnosis in the general population of those areas. In Europe and United States the predominant histologic subtype is adenocarcinoma. The role of early diagnosis of adenocarcinoma in Barrett's esophagus remains controversial. The differences in the therapeutic management of early esophageal carcinoma (high-grade dysplasia, T1a, T1b, N0) between different parts of the world may be explained by the number of cancers diagnosed at an early stage. In areas where the incidence is high (China and Japan among others) early diagnoses is more frequent and has led to the development of endoscopic techniques for definitive treatment that achieve very effective results with a minimum number of complications and preserving the functionality of the esophagus.

Key words: Oesophageal cancer; Adenocarcinoma; Squamous cell carcinoma; Epidemiology; Barrett's oesophagus; Screening; Early stage; Endoscopic mucosal resection; Endoscopic submucosal disection

Core tip: Esophageal cancer is a disease with a non-negligible impact, being the 6th leading cause of death from cancer, and with a very high morbidity and mortality due to diagnosed in advanced stages. A better understanding of the epidemiology, the natural history,

and the risk factors could lead to an earlier diagnosis and treatment by endoscopic methods or by other less aggressive techniques. As a result, we could improve treatment outcomes, even though less aggressive modalities. This article provides a global perspective by comparing the management of esophageal cancer in Western and Eastern countries with particular emphasis on current prevention strategies.

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EPIDEMIOLOGY OF ESOPHAGEAL CANCER

Esophageal cancer is the 6th leading cause of death from cancer and the 8th most common cancer in the world. The 5-year survival is around 15%-25% and the best results are related to early diagnosis, which is commonly known as "early stages"[1].

Esophageal squamous cell carcinoma is the predominant histological type worldwide. However, at present, in countries like United States, Australia, United Kingdom and Western Europe (Finland, France, Norway), there is a preponderance of adenocarcinoma subtype, having squamous carcinoma moved to second place[1]. The so-called "Asian Esophageal Cancer Belt" encompasses areas such as Turkey, Iran, Kazakhstan and northern and central China, with an estimated esophageal squamous carcinoma of more than 100 cases/100000 person-years. Another area with high incidence of squamous cell carcinoma is southeastern Africa, with similar rates to those observed in Eastern countries. In the United States, from 1975-2004, the age-adjusted incidence in white males has increased from 5.76 to 8.34 cases/100000 person-years at the expense of the adenocarcinoma histological subtype. Nevertheless squamous carcinoma remains the most common subtype in American black males, but still adenocarcinoma, is one of the few cancers that contributes to an increased mortality from cancer among American men[2]. The trend towards dominance of adenocarcinoma subtype is not limited only to North America. In European countries like the United Kingdom, France or Norway the age-adjusted incidence increased by 39.6% for men and 37.5% for women in the last five years[1]. There is also a significant difference between gender distributions; the incidence of this disease is about 2-4 fold higher among males compared to females[3]. The incidence rates of squamous neoplasia in men in the territory of "Asian Esophageal Cancer Belt" are around 23 cases/100000 person-years and 16 cases/100000 person-years for females. In South Africa similar rates for males have been estimated[4]. Mortality rates follow, overall, a major parallelism with incidence rates in each country[5] (Figure 1). Regarding race, age-adjusted mortality for black individuals have a tendency to decrease, but still it is two-fold higher compared to Caucasians (7.79 *vs* 3.96, $P < 0.05$)[2].

RISK FACTORS

Risk factors of esophageal cancer are slightly different between the two major subtypes.

Risk factors for squamous carcinoma

Gender and race: Squamous cell carcinoma is the most frequent histological type in black individuals and white women, while adenocarcinoma is predominant in white men ($P < 0.001$)[2]. The incidence of esophageal squamous cell carcinoma is generally higher in men than women in most countries, and black men, compared to whites in the United States[4].

Smoking: Smoking is one of the major risk factor for developing esophageal squamous carcinoma. Smokers have a 5-fold risk of developing this disease compared to non-smokers[4]. However, there are parts of the world where smoking is not such an important risk factor and racial differences could account for these geographical differences. In a prospective study of risk factors for esophageal cancer in the province of Linxia, China, smoking was not an important risk factor compared to other parts of the world, while diet-related factors seem to play a major role in esophageal carcinogenesis. A study from Taiwan compared current and former smokers to people who never smoked and found that the OR was 4.2 and 3.4 respectively for smokers and former smokers compared to people without this habit[4].

Alcohol: Alcohol is a clear risk factor for squamous carcinoma. The relative risk (RR) increases with the amount of alcohol ingested varying between 1.8 and 7.4 depending on the weekly volume[4]. The intake of certain types of drink creates worldwide "hot spots" of squamous cell carcinoma of the esophagus in areas of Northern France consuming Brandie, corn beer in Southeast Africa, distilled sugary drinks in Puerto Rico, or certain whiskies in Carolina, United States. In Northern China, alcohol is not consumed regularly and therefore the risk associated with this habit is not relevant[4].

Diet and nutrients: Tea, mate and coffee have been extensively studied as potential risk factors associated with esophageal carcinoma and its geographical distribution, particularly in regions of South America. There is little evidence for carcinogenicity relationship

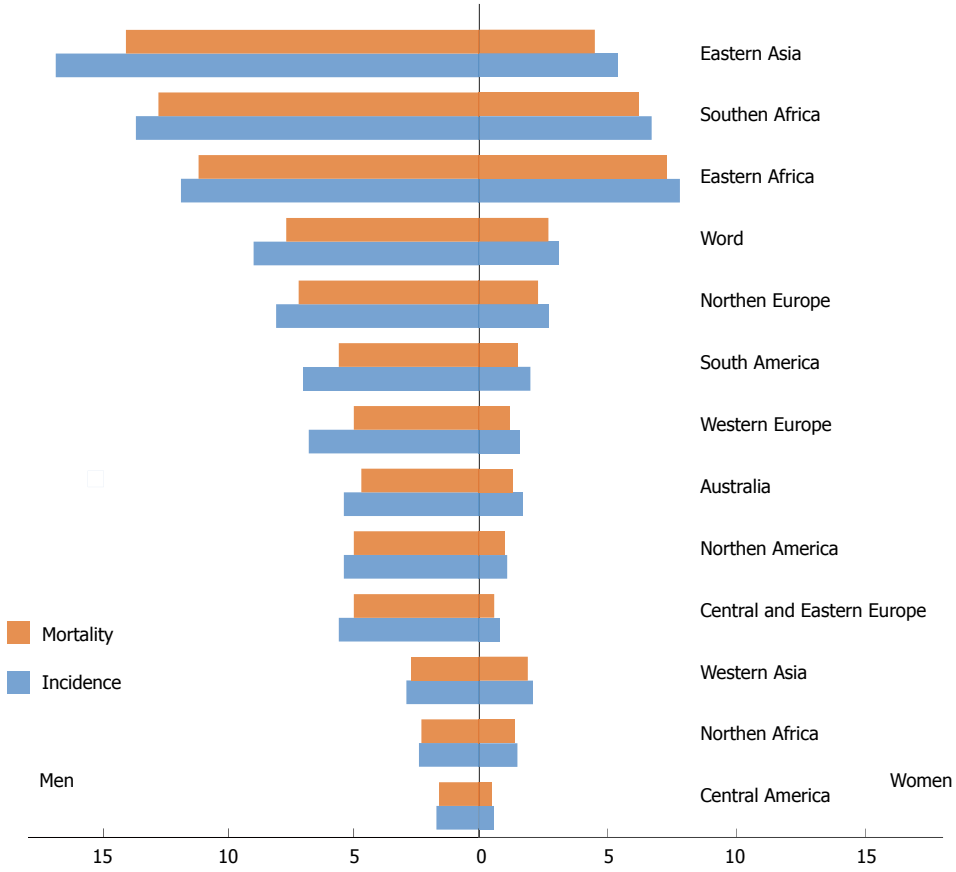

Figure 1 Estimated age-standardized rates per 100000. Year 2012. Modified from IARC, GLOBOCAN[5].

through its components except for mate, which has been linked for both amount consumed and temperature[4]. Foods rich in nitrogenous components are historically related to the high incidence of squamous cell carcinoma in certain regions of China. A meta-analysis published in 2003, shows an OR of 2 for individuals who eat foods rich in such compounds compared to those who do not[4].

The "chewers of areca nut" (often mixed with tobacco), are common in regions such as Southeast Asia and India and have been linked to the development of squamous carcinoma. In Taiwan, where the tobacco is not included in the chewable mixture, the OR for chewers is 2.3[4]. Similarly, people living in developing countries that have significant deficits of minerals and vitamins, mainly due to low intake of foods like fruits and vegetables also have an OR of 2[4].

Genetics: There are conditions with a genetic basis, such as Tylosis, an autosomal dominant disease, that are clearly related to the development of esophageal squamous carcinoma. Familial aggregation in population of high incidence of esophageal carcinoma, such as northern regions of China has also been reported[4]. Four genome-wide association studies (GWAS), three of them conducted in Chinese population and one in Japanese population have shown genetic susceptibility factors in the development of squamous carcinoma, especially in heavy alcohol and tobacco users. Two nucleotide polymorphisms (SNPs, single nucleotide polymorphisms) deserve special attention because encode enzymes metabolizing alcohol: alcohol dehydrogenase 1B (rs1229984, OR = 1.79) and aldehyde dehydrogenase 2 family (rs671, OR = 1.67). Other GWAS found association at two loci, one located in the enzyme phospholipase C and another in a particular region of chromosome 20 (C20orf54)[3]. Regarding association with squamous carcinoma a GWAS dataset that included 453852 SNPs from 1898 squamous carcinoma patients and 2100 control subjects of Chinese population was reviewed. The authors identified candidate causal SNPs, and pathways (ICSNPathway) analysis identified seven candidate SNPs, five genes, and seven pathways, which together revealed seven hypothetical biological mechanisms. The three strongest hypothetical biological mechanisms were rs4135113, rs1800450 and rs3769823[6].

Risk factors for esophageal adenocarcinoma

Gender and race: The incidence of esophageal adenocarcinoma is 8-fold more common in men than in women and 5-fold more common in whites than in blacks in the United States[4].

Gastroesophageal reflux disease and Barrett's esophagus: The prevalence of gastroesophageal reflux disease (GERD) in the Western population is about 10%-20%, and about 30 to 60 million people in the United States. This entity is capable of producing esophageal adenocarcinoma directly or, more commonly, through an intermediate pre-neoplastic lesion, the Barrett's esophagus (BE). The increased incidence of BE in the last 30 years, is correlated with an increased incidence of adenocarcinoma in the same period. Barrett's esophagus is a pre-malignant lesion that develops in 6%-14% of patients with GERD and of which, around 0.5%-1% will develop adenocarcinoma[4]. In a study performed in Spain, the incidence of adenocarcinoma during follow-up of patients with BE was 0.48% per year (95%CI: 0.006%-2.62%), for an incidence of 1 per 210 patient-years[7]. The largest study is a nationwide, population-based, cohort study conducted in Denmark, involving all patients with BE during the period from 1992 through 2009, using data from the Danish Pathology Registry and the Danish Cancer Registry. The study included 11028 patients with BE for a median of 5.2 years. The incidence rate for adenocarcinoma was 1.2 cases per 1000 person-years (95%CI: 0.9-1.5). As compared with the risk in the general population, the RR of adenocarcinoma among patients with BE was 11.3 (95%CI: 8.8-14.4). However, the annual risk of esophageal adenocarcinoma was 0.12% (95%CI: 0.09-0.15). Current surveillance guidelines assume a risk for adenocarcinoma of 0.5%-1%, far from the results obtained in this study. Detection of low-grade dysplasia was associated with an incidence rate for adenocarcinoma of 5.1 cases per 1000 person-years compared to 1.0 case per 1000 person-years among patients without dysplasia. These data question the rationale for ongoing surveillance in patients who have Barrett's esophagus without dysplasia[8].

Obesity: Obesity is a major and consistent risk factor for the development of esophageal adenocarcinoma. It has become a serious public-related disease in developed countries. By 2015, an estimated 75% of the American people will be overweight (BMI > 25) and 41% obese (BMI > 30). The OR of developing adenocarcinoma is 1.52 (95%CI: 1.33-1.74, $P < 0.0001$) for those with BMI in the 25-30 rank compared with those who have normal-weight. A high BMI (> 25) was associated with an increased risk of oesophageal adenocarcinoma (males, OR = 2.2; 95%CI: 1.7-2.7; females, OR = 2.0; 95%CI: 1.4-2.9)[9]. Higher levels of BMI were associated with increased risk of oesophageal adenocarcinoma (overweight males, OR = 1.8; 95%CI: 1.5-2.2; obese males, OR = 2.4; 95%CI: 1.9-3.2)[10]. Two main mechanisms have been proposed for the development of esophageal adenocarcinoma in obese patients. First, a physical mechanism involving an increase in the incidence of GERD, and second a hormonal-dependent mechanism mainly mediated by inflammatory markers that are secreted by adipocytes[4].

Tobacco, alcohol and nutritional deficit: Alcohol is not related to the presence of adenocarcinoma, but smoking tobacco is a known risk factor, with an OR of 2.7 (95%CI: 1.64-4.45) relative to non-smokers[11].

In a Swedish population study, an inverse relationship was found between intake of total dietary fiber and the presence of adenocarcinoma of the gastro-esophageal junction. Similarly, in a United States case-control study it was found that a diet rich in vitamins, fruits and vegetables protect against the development of this disease[4].

Drugs: Observational studies with a large number of patients showed that the use of non-steroidal anti-inflammatory drugs (NSAIDs), proton pump inhibitors (PPIs) and statins in patients with BE, reduced the progression to adenocarcinoma[4]. The most studied agents have been acid suppressants. A systematic review with meta-analysis of studies evaluating the association between PPIs and histamine receptor antagonists (H2RAs) and risk of esophageal adenocarcinoma or high-grade dysplasia (HGD) in patients with BE has been recently published. The authors identified seven observational studies (2813 patients with BE, 317 cases of esophageal adenocarcinoma or HGD, 84.4% PPI users). On meta-analysis, PPI use was associated with a 71% reduction in risk of esophageal adenocarcinoma and/or HGD in patients with BE (adjusted OR = 0.29; 95%CI: 0.12-0.79). There was a trend towards a dose-response relationship with PPI use for > 2-3 years protective against esophageal adenocarcinoma or HGD [three studies; PPI use > 2-3 years *vs* < 2-3 years: OR = 0.45, (95%CI: 0.19-1.06) *vs* OR= 1.09 (95%CI: 0.47-2.56)]. Considerable heterogeneity was observed. Two studies reported the association between H2RA use and risk of esophageal adenocarcinoma and/or HGD (1352 patients with BE, 156 cases of esophageal adenocarcinoma, 25.4% on H2RAs), and both studies did not show a significant effect[12]. The largest study was published short after and challenged these results. In such nationwide case-control study carried out in Denmark, no cancer-protective effects from PPI's were seen. In fact, among 9883 patients with a new diagnosis of BE the authors identified 140 cases with incident esophageal adenocarcinomas and/or high-grade dysplasia, with a median follow-up time of 10.2 years. The relative risk of esophageal adenocarcinoma or high-grade dysplasia was 2.2 (95%CI: 0.7-6.7) and 3.4 (95%CI: 1.1-10.5) in long-term low- and high-adherence PPI users respectively. Such results could partly be due to confounding by indication or a true negative effect from PPIs. Based on these results and until the results from future studies can elucidate what

Table 1 Risk factors for squamous cell carcinoma and adenocarcinoma of the esophagus

Risk factor	Squamous cell carcinoma	Adenocarcinoma
Geography	Southeastern Africa, Asia, Iran, South America	Western Europe, North America (United States), Australia
Race	Black > White	White > Black
Gender	Male > Female	Male > Female
Alcohol	++++	-
Tobacco	++++	++
Obesity	-	+++
GERD	-	++++
Diet: Low fruits and vegetables	++	+
Socioeconomic conditions	++	-
Genetic aspects	++	+

GERD: Gastroesophageal reflux disease; +: Associated risk; -: No risk associated. Modified from[4].

the association might be, continuous PPI therapy might not be necessary in all patients and could be directed at symptom control[13].

Genetic aspects: Very recently, it has been demonstrated using GWAS, that risk of BE and esophageal adenocarcinoma is influenced by many germline genetic variants of small effect and that shared polygenic effects contribute to the risk of these two diseases. In fact, the authors found that the genetic correlation between BE and esophageal adenocarcinoma was high (rg = 1.0; SE = 0.37) and estimated a statistically significant polygenic overlap between BE and esophageal adenocarcinoma [one-sided P = 1 × 10(-6)]. These data strongly suggest that shared genes underlie the development of BE and esophageal adenocarcinoma[14].

GWAS type studies have also been conducted to elucidate susceptibility loci. The first genome-wide association study of esophageal adenocarcinoma, together with BE has been recently published. The most significant results were for cancer and pre-cancer combined suggesting that much of the genetic basis for esophageal adenocarcinoma lies in the development of BE, rather than its to esophageal adenocarcinoma. The authors found three novel genome-wide significant loci for esophageal adenocarcinoma and BE combined, and extended existing findings at the *FOXF1* and *HLA* loci. One of the novel regions is chromosome 3p13, near FOXP1, a gene encoding a transcription factor, which regulates esophageal development. Interestingly, two of the other regions (*BARX1*/9q22.32 and *FOXF1*/16q24.1) contain risk associated SNPs which disrupt binding of FOXP1. Further dissection of these loci is likely to lead to insights into the etiology of this rapidly fatal cancer[15] (Table 1).

EARLY DIAGNOSIS AND SCREENING OF ESOPHAGEAL CARCINOMA

Esophageal cancer is a health problem worldwide with high mortality due to its natural history and the common diagnosis in advanced stages. Therefore, its detection at an early stage would improve outcomes of mortality significantly. Squamous dysplasia is the precursor lesion of esophageal squamous cell carcinoma; Barrett's esophagus is the pre-neoplastic lesion preceding adenocarcinoma[16].

Screening and surveillance of esophageal adenocarcinoma

Screening of BE-associated adenocarcinoma by endoscopy is a worldwide clinical practice although it has not been proven cost-effective. According to current guidelines, random endoscopic biopsies should be taken in all 4 quadrants and each 2 cm of columnar epithelium, and ideally performed with high-resolution endoscopes and NBI (narrow banding imaging)[17].

The results of large cohort studies suggest that the annual cancer risk for patients with non-dysplastic Barrett's esophagus is low in European populations (0.12%-0.40% per year)[8]. Dysplasia within BE lesions signals a marked increase in cancer risk: the annual risk is approximately 1% for patients with low-grade dysplasia and more than 5% for patients with high-grade dysplasia. However, 80% to 90% of cases of esophageal adenocarcinoma are diagnosed in patients without known BE. Endoscopic screening results in detection of BE in 6% to 12% of patients with prolonged GERD symptoms, most frequently white men older than 50 years of age[18].

A recent review by Spechler and Souza suggests that people with chronic GERD symptoms and at least 1 risk factor for esophageal carcinoma are suitable for active endoscopic screening for Barrett's esophagus and early adenocarcinoma with adequate surveillance depending on the lesion found on the index endoscopy and pathology (Figure 2). The main caveat of such strategy is that the target population focuses on GERD patients although around 40% of esophageal adenocarcinomas have no prior history of GERD[19].

In 2006, a systematic review, expert workshop and economic modelling was performed focused on Surveillance of BE. Such study identified 3 cost-utility analyses of surveillance of BE that used Markov modeling and confined their analysis to 50- or 55-year-

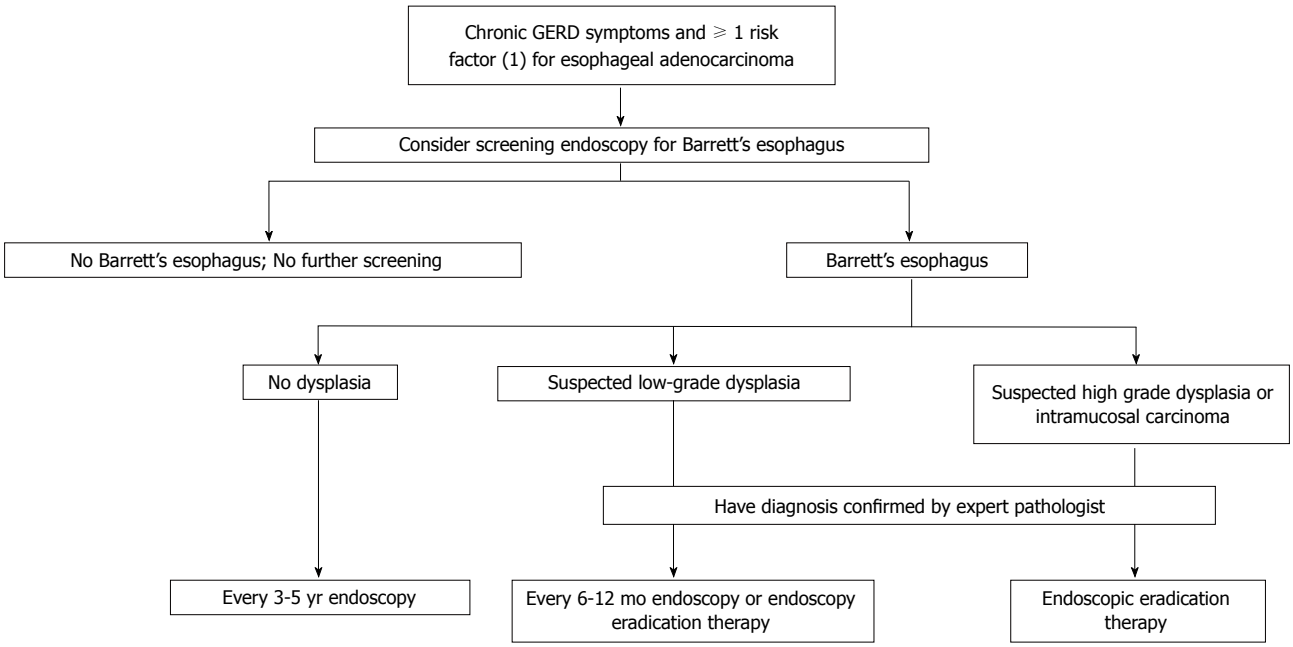

Figure 2 Algorithm for the screening surveillance, and management of Barrett's esophagus. Risk factors for esophageal adenocarcinoma: age > ó = 50 years, male sex, white race, hiatal hernia, elevated body-mass index, intra-abdominal body-fat distribution, or tobacco use. Modified from reference[19].

old white men with GERD symptoms. In one study, the authors concluded that the only cost-effective strategy was once in a lifetime screening of 50-year-old white men with GERD, followed by surveillance of those with dysplasia only. In the other 2 studies (performed by the same group) surveillance of BE every 5 years compared with no surveillance was cost-effective; however the model was very sensitive to the incidence of adenocarcinoma and quality of life (utility value) in the post-esophagectomy state. Moreover, the incremental cost-effectiveness ratio for 5-yearly surveillance was no longer within the range usually considered cost-effective[20].

These models are American, so there are almost certainly differences in practice from Europe and possible underlying differences in the epidemiology and natural history of the disease. In European public services there is a major difficulty in knowing what proportion of patients with GERD have an endoscopy and at what stage of the disease, whereas in the United States, those who present to health services are more likely to be investigated at an earlier stage. The costs of the procedures involved are also likely to be very different.

They key of surveillance may underlie on what patients may benefit from it. Is dysplasia a good marker? Should genetic markers be used? A recent cost-utility analysis from Australia compared (1) No surveillance; (2) 2-yearly endoscopic surveillance of patients with non-dysplastic BE and 6-monthly surveillance of patients with low-grade dysplasia; and (3) a hypothetical strategy of biomarker-modified surveillance. In a total of 2040 patient-years of follow-up and by using best available estimates of the malignant potential of BE, endoscopic surveillance of patients with non-dysplastic BE is unlikely to be cost-effective for the majority of patients and depends heavily on progression rates between dysplasia grades. However, strategies that modify surveillance according to cancer risk might be cost-effective, if high-risk individuals can be identified and prioritized for surveillance[21]. However, unless newly emerging technologies improve the quality-adjusted survival benefit conferred by endoscopic surveillance, current strategies are unlikely to be cost-effective in Europe. Obsolete assumptions and incomplete analyses reduce the quality of published evaluations. For these reasons new evaluations are required that encompass the growing evidence base for new technologies, such as new endoscopic therapies for high-grade dysplasia and intramucosal cancer[22].

Another fact that should be added in the evaluation is that, despite the absence of direct evidence from randomized trials, most but not all observational studies have shown that patients in whom adenocarcinoma is detected during endoscopic surveillance for BE are more likely to have early-stage cancer, receive curative therapy, and survive longer than symptomatic patients in whom adenocarcinoma is detected during the clinical workout[18].

Squamous cell carcinoma: Need or not a screening program?

Esophageal squamous cell carcinoma is the predominant histologic subtype in Asia and the incidence and mortality are higher in China than in Japan. In Japan, the incidence of this disease is declining from the late 90 s to the present. By contrast, in China, esophageal

cancer is the 4th most frequently diagnosed cancer and the 4th leading cause of death from cancer. Incidence rates are higher in rural areas of China compared to urban areas, especially in regions such as Henan, Hebei, Linxia and Shanxi[23]. As mentioned before, squamous dysplasia is a precursor lesion of squamous carcinoma. It is hardly detectable in asymptomatic individuals and there is no standardized screening program to detect this condition[23].

In Japan there are controversies about whether dysplasia should be actively detected by gastroenterologists. There are no reliable data on the actual prevalence of dysplasia in Japanese asymptomatic patients, but a recent study of 1345 asymptomatic individuals, who underwent endoscopy during a health check, found a prevalence of dysplasia of 3% in this population. There are no prospective studies and the relationship between dysplasia and squamous carcinoma development in this population is still unknown[23].

In China, endoscopic screening in high-risk areas (defined as an incidence higher than 30 cases per 100000 inhabitants per year) has been shown to detect precursor lesions in asymptomatic patients with dysplasia, with high rates of what is known as "esophageal early cancer". The main dysplastic lesion associated with esophageal squamous cell carcinoma in prospective population studies in the Chinese region of Linxia is the high-grade dysplasia, which is associated with an RR of 28.3 (95%CI: 15.3-52.3) for developing the disease compared to patients who have a normal esophageal mucosa[23,24].

Endoscopy: Endoscopy is the gold standard for the diagnosis of pre-cancerous squamous lesions. Squamous dysplasia may go undetected when using standard endoscopy and therefore chromoendoscopy techniques have been suggested to improve the performance of the test. The most simple and effective for the detection of squamous dysplasia is Lugol staining. The sensitivity and specificity of white-light endoscopy for the detection of high-grade dysplasia and cancer is 62% and 79% respectively, compared with a much higher sensitivity of 96%, at the expense of a slight loss of specificity of 63%, when using Lugol chromoendoscopy[16].

Most of the studies, if not all, have been performed in Asia where the incidence of squamous carcinoma is high. The prevalence of low, medium, high grade and invasive carcinoma, using Lugol chromoendoscopy, are 28%, 21.9%, 6.3% and 0% to 9.5% respectively in expert hands, and many of these lesions can be treated with endoscopic resection[16]. In this regard, a prospective population study was conducted in 2014 in Henan, one of the areas of Northern China with high incidence of esophageal carcinoma, in the context of a screening program with biopsies taken and guided by chromoendoscopy. A total of 36154 people between 40 and 69 years were examined. The study detected 7.1% of people with low-grade dysplasia, 2.3% with intermediate grade dysplasia and 1.6% with cancerous lesions, being 87.32% of them early carcinomas (high-grade dysplasia, carcinoma mucosa-submucosa) cases[25].

The results of several cost-benefit studies about endoscopic screening of esophageal squamous carcinoma have shown that such strategy is only cost-effective in areas of high incidence of squamous cell carcinoma, such as in Northern and rural areas of China. However, some variations may occur even in high-risk areas. The geographical and the economic status of the region have a great impact in the onset of esophageal carcinoma regarding the age of onset, the number needed to screen, the precursor lesions that have to be identified and the intervals for a proper surveillance in people with such lesions[26,27].

A recent study, based on economic parameters and management, made a comparison between 12 different existing screening methods in high-risk/high incidence of squamous cell carcinoma in China. The two key strategies to be followed to ensure cost-effective programs taking into account the acceptance of the population and the distribution of wealth in different regions were: (1) screening once throughout life and starting at the age of 50, following up after 5 years of detecting low-grade dysplasia and 3 years after intermediate-grade dysplasia, for areas with limited access to healthcare, impoverished and with a difficult track the target population economy[26]; and (2) screening three times over life, starting at the age of 40, and monitoring low-grade dysplasia and intermediate-grade dysplasia as above, for areas with appropriate access to health care, and economies that are more advanced and good monitoring program by the target population[26].

One of the questions is whether these results can be applied to Western countries. There are no European studies suggesting that endoscopic screening for squamous esophageal carcinoma is either necessary or cost-effective. The low incidence of squamous esophageal carcinoma in the European population and the predominance of public health systems might be some of the main reasons why screening of this condition is not an option even in individuals with risk factors.

Other screening techniques: There are areas in the world with high incidence of squamous carcinoma, beyond those already mentioned, where screening program using the gold standard technique with Lugol chromoendoscopy have not been shown to be cost-effective. An Iranian review published in 2013 suggested that new screening strategies, cheaper and more effective, should be tracked. They propose combining the individual risk factors of patients with cytology techniques without endoscopy and/or tissue or serum markers of risk detected

Table 2 TNM esophageal cancer

T: Primary tumor:
Tx: It can not be evaluated
T0: No evidence of primary tumor
Tis: High-grade dysplasia (intra-epithelial neoplasia noninvasive)
T1: Tumor invades own lamina, muscularis mucosae and submucosa:
T1a: Tumor invades own lamina or muscularis mucosae
T1b: Tumor invades the submucosa
T2: Tumor invades the muscularis
T3: Tumor invades the adventitia
T4: Tumor invades adjacent structures:
T4a: Tumor invades resectable pleura, pericardium, or diaphragm
T4b: Unresectable tumor that invades other adjacent structures: aorta, vertebral body, trachea, *etc.*
N: Regional lymph nodes:
Nx: They can not be evaluated
N1: Metastasis in 1-2 regional lymph nodes
N2: Metastasis in 3-6 regional lymph nodes
N3: Metastasis in 7 or more regional lymph nodes
M: Distant metastasis:
M0: None
M1: There are distant metastases

Modified from AJCC 2010[29].

enzimoimmunoassay techniques or micro-RNA[28].

There is a relatively large number of extraction techniques without endoscopic for esophageal cytology which include inflatable balls and sponges, recently developed, but these techniques have a sensitivity of only 24%-47% for dysplasia-cancer and 18%-44% for cancer, despite having good specificity of 81%-92% and 99%-100% for dysplasia-cancer and cancer respectively. The low number of suitable samples and low sensitivity makes them unsuitable for effective screening[16].

Very few studies looking at blood biomarkers on people of countries with high incidence of squamous carcinoma have been performed, but most suggest that these should be used in the future in combination with other screening techniques to optimize the results[16].

TREATMENT OF EARLY ESOPHAGEAL CARCINOMA

Early esophageal carcinoma (EEC) is defined as those early stages in which the neoplastic involvement does not exceed the submucosa, and there are no nodes involved (DAG, T1a, T1b, N0)[29,30] (Table 2, Figure 3).

There are big differences among treatment for early esophageal cancer between Western and Asian countries. In fact, the Asian attitude is more aggressive in managing these patients.

Management of ECC in Western countries

Most Western studies convey the idea that the rate of lymph node metastasis in T1b tumors is too high to be considered a safe endoscopic therapy as a definitive treatment for this neoplastic disease. It is estimated that the risk of nodal metastases in tumors confined to the mucosa (T1a), mainly adenocarcinomas in clinical practice, is 1%-2%, therefore, an endoscopic local treatment may be considered sufficient as definitive treatment. In tumors invading the submucosa (T1b), the risk of nodal metastases exceeds 10%, therefore a definitive endoscopic treatment is not feasible in principle[31]. In this type of tumor stages (high-grade dysplasia, T1a) the most common therapeutic approach is the combination of endoscopic resection techniques by means of mucosal resection (EMR) to remove the neoplastic tissue associated with ablative techniques such as radiofrequency to remove the remaining metaplastic/dysplastic residual tissue. Its therapeutic efficacy is up to 98%, and its potential complications include bleeding, perforation and residual stenosis[31,32].

For stage T1a, esophagectomy is seen today as a second treatment option, with a success rate similar to endoscopic cancer but with a much larger treatment morbidity. However, esophagectomy should be considered in patients in whom the risk of recurrence is considered high (7%-30%), such as multifocal lesions and long BE segments associated with neoplasia where it is not possible to associate ablative techniques[33].

A review of 46 studies involving 7645 patients with esophageal cancer T1N0 concluded that in T1b sm1 adenocarcinomas, well or moderately differentiated without lymphovascular invasion or lymph node metastasis, endoscopic treatment is the preferred option because the rate of lymph node involvement is lower than suspected (6% in sm1). However, in m3 T1a squamous carcinomas, lymph node involvement is higher than previously presumed and esophagectomy with lymphadenectomy should be considered[31,32].

Management of EEC in oriental countries

A number of articles from Asia, mainly Japan and China, have a more aggressive approach from the point of view of endoscopic management of early esophageal cancer. T1a and T1b lesions, regardless of histological type, with confirmed no lymph node metastases, are managed by endoscopy resection, since it is considered that this technique has the same efficacy as esophagectomy. A recently published population-based study comparing the survival of both techniques for T0/T1 stages, with a total of 430 patients who received endoscopic treatment over 1586 patients who received surgical treatment showed no differences in mortality after 2 (endoscopy: 10.5% *vs* 12.7% surgery, $P = 0.27$) or 5 years (endoscopy: 36.7% *vs* 42.8% surgery, $P = 0.16$) of follow-up[34]. The fundamental treatment of neoplasia at this stage is suggested to be the combination of definitive endoscopic treatments such as EMR or ESD (endoscopic submucosal dissection) with ablative treatments to eradicate the rest of metaplastic/dysplastic tissue if necessary[35].

The main objective of this approach is to preserve

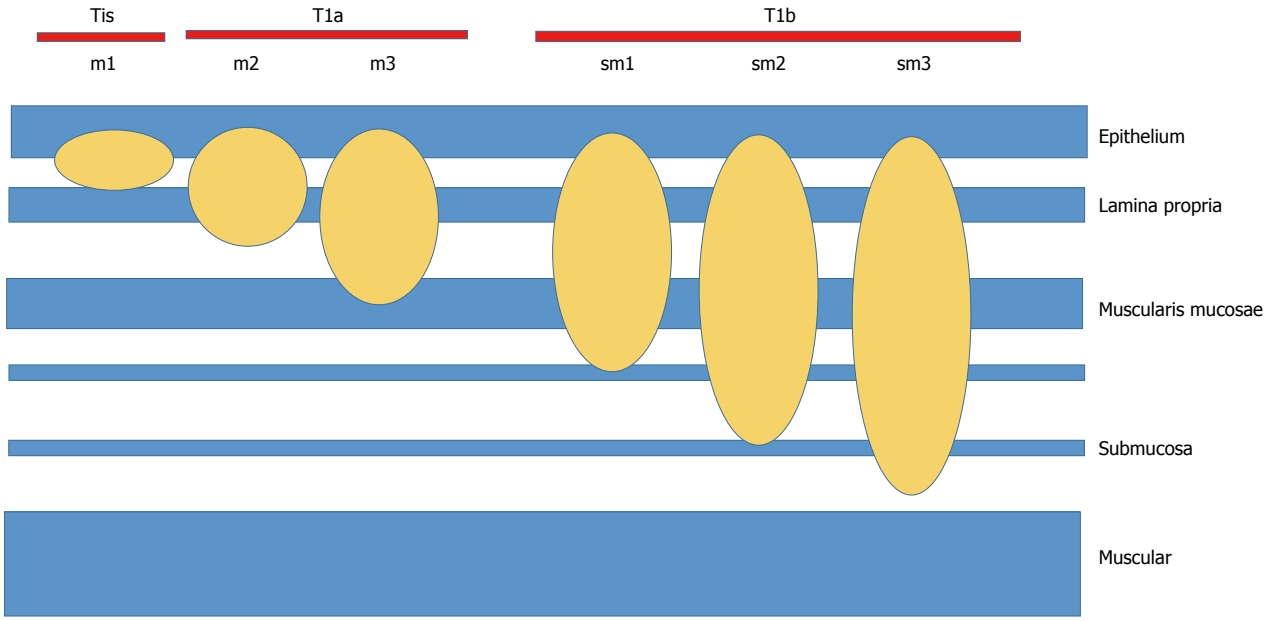

Figure 3 TNM classification. m: Mucosa; sm: Submucosa; 1,2,3: The tumor affects the upper third, middle third or lower third, respectively. Modified from reference[30].

the esophagus as a functional organ and avoid the morbidity of surgery at that level. The EMR was the first endoscopic technique developed. However, it has its limitations. In a meta-analysis of five case-control studies that included 319 lesions treated with ESD and 476 lesions treated with EMR it was observed that ESD shower better "*en bloc*" and histologically resection rates, and lower recurrence, without increasing the incidence of procedure-related complications but at the cost of a longer process and higher costs[36]. In fact, in a similar meta-analysis of 21 studies, 1152 patients and 1240 lesions treated with ESD, with an average follow-up period between 12 and 53 mo, it was observed that the rates of resection as a whole were 99% (95%CI: 99%-100%), and R0 resection rate of 90%. In lesions less than 25 mm higher a percentage of R0 resections (92% *vs* 85%, $P < 0.001$) was achieved. The complication rate was very low, the most significant being stenosis, with an incidence of 5% (95%CI: 3%-8%). The authors conclude that it is a safe and effective technique[37].

SUMMARY AND CONCLUSIONS

Squamous cell carcinoma is still the most common histologic type in the world. The areas with the highest incidence are found in Africa and the Middle East. The risk factors most frequently involved, are the abuse of tobacco and alcohol, as well as mutations in metabolizing pathways of these substances, and nutritional deficits. In areas of high incidence, defined as 30 or more cases per 100000 person-years, justified the mass screening of squamous carcinoma, a fact that improves detection rates of early squamous cell carcinoma and its management without surgery, with a high proportion of patients treated with endoscopic resection strategy.

There has been a shift from squamous carcinoma to adenocarcinoma as the most frequent histological type of esophageal carcinoma in fundamental areas of Europe such as Norway, United Kingdom, in the United States and in Australia. Differentiating risk factors are fundamentally obesity, GERD and BE as well as the influence of toxics such as tobacco. BE is a precursor of adenocarcinoma, but the rate of cancer transformation in European and United States populations is low, which questions surveillance programs and the search for an early diagnosis of adenocarcinoma in BE, which is common clinical practice today. In any case, the rate of detection of early stage adenocarcinoma is lower in Western countries and treatment, therefore, is less conservative, with high proportion of patients treated with surgical techniques to achieve eradication of the disease.

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EDITORIAL

Familial colorectal cancer screening: When and what to do?

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Abstract

Colorectal cancer (CRC) is the third leading cause of death worldwide and represents a clinical challenge. Family members of patients affected by CRC have an increased risk of CRC development. In these individuals, screening is strongly recommended and should be started earlier than in the population with average risk, in order to detect neoplastic precursors, such as adenoma, advanced adenoma, and nonpolypoid adenomatous lesions of the colon. Fecal occult blood test (FOBT) is a non invasive, widespread screening method that can reduce CRC-related mortality. Sigmoidoscopy, alone or in addition to FOBT, represents another screening strategy that reduces CRC mortality. Colonoscopy is the best choice for screening high-risk populations, as it allows simultaneous detection and removal of preneoplastic lesions. The choice of test depends on local health policy and varies among countries.

Key words: Colonoscopy; Colorectal cancer screening; Fecal occult blood test; Advanced adenoma; First-degree relative; Sigmoidoscopy

Core tip: One-fifth of people who develop colorectal cancer (CRC) have a first-degree relative (FDR) affected by this malignancy. Screening is an efficient method to reduce mortality for CRC and should be started in FDRs earlier than in the population at average risk. There is a large disparity in guidelines for screening in familial CRC, therefore, here we address the principal indication and methods for screening in this population at increased risk. Recent or emerging methods to improve the participation rate in screening programs are described. Ongoing trials on CRC screening are also reported.

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INTRODUCTION

Colorectal cancer (CRC) remains a major health problem in industrialized countries, being responsible for > 550000 deaths annually, and representing the third leading cause of cancer mortality worldwide[1,2]. In Europe in 2012, it is estimated that 3.45 million new cases of cancer were diagnosed and 1.75 million patients died from malignant diseases. Concerning CRC, the annual number stands at 447000 new cases[3].

The incidence of CRC increased from 22 cases per 100000 individuals in 1960 to 34 per 100000 in 2007[2] in Northern Europe. From 1998 to 2002, the incidence in the United States and Europe was similar, being, respectively, for men 38.6 and 38.5 and for women 28.3 and 24.6 world age standardized rate (ASR-W), as calculated per 100000 inhabitants[4]. However, mortality both for men and women, over the same period, was higher in Europe than in the US, being 18.5 and 10.7 *vs* 13.5 and 9.2 ASR-W, respectively, as calculated per 100000 inhabitants[5]. The estimated Italian median annual incidence rate in 2010 was 88.8 cases per 100000 individuals among men and 70.3 cases per 100000 among women per year[6].

The lifetime risk of CRC for average-risk subjects in industrialized countries is about 5%[1,7], but it increases 2-4-fold if there is a family history of CRC[8]. Studies in kindred and twins estimated that approximately 30% of all cases of CRC occur in patients with a family history of CRC, but only 2%-5% of cases of inherited CRC are caused by a syndrome-related to a Mendelian pattern of inheritance[8-12]. These rare syndromes are associated with a known gene mutation (Table 1). Familial adenomatous polyposis (FAP) syndrome is the most common hereditary condition, with a prevalence of 1 in 10000 individuals. Young adolescents with FAP develop hundreds to thousands of colonic adenomas and CRC is inevitable before age 40 years if preventive surgery (colectomy) is not performed. Attenuated FAP is a less severe form of the disease, characterized by an average 69% lifetime risk of CRC and an average of approximately 30 colonic adenomatous polyps (range 0 to 100 s). Both FAP and attenuated FAP result from germline mutations in the APC gene[10]. In the absence of these inherited syndromes, occurrence of CRC in family members of a CRC patient is nowadays considered to be a heterogeneous condition, including a cluster of patients with undefined hereditary syndromes that have not yet been completely defined in terms of molecular pathogenesis. A study in sibling pairs and parent/child pairs reported the presence of chromosomal regions containing low penetrance susceptibility genes possibly associated with high risk of familial CRC[10]. Together with genetic conditions, a combination of different environmental factors plays a role in the development of familial CRC. As in the average-risk population, in familial CRC, several environmental and lifestyle factors may increase the risk of malignancy, such as obesity, high intake of alcohol, cholesterol-rich diet, low consumption of green vegetables, low level of physical exercise, and smoking[13,14]. In contrast to what would be expected, family members of a CRC patient often do not seem to change their lifestyle, including physical exercise, smoking and eating/drinking habits[14].

Table 1 Hereditary syndromes associated with high risk of colorectal cancer and principal involved genes

Syndrome	Mendelian pattern	Gene
Lynch syndrome	Autosomal dominant	*hMLH1*
Familial adenomatous	Autosomal dominant	*APC*
Attenuated FAP	Autosomal dominant	*APC FAP*
MUTYH-associated polyposis	Autosomal recessive	*MUTYH*
Peutz–Jeghers syndrome	Autosomal dominant	*STK11*
Juvenile polyposis syndrome	Autosomal dominant	*SMAD4*

CRC in subjects with a family history seems to have a better prognosis, with a greater overall 5-year survival rate and an 11% reduction in the risk of death compared with those with no family history[15]. Further studies support a better prognosis in patients with a family history of CRC[16,17]. The reason for the survival advantage associated with familial CRC is not known. It could be that a family history of CRC leads to earlier detection of tumor and therefore a better prognosis. Indeed, the survival difference persisted when patients with or without a family history were matched by stage at diagnosis. An alternative explanation suggests a deficit of mismatch repair mechanisms in patients with familial CRC[15], which has been linked to a longer survival rate in CRC[18]. This hypothesis is based on the finding that patients with a family history of CRC have a high proportion of right-sided tumors, which frequently are associated with deficient mismatch repair mechanisms[18].

A first-degree relative (FDR), namely a family member who shares at least 50% of genes with a particular individual in the same family, such as parents, offspring and siblings, of a CRC patient is at higher risk of developing CRC[8]. Additional risk factors are age of tumor occurrence in the index case and the number of affected relatives[12,19], which contribute to increasing the CRC risk from moderate (1.5-2.5 times), when only one FDR is affected by CRC, to high (4-6 times), when two or more FDRs are affected or when cancer is diagnosed before age 50 years[8]. In a large population study[19] from the Utah database, including persons with a family history of CRC of ⩾ 3

generations, an increased number of affected FDRs was demonstrated to influence the risk much more than an affected second-degree relative (SDR) or third-degree relative (TDR). However, when combined with a positive FDR history, a positive SDR and TDR family history represents a further increase of risk.

An increased rate of colonic adenoma detection is also reported in individuals with a family history of CRC in comparison with average-risk subjects[20-26]. Colorectal adenoma > 10 mm, with high-grade dysplasia and/or a villous component, termed as advanced adenoma (ADA), is a precursor of CRC. Several colonoscopy-based screening studies[21-25] reported an increased prevalence of ADA in FDRs of CRC patients, ranging from 3.3% to 21.3%, in relation to average-risk subjects in whom it was defined as 1.9%-11.5%. A high prevalence of ADA has also been described among young FDRs aged 40-45 years, which increased with age[25]. Additional risk factors are male sex and the strength of family history, increasing the risk of developing CRC or ADA by 1.5-3.0-fold[27]. The number of FDRs affected also influences the risk of ADA, being higher in asymptomatic subjects with two FDRs with CRC diagnosed at any age in comparison to asymptomatic subjects with only one FDR with CRC at age < 50 years[22]. All these risk factors have to be taken into consideration in a screening program, in order to select a subpopulation of patients with highest risk, and in whom screening investigations could be indicated earlier than in subjects without these risk factors. According to these studies, United States scientific societies[28-32] suggest a different and more aggressive screening program in subjects with familial CRC in comparison to that recommended in average-risk populations.

Data on familial CRC screening from Asia confirm the increased risk in FDRs of CRC patients. A study from Taiwan[33] reported that among FDRs of patients with CRC, the risk of adenoma detected by colonoscopy was 2.5-fold and the risk of ADA was 4.5-fold higher compared with that in control subjects without a family history of CRC. Another study from Hong Kong[24] reported that the risk of detecting adenoma and advanced neoplasms in asymptomatic FDRs of patients with CRC was, respectively, 2.19-fold and 3.07-fold higher than in those with a negative family history of CRC. The increased risk is more marked if the index case were diagnosed with CRC before the age of 50 years.

FAMILIAL CRC SCREENING: WHEN TO DO

Screening programs are based on the assumption that the vast majority of CRCs develop from a benign precursor lesion, such as adenoma, through a series of genetic changes over a long-time period (adenoma-carcinoma sequence)[7,34]. It has been estimated that a small adenoma needs at least 10 years to become a cancer[7]. Thus, screening programs are aimed to identify these preneoplastic lesions using different tools, such as fecal occult blood test (FOBT), sigmoidoscopy, and colonoscopy. Screening recommendations take into consideration the so-called anticipation phenomenon, suggesting that CRC arises 10 years earlier in FDRs of CRC patients than in subjects without a family history[7,35]. Therefore, according to United States recommendations[29-32], screening interventions should be offered to individuals with a family history of CRC earlier than for the average-risk population. Subjects with a single FDR with CRC diagnosed at age > 60 years should receive a standard CRC screening, namely every 10 years, but starting at age 40 years. Individuals having one FDR with CRC before 60 years or two FDRs with CRC should be screened every 5 years, preferably by colonoscopy, starting at age 40 years, or at 10 years younger than the earliest case in the family[36]. In individuals with SDRs or TDRs with CRC, colonoscopy every 10 years is recommended, as in subjects at average risk. In contrast to United States recommendations, European guidelines[37] suggest performing an immunochemical FOBT every 1 or 2 years in subjects at average risk, and high-risk individuals should be referred for high-risk protocols. Although CRC screening is generally considered to be an effective way to reduce the incidence and mortality of CRC, the optimal screening strategy in high-risk populations is still debated, especially regarding the appropriate age at which to start screening colonoscopy, the time interval for repeat colonoscopy, and which diagnostic tool is preferred, according to different health policy organizations in different countries[29-32,37].

Asia-Pacific guidelines also recommend earlier screening in FDRs of CRC patients, that is, before 50 years of age[38]. A scoring system, based on several risk factors, such as age, sex, family history and smoking habit, has been developed by the Asia-Pacific Working Group for stratifying risk and prioritizing high-risk individuals for earlier screening[39]. According to this scoring system, validated in a 15-country multicenter Asian study on asymptomatic subjects, moderate-to-high-risk individuals should undergo colonoscopy, while those classified as average risk should undergo a fecal immunochemical test (FIT) followed by colonoscopy in case of a positive result[38].

FAMILIAL CRC SCREENING: WHAT TO DO

An ideal biochemical test for population screening should be specific and sensitive for both cancer and preneoplastic lesions, on easily collected samples, safely and cheaply transported to a centralized laboratory for accurate, reproducible, and cheap automated analysis. Unfortunately, no investigation

fulfills those criteria. Screening tests can be grouped into those detecting cancer, such as FOBT, and those revealing cancer and adenomatous polyps or nonpolypoid lesions, such as sigmoidoscopy and colonoscopy, which allow simultaneous removal of neoplastic precursors, providing greater potential for secondary prevention. Colonoscopy has been proposed as the preferred screening method, especially in high-risk populations[40-42], while both colonoscopy and FOBT have been recommended in CRC screening program in expert panel recommendations from various countries[30,37,43].

FOBT

Screening by FOBT has been tested in large, prospective, case-controlled studies in average-risk subjects, showing a significant reduction in CRC mortality[44-46]. In an Italian screening population study based on FOBT, an increased risk of ADA (OR = 1.53) was reported in subjects with familial CRC compared to those without a family history[26]. The rationale for the use of FOBT as a screening tool in the clinical diagnosis of CRC is based on the observation that small, macroscopically invisible traces of blood (occult blood) are released into the bowel lumen by colonic neoplastic tissue. However, FOBT cannot detect nonbleeding colonic preneoplastic lesions. The main limit of FOBT is the high number of false-positive results due to gastrointestinal bleeding associated with several causes other than colonic neoplasia, such as erosions, ulcers, inflammatory bowel diseases, or therapy with antiplatelet agents, anticoagulants or nonsteroidal anti-inflammatory drugs.

Two types of FOBT are available, guaiac-based tests (gFOBTs) and immunochemical tests (FITs). gFOBT is unable to distinguish human from non-human blood, contained in raw meat, and requires a restricted diet before stool collection. gFOBT is available in rehydrated and non-rehydrated form, according to the mechanism of the hydration of stool samples. The mechanism of rehydration increases the sensitivity, but decreases specificity, leading to more false-positive results. FIT is based on the use of monoclonal or polyclonal antibodies against the protein component of human globin, therefore, it does not require a specific diet. Several recent studies[36,47-50] on average- and high-risk population screening programs demonstrated a higher sensitivity but lower specificity of FIT in comparison to gFOBT (61%-69% and 91%-98% *vs* 25%-38% and 98%-99%, respectively) in detecting CRC.

Different cut-off values for fecal hemoglobin detection have been proposed to increase further the diagnostic capability of FIT in identifying early neoplastic lesions and ADA. Good sensitivity of FIT was demonstrated when the cut-off level for fecal hemoglobin detection was reduced from 250 to 50 ng/mL buffer[48]. FIT with a low cut-off level repeated annually for 3 years seems to have sensitivity in detecting both ADA and CRC in FDRs of CRC patients similar to that of a single colonoscopy[51]. Thus, FIT could increase screening acceptability in high-risk subjects and reduce the number of negative screening colonoscopy results[51]. The disadvantage of FIT is the cost, even if it is now approaching that of gFOBT, particularly for qualitative tests[52].

Few data regarding diagnostic accuracy of FOBT in familial screening programs are available. In a cohort study of asymptomatic high-risk patients with a personal history of adenomas/CRC or family history of CRC, sensitivity, specificity, positive predictive value and negative predictive value of single FIT sampling were 80%, 89%, 3% and 99.9% for CRC and 28%, 91%, 24% and 92% for ADA, respectively[53]. High accuracy of FIT was confirmed in a multicenter study among FDRs of CRC patients, in which AUC was 0.96 (95%CI: 0.95-0.98) for CRC and 0.74 (95%CI: 0.66-0.82) for ADA[54].

European guidelines[37] recommend the use of FIT as test of choice for population screening, although gFOBT could be more practicable and affordable than FIT, considering the local labor costs and the mechanism of kit distribution and collection.

Advantages and disadvantages: gFOBT and FIT are both simple noninvasive screening methods, cheaper with respect to other screening tests such as colonoscopy, and easy to perform in the general screening population. The only disadvantage of gFOBT or FIT is the low sensitivity for detecting cancerous and preneoplastic lesions.

Fecal DNA test

Fecal DNA test is a new screening method based on finding several specific tumor-related DNA changes in cells shed from colonic neoplastic lesions into the bowel[55]. Most studies published to date have focused on the feasibility and characteristics of the test rather than on the real impact on reduction of CRC incidence and mortality. Fecal DNA test has higher sensitivity but lower specificity than gFOBT for CRC detection. A stool-based test for methylation analysis of the vimentin (VIM) gene has been developed recently in the United States, showing a specificity and sensitivity of almost 80%. Several additional hypermethylated genes, including APC, p16, hMLH1, MGMT, SFRP1, SFRP2 and VIM, have been isolated from stool samples and utilized as biomarkers for detecting CRC or colorectal adenomas with a sensitivity of 62%-75%[56]. In another study[57] hypermethylation of fibrillin-1 (FBN1), detected in stool samples, showed a sensitivity of 72% and a specificity of 93% for detecting CRC.

Whether ADA can be reliably detected by fecal DNA test remains to be fully clarified. Despite a recommendation for its use by the United States Multi-Society Task Force on Colorectal Cancer[28], fecal DNA test has not yet achieved wide application, probably

due to its considerable cost.

Advantages and disadvantages: Fecal DNA test offers the same advantages but is more expensive than FOBT. How frequently fecal DNA test should be done to screen adequately for CRC remains to be determined.

Screening colonoscopy

The increased prevalence of CRC or ADA in FDRs of CRC patients, as mentioned above, represents the rationale for why screening colonoscopy is strongly recommended by several scientific societies[28-31] in members of families with an increased risk for CRC. The high rate of adenoma and ADA in the right colon of FDRs of CRC patients[22,58,59] and the occurrence of CRC in the right colon in 30%-40% of FDRs[60,61] indicate that an endoscopic assessment of the entire colon for screening purposes should be preferred to the limited exploration of the left colon. The usefulness of such a recommendation is confirmed by the growing evidence that colonoscopy-based screening programs are able to reduce CRC incidence and mortality. Two studies[62,63] reported that an increased use of lower gastrointestinal endoscopy led to a reduction in the incidence and mortality due to CRC in an average-risk population in the US. An Italian large population-based cohort study[64] showed that a 5-year colonoscopy-based screening for CRC in asymptomatic subjects achieved a decrease of 48% in CRC incidence and 81% in mortality. The reduction in CRC incidence was more evident in subjects who underwent complete colonoscopy[64].

However, several factors limit the use of colonoscopy as screening procedure, such as a high cost, possible occurrence of complications, and low acceptability. In a cost-effectiveness analysis[65] of different screening methods, such as FOBT, sigmoidoscopy, and colonoscopy, considering the number of prevented cases of CRC and the costs spent per life-year saved from cancer-related mortality, annual screening with FOBT was less expensive but saved fewer life-years than colonoscopy. A screening strategy based on sigmoidoscopy every 5 or 10 years is less cost-effective than FOBT and colonoscopy[66]. In prospective cohort studies[40,67-69] on asymptomatic adults undergoing colonoscopy, for screening or surveillance due to a history of CRC or adenoma, reported complication rates ranged from 0.79 to 8.4 per 1000 colonoscopies. Thus, the absolute risk of serious complications is low, even if it is higher than for FOBT or sigmoidoscopy. Finally, low acceptance of colonoscopy is still the main barrier to widespread dissemination for screening. Adherence to colonoscopy screening programs is low even in members of high-risk families, and varies from 18% to 78% in different countries. This low acceptability of colonoscopy in FDRs may have several reasons, such as invasiveness of the method, fear of feeling pain, and lack of information about the possibility to prevent CRC by simultaneous detection and removal of preneoplastic lesions. Therefore, more detailed information should be provided to subjects with a family history of CRC regarding the safety of colonoscopy and the possibility of performing the procedure under sedation. In this regard, general practitioners play a decisive role, especially in less-educated people who are less likely to obtain information in other ways[22,58,70,71].

High-quality colonoscopy is crucial to achieve good CRC screening, therefore several technical factors have to be taken into account[37]. Colonoscopy should be completed to the cecum, and withdrawal of the endoscope should be slow. The number of adenomas and ADA found during colonoscopy with a withdrawal time of $\geqslant$ 6 min is about twofold, or more, than that found with a shorter withdrawal time[72]. A 6-min withdrawal time is currently considered a standard of care. Screening colonoscopy has to be performed under conditions of good bowel cleansing, which means that, in the absence of completely removable residual tumor, the examination has to be repeated following a more intensive cleaning procedure. Of course, screening colonoscopy has to be performed by an expert, high-volume operator (> 300 colonoscopies per year), and photographic documentation of the ileocecal valve and cecum should be auditable[36,73].

Advantages and disadvantages: The main advantage of colonoscopy is the possibility to examine the entire colon and immediately remove a preneoplastic lesion. Disadvantages include the need for colonic lavage, which requires a low-residue diet on the days before the examination and oral intake of laxatives with a large amount of water. It is an invasive screening method and, therefore, is not easily accepted by asymptomatic subjects if not proposed under sedation.

Sigmoidoscopy

Flexible sigmoidoscopy (FS) is an endoscopic examination with maximum reach to the splenic flexure. When compared with no screening in average-risk populations, CRC mortality was lower with FS in comparison to FOBT[74]. In a systematic review and meta-analysis[75] of five randomized controlled trials, FS screening achieved a 18% relative risk (RR) reduction in the incidence of CRC (RR = 0.82, 95%CI: 0.73-0.91; $P < 0.001$), a 33% reduction in the incidence of left-sided CRC (RR = 0.67, 95%CI: 0.59-0.76; $P < 0.001$), and a 28% RR reduction in the mortality of CRC (RR = 0.72, 95%CI: 0.65-0.80; $P < 0.001$).

However, FS has no effect on the incidence of proximal colonic malignancy[76]. The combination of FS every 5 years with annual FOBT is better than either test used alone[29-31].

Advantages and disadvantages: FS is a less-invasive procedure than colonoscopy and requires easier preparation. The main disadvantage is that FS evaluates only the distal segments of the colon and, in case of a positive result, complete colonoscopy is necessary to examine the proximal colonic tracts.

Potential screening methodologies

Computed tomography colonography: Computed tomography colonography (CTC), also known as virtual or CT colonoscopy, is a low-invasive radiological method to study the colon with a low risk of complications. Thus, CTC could be an alternative to colonoscopy in CRC screening. Indeed, CTC is already used for screening purposes in patients with a positive FOBT result when colonoscopy is contraindicated or fails to reach the cecum for anatomical reasons[77]. CTC has a high sensitivity (approximately 95%) in detecting CRC[78] and colonic polyps > 10 mm[79], but sensitivity drops to 75%-80% for nonpolypoid adenomas ⩾ 5 mm[80]. Patients undergoing CTC are exposed to ionizing radiation, raising concerns about a possible increased risk for malignancy, and the need to perform colonoscopy if polyps or other possible neoplastic lesions are detected, with increased screening costs. To reduce the discomfort associated with bowel preparation, noncathartic CTC has been proposed as a screening method for CRC in FDRs, with good sensitivity and specificity for small adenoma (77% and 99%) and ADA (89% and 96%)[81]. Bearing in mind all these considerations, CTC is not yet considered for population screening programs.

Electronic nose: It is a new technology based on an array of nanosensors reacting to volatile organic compounds by a sensor-specific change in resistance. Volatile organic compounds are gaseous carbon-based chemicals derived from biochemical metabolism in the body, and in the bowel they are mainly produced by the intestinal microbiota and excreted by the feces[82]. Electronic nose has already been proposed as a potential noninvasive diagnostic biomarker test for lung cancer, breast cancer and malignant melanoma[83,84], and recently[85], it was shown to discriminate healthy subjects from patients with CRC (sensitivity and specificity: 85% and 87%, respectively) and patients with ADA (sensitivity and specificity: 62% and 86%, respectively). If diagnostic accuracy is confirmed, electronic nose could represent a new noninvasive method of screening for CRC and its adenomatous precursors.

DNA methylation blood analysis: It could be a valuable noninvasive diagnostic tool for CRC screening. Aberrant patterns of DNA methylation from CRC cells can be detected in blood and reflect DNA methylation profiles present in CRC tissue. The presence of aberrantly methylated septin 9 in plasma is a valuable and minimally invasive blood-based PCR test, showing a sensitivity and a specificity of almost 90% in detecting CRC[86].

Soluble CD26: Soluble CD26 (sCD26) is a transmembrane glycoprotein expressed in a variety of cell types and associated with neoplastic transformation. Being present in plasma, serum and other biological fluids, sCD26 has been proposed as a blood screening tool, showing a sensitivity of 39.6% for ADA and 42.1% for advanced neoplasms, achieving specificity of 90%. The combination of sCD26 and FIT increases the sensitivity for ADA and advanced neoplasms up to 52.8% and 56.1%, respectively, corresponding to 93.5% specificity[87].

ONGOING TRIALS ON COLORECTAL CANCER SCREENING

Two Italian trials are ongoing to compare colonoscopy or sigmoidoscopy *vs* CTC for CRC screening[88,89]. Data regarding acceptability, diagnostic yield, and costs of the methods emerging from these two studies will be helpful to understand better whether CTC may play a role in screening for CRC. An interesting trial[90] is ongoing to evaluate the importance of an enhanced family communication about genetic testing and hereditary risk information. The trial will evaluate the effectiveness of additional support using a randomized controlled design based on motivational interviewing; will apply an intervention for mutation carriers and counselees with relatives with an increased risk of developing cancer; and will involve relatives in the study.

CONCLUSION

CRC screening can reduce mortality and is cost-effective. Therefore, it is mandatory that clinicians and health organizations implement strategies to improve adherence to screening programs in subjects at average risk, but first of all in those having an increased CRC risk. To date, colonoscopy represents the best choice for a screening program. General practitioners and physicians should make efforts in counseling individuals at high risk of CRC to undergo this procedure, starting at 40-45 years of age. If not accepted, FOBT, preferably associated with sigmoidoscopy, has to be prescribed.

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EDITORIAL

Multimodal treatment of gastric cancer in the west: Where are we going?

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Abstract

The incidence of gastric cancer (GC) is decreasing worldwide, especially for intestinal histotype of the distal third of the stomach. On the contrary, proximal location and diffuse Lauren histotype have been reported to be generally stable over time. In the west, no clear improvement in long-term results was observed in clinical and population-based studies. Results of treatment in these neoplasms are strictly dependent on tumor stage. Adequate surgery and extended lymphadenectomy are associated with good long-term outcome in early-stage cancer; however, results are still unsatisfactory for advanced stages (Ⅲ and Ⅳ), for which additional treatments could provide a survival benefit. This implies a tailored approach to GC. The aim of this review was to summarize the main multimodal treatment options in advanced resectable GC. Perioperative or postoperative treatments, including chemotherapy, chemoradiotherapy, targeted therapies, and hyperthermic intraperitoneal chemotherapy have been reviewed, and the main ongoing and completed trials have been analyzed. An original tailored multimodal approach to non-cardia GC has been also proposed.

Key words: Epidemiology; Hyperthermic intraperitoneal chemotherapy; Chemotherapy; Radiotherapy; Gastric cancer; Targeted therapy

Core tip: In advanced gastric cancer (GC), multimodal treatment is currently an option in the west. Adequate surgery and extended lymphadenectomy, together with

modern chemotherapy, radiotherapy, targeted therapies, and a combination of all could possibly improve survival in advanced GC. A tailored multimodal approach is strictly necessary in the light of treatment results and recent epidemiological trends, which indicate a relative increase of more aggressive forms, such as proximal location and diffuse Lauren histotype in the west. The main ongoing and completed clinical trials regarding multimodal approach to GC have been reviewed, and an original tailored multimodal protocol to non-cardia GC has been proposed.

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CHANGING EPIDEMIOLOGY OF GASTRIC CANCER

Despite the reported declining incidence, gastric cancer (GC) is one of the most common causes of cancer mortality worldwide[1-3]. It represents the fourth most common cancer after lung, breast and colorectal cancer, and the second most common cause of cancer-related death after lung cancer. Geographic variability of GC is also well known: highest incidence rates are observed in East Asia, Central Asia, Eastern Europe, and the Pacific Coast of South and Central America, whereas the lowest incidence rates are found in Northern Europe and North America[4]. Even within the same country, there can be wide variation in geographic incidence: for example, in Italy, mortality is high in the central region, especially along the Central Apennine Mountains, and very low in Southern Italy[5,6]. Even if partly obscured by population aging, a decreasing incidence of GC has been reported worldwide in recent decades. This epidemiological trend has been attributed to several factors, such as the increased consumption of vegetables and fruit instead of cured meat, and changed methods of food conservation (refrigeration, instead of salt preservation)[7]. The decreased prevalence of *Helicobacter pylori* (*H. pylori*) infection has also had a role. However, decreasing rates are more evident in high-risk areas, whereas in low-risk areas, the rates have fallen slowly, with a trend to become stable over time[5,6,8,9].

Certain subtypes of GC demonstrate different epidemiological features. Tumors located in the distal third of the stomach have shown the most evident decrease in incidence, whereas proximal tumors are stable or even increasing[10,11]. This trend has been confirmed in some recent studies: the incidence decreased among men and women, but the proportion of cardiac tumors remained stable over time; 5-year survival worsened over time for patients with non-cardiac tumors, whereas the risk of death decreased for patients with cardiac tumors[12].

Different epidemiological trends in the intestinal type (IT) and diffuse type (DT) Lauren histotypes have also been observed. The declining incidence of GC has been linked to the decreasing number of ITs; on the contrary, the incidence of DT is generally stable throughout the world[10,13-15]. As most proximal tumors are IT, it is important, when evaluating epidemiological trends, to group data according to histotype and location. In a recent study from the Italian Research Group for Gastric Cancer (GIRCG), a decreasing number of IT tumors of the distal stomach was observed; on the contrary, IT located in the proximal third, and DT, at any location, were stable over time[9]. As a consequence, the DT neoplasms showed a relative increase with time (Figure 1).

Recent studies have also reported different trends of GC incidence in young patients; declining rates were observed for subjects aged 40-84 years, whereas for younger cohorts, the incidence rates increased over time[16]. Recent reports from Europe also confirm these findings[17]. The higher prevalence of DT in young patients may explain the epidemiological trends described for specific histotypes of GC.

As for GC prevention, two potential strategies are proposed. Primary prevention is possible due to eradication of *H. pylori*, and secondary prevention by detection of GC in mass screening[4]. Primary prevention is based on the fact that *H. pylori* is the strongest known factor associated with distal IT GC. It is possible to eradicate the infection using antibiotics in association with an antisecretory agent. It is proposed to offer prophylactic eradication for high-risk individuals, or for patients in high-risk areas.

For secondary prevention, mass screening is performed in countries with the highest incidence of GC. In Japan or South Korea the screening programs seem to be effective, with the higher rate of early GC detection, improved 5-year survival, and improved proportion of localized GC at diagnosis[4,17]. The main screening methods are barium X-ray, combination of barium digital radiography together with serum pepsinogen testing, and endoscopy with photofluorography. However, mass screening is hard to promote and organize in low-risk areas, where few but more advanced GC cases, mainly with proximal location or DT tumors, are generally observed in clinical practice[4].

CLINICAL IMPLICATIONS OF CHANGING EPIDEMIOLOGY

The above-mentioned epidemiological trends could have important clinical implications. Indeed, the overall number of newly diagnosed GC cases is decreasing, but the relative percentage of proximal locations and

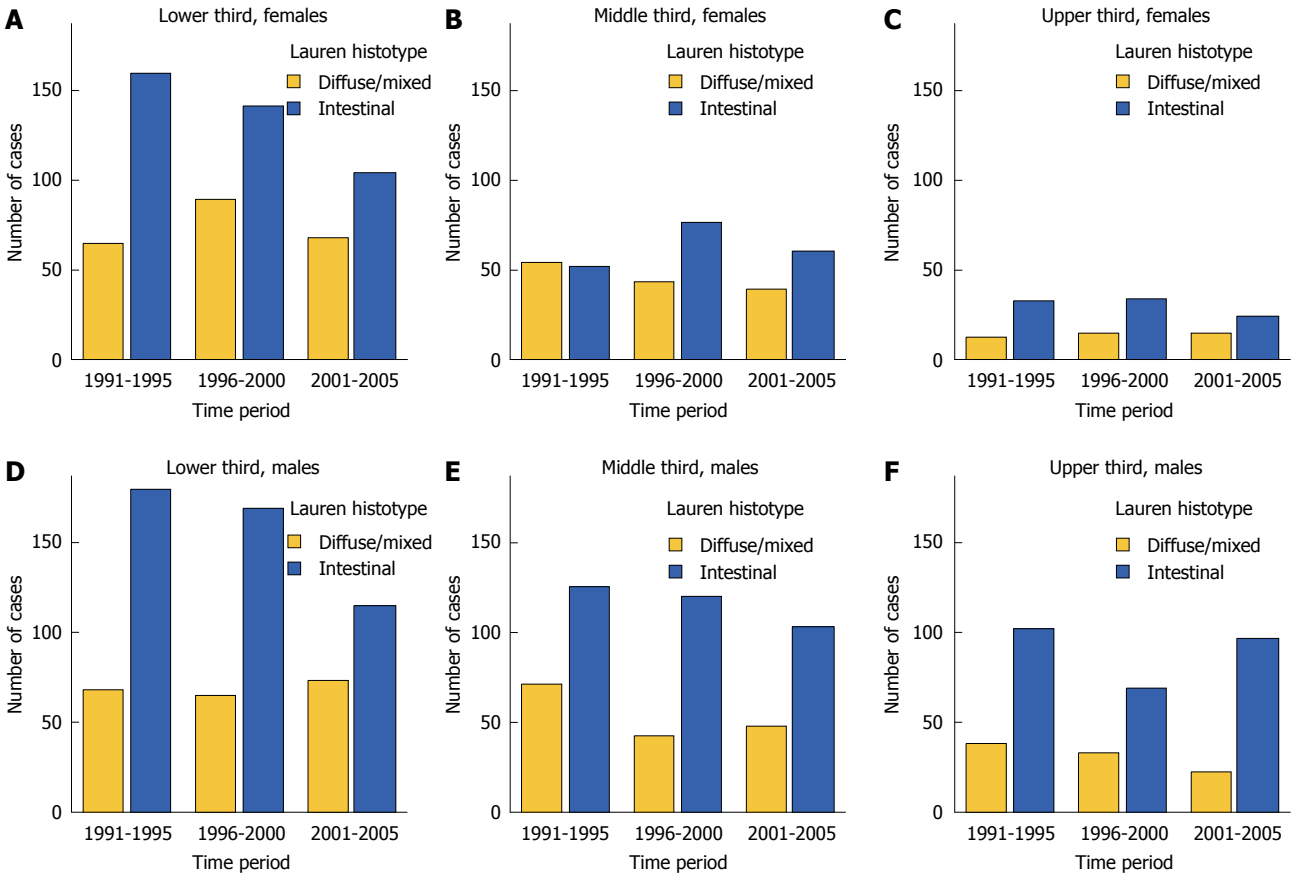

Figure 1 Changing number of patients in three subperiods, stratified according to tumor location and Lauren histotype (GIRCG database).

DT is increasing. Proximal tumors, including those involving the esophagogastric junction (EGJ), are associated with higher clinical aggressiveness and worse prognosis[9,18-21]. The relative increase in the proportion of proximal tumors could lead to a general decrease in survival probability.

Another important consequence of epidemiological trends is the relative increase in DT tumors (Figure 2). Besides histomorphometrical characteristics, IT and DT histotypes show evident differences in epidemiological, clinical and molecular features[22]. IT type is more common in males and older patients, whereas DT type usually affects younger patients with a lower male-female ratio. Environmental factors seem to be involved in the pathogenesis of IT tumors, and they usually follow the sequence of chronic atrophic gastritis, intestinal metaplasia, and dysplasia. On the contrary, DT tumors usually originate from healthy gastric mucosa or non-atrophic gastritis, and are more related to genetic factors. A further characteristic of the DT is their greater biological aggressiveness. The risk of lymph node metastasis is higher in the DT than the IT, at the same T stage. Indeed, the DT is a strong risk factor for lymph node metastasis in early GC[23], but an increased risk is also present in more advanced pT stages. The correlation between lymph node metastasis and Lauren histotype, stratified for pT stage, has been evaluated in 2090 non-cardia GC patients from the GIRCG database (Figure 3). The incidence and number of lymph node metastases were notably higher in the DT than IT groups at the same pT stage. Furthermore, DT is also a risk factor for lymph node metastases in extra-regional nodal locations (such as para-aortic nodes)[24,25].

The higher probability of lymph node metastases in DT could be an indication for more extended lymphadenectomy or neoadjuvant treatment. In contrast, clinical diagnosis, by radiological imaging, of lymph node metastasis may be more difficult in the DT. It has been reported that, in this histotype, the size of involved nodes may be smaller than the commonly used cut-off values[22].

Besides the lymph node involvement, DT tumors also show a greater propensity to peritoneal spread. Indeed, several studies have demonstrated a higher risk of peritoneal recurrence in DT tumors; mainly when the tumor has serosal involvement[22,26].

In a GIRCG follow-up study, the 5-year risk of peritoneal recurrence has been calculated to be 69% in DT GC with serosal involvement, *vs* 20% for IT cases at the same pT stage. It has been demonstrated that the clinical impact of extended surgery, including D2/D3 lymphadenectomy, is of low value in serosally exposed forms at risk of peritoneal recurrence[27,28].

The chance of cure in patients with peritoneal recurrence of GC is low: in a GIRCG follow-up study,

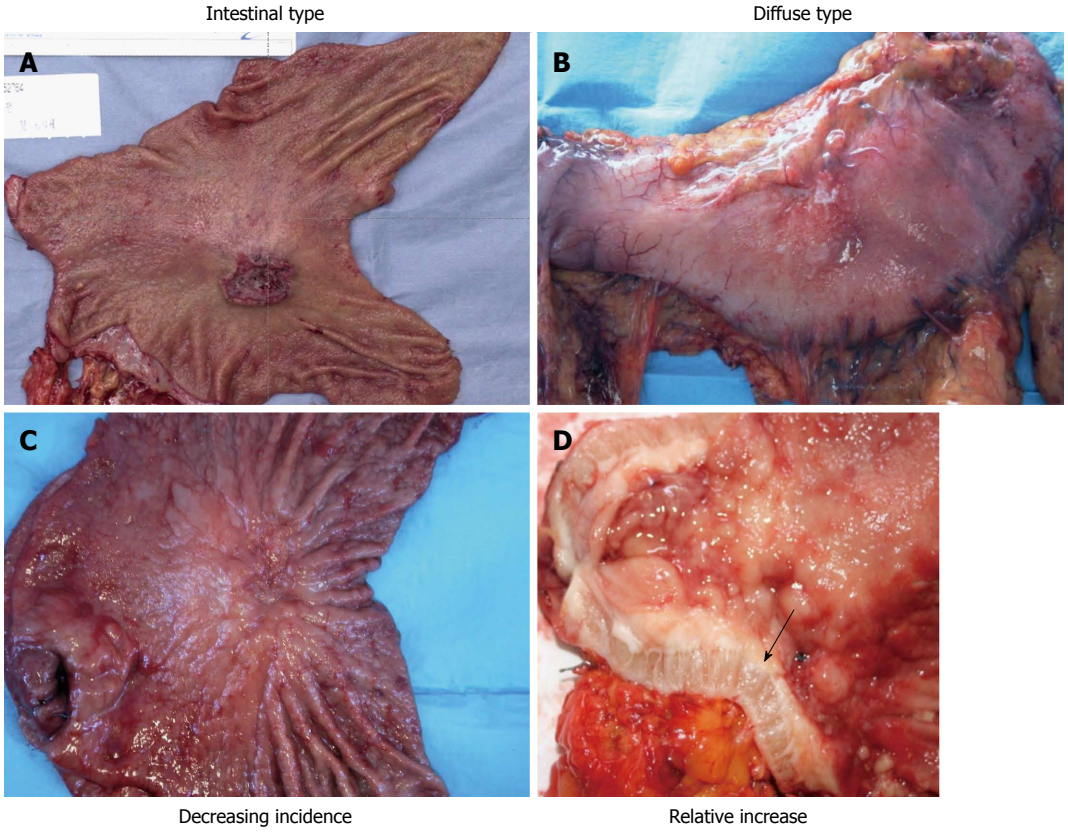

Figure 2 Images of intestinal (A, C) and diffuse type (B, D) tumors of the stomach. The arrow in D indicates the infiltrative growth of the diffuse histotype in the gastric wall.

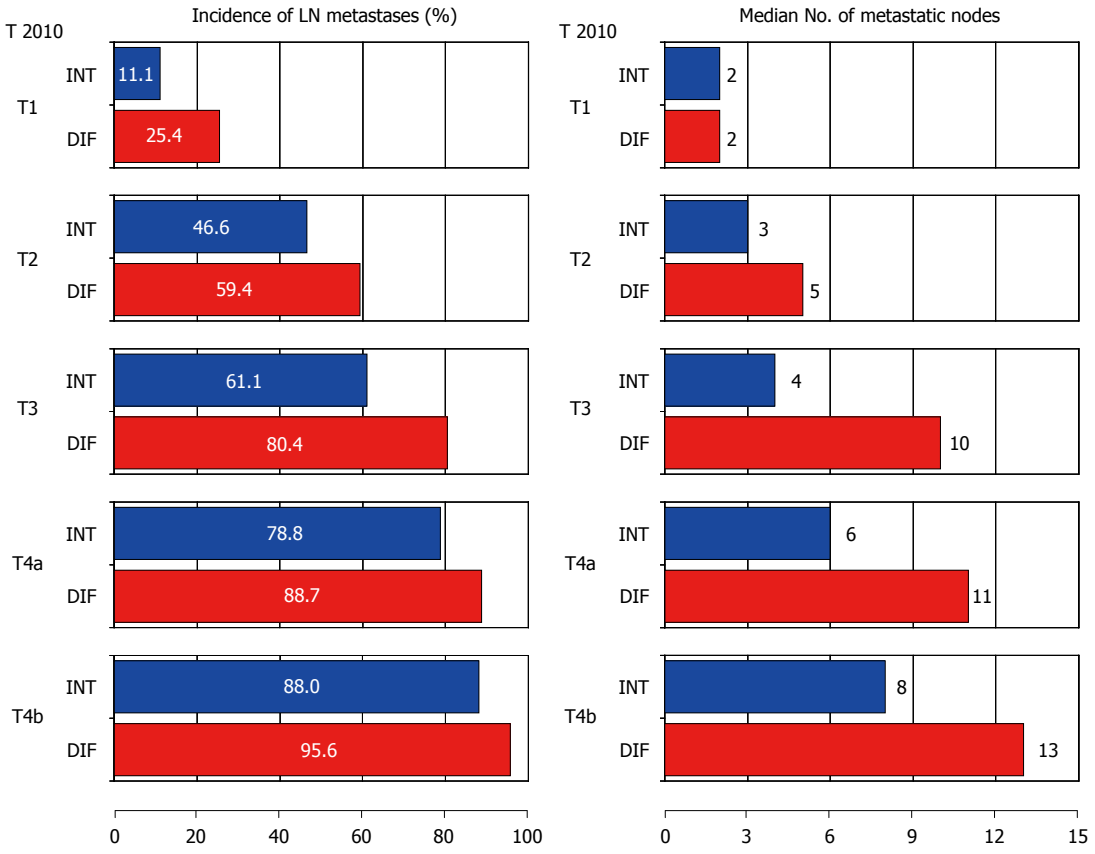

Figure 3 Incidence of lymph node metastases according to Lauren histotype stratified for pT stages (GIRCG database).

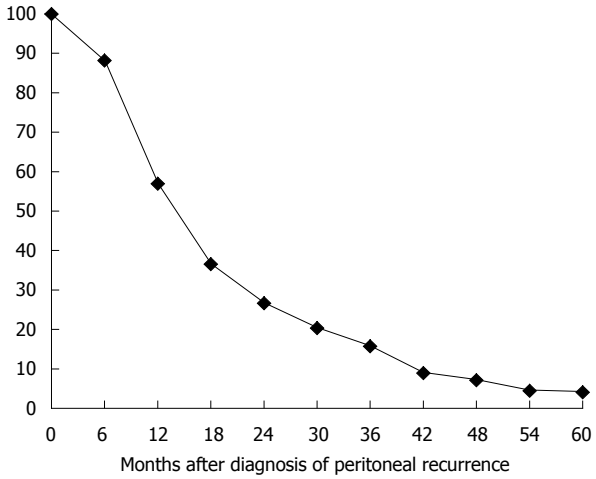

Figure 4 Survival rate of patients with peritoneal recurrence of gastric cancer.

5-year survival probability in 221 patients with metachronous peritoneal carcinomatosis (PC) was only 3% (Figure 4)[29]. As such, prevention of peritoneal recurrence, more than treatment after its occurrence, may be the only potential chance of cure in high-risk cases[30].

Late-phase DT GC can evolve into diffuse infiltration, thickening and stiffening of the gastric wall with reactive fibrosis, also named gastric linitis plastica. This is a subset of GC with a large propensity to diffuse infiltration, massive lymph node metastasis, and peritoneal seeding[31]. The rate of radical resection in this form of GC is < 30%, and, even after R0 resection, the 5-year survival probability does not exceed 5%. Some population-based studies from Europe, along with the decreased incidence of GC, have reported a significant increase of gastric linitis plastica with time[32]. These data are consistent with previously mentioned epidemiological trends.

Pathological characteristics of different histotypes of GC may explain epidemiological and survival data reported in large European studies. Recent data from 49 cancer registries in 18 European countries (EUROCARE-4 Working Group) have revealed a notable survival increase in Europe over the period 1988-1999 for several cancer sites, in particular, for prostate, colorectal and breast cancer. However, for GC, the increase was small (from 22% to 24%), despite potential time-related improvements in diagnosis, surgical and medical treatment[33]. Survival improvement was higher for men (4.1%) than women (1.4%). The declining incidence of cancers of the distal stomach could help to explain these survival trends. Indeed tumors of the cardia or fundus are usually diagnosed in older patients, at an advanced stage, and with diffuse/signet ring cell morphology. Other population-based and clinical studies reported similar results. In the previously mentioned French study, the global prognosis of GC did not improve significantly over a 12-year period of observation[32]. Recent studies from the Netherlands have also confirmed the decreasing incidence of GC but stable survival rates over time[34].

These data seem to be consistent with the findings of a previous GIRCG study: along with the decreasing number of distal IT tumors and the relative increase of DT forms with time, a lack of improvement of cancer-related survival probability, and a significant increase of peritoneal recurrence after surgery were observed[9]. In particular, survival rates decreased in the more recent period in the group of patients with serosal involvement, in women, and in distal tumors, whereas an increasing trend was observed in proximal tumors. All these data may fit with the hypothesis that the relative increase in DT tumors may have contributed to the absence or small improvement of treatment of GC in western countries.

TREATMENT OF EARLY FORMS

Surgical treatment with adequate lymphadenectomy could offer a high probability of cure even in western patients. Survival rates in early stages reported from specialized western centers are similar to those obtained in Japan and South Korea[21,27,35].

Selected forms of early GC can be treated by endoscopic mucosal resection or endoscopic submucosal dissection, in accordance with the standard criteria described by the Japanese Gastric Cancer Association (JGCA), with acceptable results even in the west[36,37]. The resection is judged as curative when all of the following conditions are fulfilled: *en bloc* resection, tumor size not greater than 2 cm, histology of intestinal-differentiated-type, pT1a, negative horizontal (lateral) margin, negative vertical margin, and no lymphovascular invasion.

Although endoscopic approaches to early forms of GC are increasing in specialized centers in the west, they are still far from becoming a clinical standard. Early forms not treatable by endoscopic resection should be submitted to surgical resection with lymphadenectomy. According to the JGCA treatment guidelines[36], D1 lymphadenectomy may be adequate for early GC with clinically negative lymph nodes. However, we should underline that a proportion of early GC in the west is DT, which is associated with a higher risk of lymph node metastases and greater lymph node spread, especially when submucosa is involved. Furthermore, in the west, endoscopic resection, which can be considered as a treatment as well as a staging procedure, is performed less frequently than in East Asia, and the clinical diagnosis of lymph node metastasis by imaging procedures still has low accuracy[38]. As such, the Italian guidelines advise standard D2 lymphadenectomy in early forms of GC[39]. Only in selected cases (high-risk patients, early forms with favorable pathological characteristics, not treatable by endoscopic resection) should more

limited procedures be considered (D1 plus).

Early forms of GC could also be treated by minimally invasive (laparoscopic or robotic) approaches, which demonstrated non-inferior oncological results compared with open surgery[40,41]. However, it should be emphasized that oncological criteria regarding resection margin and lymph node dissection need to be carefully followed in minimally invasive procedures.

TREATMENT OF ADVANCED RESECTABLE FORMS

In advanced resectable forms of GC, it is now well established that adequate surgical treatment is a key factor in obtaining acceptable long-term results. As for the extent of resection, subtotal gastrectomy offers low postoperative morbidity and mortality risk, and better quality of life, without affecting long-term oncological results, when an adequate resection margin can be obtained (R0 resection)[42]. A proximal margin of at least 3 cm is recommended for T2 or deeper tumors with an expansive growth pattern, and 5 cm is recommended for DT and tumors with infiltrative growth pattern. In all other cases, total gastrectomy should be the preferred procedure. In early GC, a resection margin of 2 cm may be enough[39]. Total gastrectomy with splenectomy should be also recommended for tumors located along the greater curvature. Splenectomy should be performed only when macroscopic involvement of lymph nodes at the splenic hilum is present.

The extent of lymphadenectomy is crucial. Even if some randomized studies have failed to demonstrated a significant advantage for overall survival, a re-evaluation of the Dutch trial showed a reduced cancer-related survival in the long term and a higher incidence of late recurrence of GC in patients submitted to limited (D1) lymphadenectomy[43].

It is important to ensure that good early post-operative results in terms of morbidity and mortality are achieved. This is consistent with the reports of observational nonrandomized studies from specialized centers[44,45].

Nowadays, D2 lymphadenectomy is generally accepted as the standard approach in most national guidelines[39,46]. The correct procedure for lymphadenectomy involves the removal of nodal stations from 1 to 12, with some variations depending upon the extent of gastric resection[36]. Special attention should be paid upon to the complete removal of infrapyloric nodes (station 6), right paracardial nodes (station 1), left gastric artery nodes (station 7), celiac axis (station 9), hepatic artery (station 8a), splenic artery (station 11), and hepatoduodenal ligament nodes (station 12a).

More extended lymphadenectomies (D2+) can be performed in selected cases at risk of metastasis to posterior (stations 8p, 12p, 12b and 13), mesenteric (station 14) or para-aortic (stations 16a2 and b1) lymph nodes, in specialized centers and in the setting of clinical studies[22,23]. In particular, proximal or DT tumors are particularly prone to metastasis to distant nodes, and in our opinion they may benefit from super-extended lymphadenectomy[25,28]. However, it should be emphasized that in more advanced stages (UICC TNM stages ⅢA and more) the results of surgery, even with adequate lymphadenectomy, are still unsatisfactory in western patients[35]. As such, additional treatments should be planned to improve long-term survival in these patients.

MULTIMODAL TREATMENT OF GASTRIC CANCER

Neoadjuvant treatment seems to be a good option in advanced GC. The term advanced should be understood as a T3, T4 and/or N+ and/or with positive peritoneal cytology. The majority of patients who are diagnosed at this stage might receive benefits from perioperative treatment.

Even though dietary changes and the use of antibiotics to treat chronic *H. pylori* infection have helped to reduce steadily the number of new cases of GC, the progress in GC treatment is still limited[47]. Surgery remains the only treatment with curative intent in locoregional disease. From an oncological point of view the issue is to resect the cancer with a negative resection margin (R0), and with adequate lymph node dissection. The biggest problem, especially in the west, is diagnosis of patients with locally advanced disease. Advanced disease is associated with a higher rate of locoregional recurrence. For locally advanced forms, additional multimodal treatment in the preoperative, perioperative and postoperative phases has been proposed. Nowadays, we can observe geographic differences in multimodal treatment of GC. In Asia, the most commonly used treatment is adjuvant chemotherapy; in the United States, the favored treatment is chemoradiotherapy (CRT); and in Europe, neoadjuvant therapy is mostly used.

Advanced GC still has a poor survival (< 30% 5-year survival probability for stage Ⅲ). Cunningham *et al*[48] and Ychou *et al*[49] have demonstrated the advantage of starting multimodal treatment with preoperative chemotherapy over surgery alone, therefore, this seems to be a good treatment option. In the trial by Schuhmacher *et al*[50], neoadjuvant therapy improved R0 resection rate even though it did not improve overall survival (OS). In the study by Stahl *et al*[51], neoadjuvant CRT showed a higher rate of complete responders, and in the study by van Hagen *et al*[52], improved OS was observed.

In Asian countries in contrast, the greatest interest lies in postoperative oral chemotherapy, which is associated with improved OS compared with surgery alone[53,54]. However, these results have not been

reproduced in western countries.

In the United States, CRT has been used routinely since 2001, after the trial of MacDonald *et al*[55].

Neoadjuvant chemotherapy

The neoadjuvant approach is currently recommended across Europe based on the Magic and FNLCC/FFCD trial[48,49]. Other benefits of neoadjuvant chemotherapy (NC), discussed by Ott *et al*[56], for potentially resectable GC are higher rate of R0 resection achieved by downstaging of a primary tumor, and probable effect on micrometastases and isolated tumor cells in lymph nodes. Ott *et al* emphasized also that the neoadjuvant setting is more often proposed for younger patients and those in general good health.

In the Magic trial, chemotherapy consisted of three cycles of intravenous (i.v.) epirubicin, cisplatin and 5-fluorouracil (FU) preoperatively and three cycles postoperatively[48]. NC was not associated with worse postoperative complications and 30-d mortality than surgery alone, thus overturning the argument that neoadjuvant therapy may be more dangerous for patients. From the main results, 5-year survival rate was 36% *vs* 23% in favor of perioperative chemotherapy. Also, OS and progression-free survival (PFS) were significantly better. Only 49.5% of patients received the full perioperative chemotherapy treatment, therefore, this was one of the main issues criticized by some investigators. This issue was investigated in the study by Mirza *et al*[57] in which it was checked in patients using the same regimen as in the Magic trial. The full perioperative regimen had a beneficial effect on DFS but not on OS. It may be concluded that administrating the adjuvant part of this regimen postponed tumor recurrence rather than helping in prevention.

The FNLCC/FFCD trial proved the beneficial effect of perioperative chemotherapy for gastric and esophageal adenocarcinoma[49]. In the preoperative period, two or three cycles of i.v. cisplatin and 5-FU were administered, and after surgery, chemotherapy was continued when response to treatment was observed. A higher rate of R0 resection in NC in comparison with surgery alone was observed, as well as improved OS and DFS. The 5-year survival rates were 38% *vs* 24% in favor of NC.

In a meta-analysis by Ronellenfitsch *et al*[58] OS was 9% better after neoadjuvant therapy. This effect was seen 18 mo after surgery and lasted at least 10 years. R0 resection was achieved 1.4 times more often after neoadjuvant treatment. Importantly, side effects of neoadjuvant therapy, such as postoperative morbidity or mortality, as well as prolonged hospital stay, were not increased significantly compared with surgery alone. Another interesting aspect was that no benefit of neoadjuvant therapy was seen in elderly patients. The subgroup of patients with EGJ cancer had the greatest benefit in OS. One of the unanswered questions is the age of patients recruited to the trial. Most trials excluded patients aged > 70 years. This issue is currently under investigation by a study in Germany. Another subgroup of patients of particular interest is those with signet ring cell carcinoma. They seem not to benefit from neoadjuvant treatment[59]. The response rate differs also according to pathological features. In DT tenors, a good pathological response was only observed in 14.5% of patients[60].

In Asian countries, neoadjuvant treatment is also beginning to play an important role. Currently several trials (JCOG 0210, JCOG 0501, JCOG 1002, and PRODIGY) are under way. In Italy, a GIRCG phase Ⅱ trial recruited patients with non-cardiac GC who underwent accurate pretreatment clinical staging with diagnostic laparoscopy and peritoneal washing, followed in all cases by standard D2 gastrectomy. This trial aims to answer whether preoperative or perioperative chemotherapy plays a role in advanced GC treatment (NCT01876927).

Neoadjuvant CRT

As NC proved to be safe for preoperative treatment, the addition of radiotherapy to preoperative treatment has gained interest. The German POET trial compared NC *vs* CRT for locally advanced EGJ cancers[51]. In one arm, two courses of cisplatin, 5-FU and folic acid (PLF), followed by 3 wk of combined CRT (30 Gy in 3 wk with cisplatin/etoposide), and surgery were administered, *vs* 2.5 courses of PLF with surgery. This trial was closed early, and showed no significant difference in survival: 33.1 mo *vs* 21.1 mo in favor of CRT, but with higher mortality in the CRT arm: 10.2% *vs* 3.8% (P = 0.26). Results regarding 3-year survival showed an improvement from 28% to 48% in the CRT arm.

In a study by Burmeister *et al*[61] on 75 patients, the addition of radiotherapy increased the rate of pathological complete remission (13% *vs* 0%, P = 0.02), and reduced the rate of R1 resection (0% *vs* 4%, P = 0.04). Analyzing 5-year OS and PFS, only a trend was observed in favor of CRT, without statistical significance (OS 45% *vs* 36%, P = 0.6).

In the CROSS trial from the Netherlands, patients with esophageal and EGJ cancers were assigned to CRT (carboplatin, paclitaxel and 41.4 Gy radiotherapy in 23 fractions) followed by surgery, *vs* surgery alone[52]. The surgery alone arm showed R0 resection in 69% of patients with a median survival time of 24.2 mo, whereas in the neoadjuvant CRT arm, R0 resection was achieved in 92% (P < 0.001), with complete pathological response rate in up to 29% of patients; however, it is noteworthy that in case of squamous cell carcinoma the complete response rate was better (49%) than for adenocarcinoma (23%). The median survival time was 49.4 mo (P = 0.003), and 5-year survival improved from 34% to 47%. Postoperative complications rate and in-hospital mortality were similar in both arms (4%).

The neoadjuvant regimen also reduced locoregional recurrence rate (34% to 14%; $P < 0.001$), and the probability of PC (14% to 4%; $P < 0.001$). Distant metastases also showed a difference between both arms (35% *vs* 29%, $P = 0.025$). This treatment protocol is now recommended for neoadjuvant CRT in patients with EGJ adenocarcinoma in the US. The currently ongoing TOPGEAR trial is investigating CRT *vs* chemotherapy in EGJ and stomach cancers. In the chemotherapy arm, three courses of epirubicin, cisplatin and flurouracil (ECF) are given preoperatively, and in the CRT arm, two courses of ECF followed by 45 Gy, or radiation with concurrent 5-FU. Patients in both arms receive three courses of ECF after surgery.

A meta-analysis by Sjoquist *et al*[62] reviewed trials with localized gastroesophageal adenocarcinoma with preoperative CRT and chemotherapy alone. The hazard ratio for OS was 0.75.

Adjuvant chemotherapy

Analyzing data from different countries, the results of adjuvant chemotherapy after gastrectomy in western studies are less convincing than in Asian studies. In a Japanese study (ACTS-GC trial), oral fluoropyrimidine (S-1) was given after surgery for 1 year, and results were compared with surgery alone. The 5-year OS was 70.1% *vs* 61.1%[53,54]. This trial was stopped earlier because of significantly better OS in the S-1 group. It needs to be underlined that the high rates of OS in both arms were due to excellent surgery, as D2 lymphadenectomy was confirmed in all cases. The problem in translating this trial into a Caucasian population is that Tegafur, present in S-1 as a precursor of 5-FU, is transformed in the body by cytochrome P450 to 5-FU. The probable difficulties observed in Caucasians are due to polymorphism of CYP2A6 gene, and subsequent complications[63]. In the FLAGS trial, comparison of cisplatin + S-1 and cisplatin + 5-FU for palliative therpy showed significantly better tolerance in patients with the addition of S-1[64].

In the CLASSIC trial, adjuvant chemotherapy with capecitabine and oxaliplatin after curative D2 gastrectomy was compared with surgery alone[65]. This Asian trial showed significant improvements in 3-year disease-free survival (DFS; 74% *vs* 59%, $P < 0.0001$), and OS (83% *vs* 78%, $P = 0.0493$). This trial was stopped earlier as the benefit of using this chemotherapy regimen was demonstrated. In the chemotherapy arm, oxaliplatin-induced peripheral neuropathy occurred in 56% of patients, but grade 3/4 only occurred in 2% of cases. It seems that this regimen might be an alternative to the S-1 regimen. A meta-analysis on 17 trials of adjuvant chemotherapy after gastrectomy showed a small but significant benefit for 5-FU-based chemotherapy[66]. Adjuvant chemotherapy increased OS by 6%, and reduced the risk of death by 18%. A meta-analysis by Zhang *et al*[67] showed that four chemotherapy regimens may be effective: 5-FU + mitomycin C + adriamycin; 5-FU + mitomycin C; tegafur; and mitomycin C. Other proposed regimens seem to be not so effective: 5-FU + carmustine, 5-FU + methyl-semustine, 5-FU + cisplatin, 5-FU + anthracyclines, and 5-FU + mitomycin C + cytarabine. Another meta-analysis by the GASTRIC group (Global Advanced/Adjuvant Stomach Tumor Research International Collaboration) showed significant improvement in OS after 5-FU-based chemotherapy[66]. The same group in another meta-analysis on advanced GC concluded that experimental arms of chemotherapy are responsible for modest improvement in OS and DFS (hazard ratio 0.88 and 0.81). The median survival was below 1 year and none of the new regimens can be used as a standard[68].

Adjuvant CRT

The results of the INT-0116 trial by MacDonald *et al*[55] show that adjuvant CRT plays an important role in GC treatment. The problem of additional radiotherapy is the increased toxicity rate. Grade 3/4 hematological toxicity occurred in 54%, and gastrointestinal toxicity in 33% of patients. The toxic effect was also responsible for stopping the treatment in many cases. In patients with diffuse histology, the addition of radiotherapy did not confer any additional benefit. The biggest concern is about radiation of a large area of gastrointestinal mucosa. Current studies are focused on using 3D conformal and intensity-modulated radiation therapy (IMRT), and also new, safer radiotherapy techniques[69-71]. In a phase Ⅱ trial with 3D-CRT/IMRT, grade 3 or 4 nausea and vomiting (14.5%), decreased appetite (11.8%), leukopenia/neutropenia (9.1%) and fatigue (6.4%) were observed, and it proved to be a safe procedure[71]. We also need to mention that in the MacDonald *et al*[55] trial, an increased number (but not significant) of secondary malignancies after additional CRT were reported. The biggest challenge is to prove whether addition of radiotherapy to the regimen is better than chemotherapy alone. This issue was analyzed in the ARTIST trial[72]. No difference in 3-year DFS was observed between those two arms, but analyzing subgroups with lymph node metastases, 3-year DFS was improved in the CRT arm (77.5% *vs* 72.3%, $P = 0.0365$). This was also seen when adjusting for tumor stage. No difference in case of local or distal recurrence rate was observed. No OS results were reported in the 3-year analysis. The ARTIST-Ⅱ trial will investigate the influence of chemotherapy or CRT in patients with lymph-node positive GC. One particularly interesting aspect is that, in the INT-0116 trial, D2 resection was performed in only 10% of cases, whereas in most Asian studies, it is close to 100%. Indeed, local recurrences were observed in 29% of cases in the INT-0116 trial, *vs* 2.8% in the Japanese ACTS-GC trial. It seems that the addition of radiotherapy confers a potential benefit to patients with a suboptimal surgical approach. This was

Table 1 Main trials regarding adjuvant and neoadjuvant therapy for gastric cancer reported in literature

Trial name	Therapy	Treatment arms	Tumor position	OS *P* vaule	PFS/DFS *P* vaule
Neoadjuvant CT					
MAGIC[48]	CT	Perioperative Res. *vs* mult	GC + EGJ	0.009	< 0.001
FNLCC/FFCD 9703[49]	CT	Perioperative Res. *vs* mult	GC + EGJ	0.021	0.003
EORTC 40954[50]	CT	Preoperative Res. *vs* mult	GC + EGJ	0.466 NS	0.200 NS
Neoadjuvant CRT					
POET[51]	CRT	Preoperative CRT *vs* mult CT	EGJ (I, Ⅱ, Ⅲ)	0.07 NS (3 yr)	0.060 NS (3 yr)
CROSS[52]	CRT	Preoperative Res. *vs* mult CRT	Esophagus + EGJ I, Ⅱ, Ⅲ	0.003	< 0.001
Adjuvant CT					
ACTS-GC[53,54]	CT	Postoperative Res. *vs* mult	Not given	0.002	< 0.001
CLASSIC[65]	CT	Postoperative Res. *vs* mult	GC + EGJ	0.049 (3 yr)	< 0.0001 (3 yr)
Adjuvant CRT					
INT 0116[55]	CRT	Postoperative Res. *vs* mult	GC + EGJ	0.005	< 0.001
ARTIST[72]	CRT	Postoperative Res. *vs* mult	GC	Not given	0.0824 NS (3 yr)

CT: Chemotherapy; CRT: Chemoradiotherapy; GC: Gastric; EGJ: Esophageal gastric junction; OS: Overall survival; PFS/DFS: Progression-free survival/disease-free survival; Mult: Multimodal treatment; Res: Surgical resection alone.

proved by a Dutch study, showing a reduction in local recurrences after CRT in patients with D1 resection, whereas this effect was not seen in the D2 resection group[73].

The problem of GEJ region radiotherapy is described later. The main difficulty is that these patients are subgroups in esophageal cancer and GC trials. Some of these problems were mentioned above, for example, in a neoadjuvant setting as in the CROSS trial[52]. After GEJ surgery, additional CRT is based on the INT-0116 trail (approximately 20% of patients in this trial had a GEJ location)[55]. In the current AJCC staging, GEJ tumors are staged as esophageal and not as gastric. The only trial exclusively for GEJ tumors was done in Germany, analyzing neoadjuvant CRT *vs* chemotherapy alone[51]. There was a higher rate of complete pathological response (15.6% *vs* 2%), and a trend towards improved 3-year survival (47% *vs* 28%, $P = 0.07$) in favor of neoadjuvant CRT[51].

The German trial also tried to identify those patients who would benefit from neoadjuvant therapy using positron emission tomography/computed tomography[74]. The MUNICON study tried to predict response after 2 wk of NC in GEJ cancer. Non-responders to chemotherapy underwent surgery, sparing them from unnecessary toxicity, as well as undergoing surgery earlier. It should also be noted that most GEJ tumors are fluorodeoxyglucose (FDG) sensitive, but in 30% they do not take up FDG[75]. The solution might be to use radioisotopes such as fluorothymidine for GC[76]. The most important studies from multimodal GC treatment are presented in Table 1.

Targeted therapies

The new drugs that may be used in targeted therapies probably play an increasing role in modern treatment of GC. Epidermal growth factor receptor (EGFR) is overexpressed in most GC. The trials that used anti-EGFR antibody cetuximab (EXPAND trial), and panitumumab (REAL3 trial) failed to improve survival in GC patients[77,78]. In REAL3, panitumumab was shown to actually worsen survival of treated patients[78]. Another antibody tested in an adjuvant setting in GC is bevacizumab against vascular endothelial growth factor A. In the AVAGAST trial, this antibody did not improve OS when added to standard chemotherapy[79]. Overexpression of human epidermal growth factor receptor-2 (HER-2/neu) is present in > 20% of patients with GC. An antibody against this receptor - trastuzumab - showed significant improvement in OS in metastatic gastric and GEJ cancers in the ToGA trial[80]. The oral antibody lapatinib is currently being investigated for HER-2-positive GC in the LOGIC trial. Currently, we await the results of ongoing trials using molecular-targeted drugs in GC: LOGIC (lapatinib), TYTAN (lapatinib), RAINBOW (ramucirumab), GRANITE-1 and GRANITE-2 (everolimus). We also need to mention that currently many drugs are being tested in phase Ⅰ and Ⅱ trials, such as the recently finished phase Ⅱ trial of apatinib, with promising results[81,82]. From the molecular point of view, the greatest interest lies in drugs that will

be effective against VEGR2, c-MET, FGFR1, 2, HER2, HER3, and members of the PI3K/AKT/mTOR pathway.

Hyperthermic intraperitoneal chemotherapy

In advanced cases PC of gastric origin is a condition with poor prognosis, with a mean survival range of 2.2-8.8 mo and no 5-year survival probability[30]. The peritoneal surface is a preferential site of GC dissemination. The current lack of efficient systemic therapy has led many clinicians to combat this localized disease by intraperitoneal administration of cytotoxic agents (intraperitoneal chemotherapy; IPEC). Other possible delivery options have been described, like perioperative normothermic intraperitoneal chemotherapy (NIPEC), hyperthermic intraperitoneal chemotherapy (HIPEC), early postoperative intraperitoneal chemotherapy (EPIC), and delayed postoperative intraperitoneal chemotherapy (DIPEC)[83]. As Spratt in 1980 proposed HIPEC with additional cytoreductive surgery, this new therapeutic option began to play an important role in advanced GC[84]. The advantage of HIPEC in comparison with other ways of delivering IPEC is the combined effect of cytostatic drug and heat, which results in a greater cytotoxic effect on the cancer cells[30]. Neoadjuvant as well as adjuvant treatment showed a potential benefit in decreasing rates of PC[85]. Initial IPEC studies showed that patients receiving chemotherapy intraperitoneally with mitomycin C, but also cisplatin and 5-FU had better OS after curative resection of locally advanced GC[86]. After the first report by Fujimoto *et al*[87] regarding HIPEC in patients with secondary PC, others have used that technique for PC of GC origin. In one of the biggest studies on 107 patients treated with HIPEC, Yonemura *et al*[88] showed that patients who underwent complete resection had better 5-year survival than those with residual disease (13% *vs* 2%). The completeness of resection was an independent prognostic factor[89,90]. A French multi-institutional study on 159 patients showed that radical resection and HIPEC were associated with a 5-year survival rate of 23%[83]. However, it should be emphasized that only a small proportion of patients who underwent complete macroscopic cytoreduction (R0 or R1) had a chance of survival in that study.

Another issue is PC after radical gastrectomy. The peritoneal surface is the most common site of GC recurrence after surgery. After curative resection, PC may occur in 20%-50% of cases, and rises up to 80% in cases with positive peritoneal cytology[91,92]. The biggest problem is that adjuvant intravenous chemotherapy or radiotherapy does not improve survival in patients at high risk of PC. Only IPEC may prevent the development of PC, and addition of hyperthermia synergistically with some drugs increases the depth of penetration into the tissue[30].

At least two meta-analyses have studied IPEC. In the first by Xu *et al*[93] of 11 randomized clinical trials, seven compared surgery + HIPEC *vs* surgery alone. IPEC was superior after curative surgery *vs* surgery alone, and combination of HIPEC and activated carbon particles was significantly better than other drug combinations. The second meta-analysis, by Yen *et al*[94], reviewed all clinical trials of IPEC. Among 13 trials, four of them investigated the efficacy of HIPEC, five NIPEC, two EPIC, two combined HIPEC and EPIC, and finally, two trials reported the combined effects of DIPEC. All data form 1648 patients showed a significant difference in survival of patients treated with HIPEC, or HIPEC together with EPIC. A trend toward survival improvement was observed with NIPEC. No benefit was seen using EPIC or DIPEC. In our opinion, the addition of HIPEC may provide a survival benefit in patients at high risk of PC after gastrectomy, such as patients with diffuse-mixed type, serosal invasion, or positive peritoneal cytology. HIPEC is an effective treatment in patients with free cancer cells and cancer microfoci, but becomes less effective as the tumor size increases, and the disease is disseminated[30]. A new trial is ongoing to prove the effectiveness of HIPEC during curative gastrectomy in case of positive peritoneal cytology (GASTRICHIP trial). This new perspective can probably assist wider usage of HIPEC to prevent further PC.

Metastatic GC

GC is often diagnosed as an advanced disease, especially in western countries where no screening for early diagnosis is used. The surgical resection of all pathological tissues is essential for curative treatment, and in most cases of advanced disease, it is not possible. Palliative chemotherapy for stage Ⅳ GC is a treatment of choice. Because of improvement of modern chemotherapy, better response, and usage of surgical techniques, survival of stage Ⅳ GC has improved during recent decades. The biggest question is who will benefit from more aggressive treatment, especially keeping in mind that extended survival is important, as well as patients' quality of life (QoL)[95]. The role of surgery even in primarily incurable disease has increased because some patients who respond well to chemotherapy might be restaged and eventually undergo surgery. Unfortunately, the outcomes measured in most studies are limited to survival. Surgical palliation should be defined as a treatment that relieves symptoms or improves QoL[96]. Surgical resection that does not remove all pathological masses should be named as noncurative rather than palliative. In the SEER database of 23830 patients with stage Ⅳ disease, surgery was offered to 45.7% of patients. Overall, the median survival was only 4 mo. The surgical approach is associated with some survival advantages compared with other palliative treatments. In the study by Li *et al*[97] on a group of 253 synchronous GC metastases, 5-year survival was 6.5% for patients with resection *vs* 0% without surgery. Multivariate analysis proved that patients

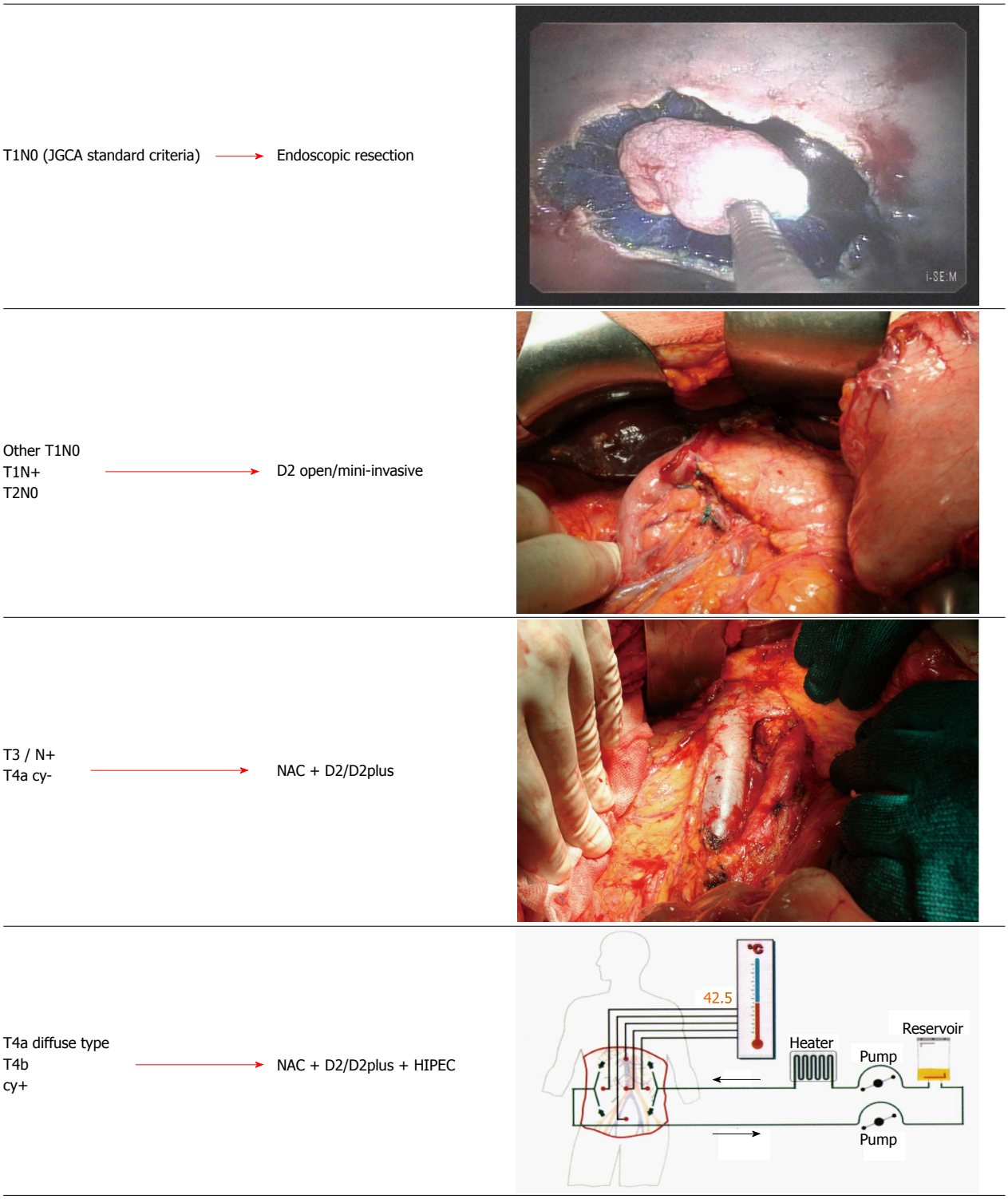

Figure 5 Proposal of a tailored multimodal approach in resectable non-cardiac gastric cancer. JGCA: Japanese Gastric Cancer Association; NAC: Neoadjuvant chemotherapy; HIPEC: Hyperthermic intraperitoneal chemotherapy.

with liver metastases, peritoneal dissemination, and those without resection deteriorated. The survival difference between groups with or without resection was only seen with those who had single site peritoneal dissemination. The Cochrane review found that chemotherapy improved survival over best supportive care in patients with incurable GC[98]. The authors also stated an advantage for combination chemotherapy over single agent approaches. The improvement in tumor response after multimodal treatment again raises a question about the surgical approach. In a Japanese study of 28 patients who responded well to S-1-based chemotherapy, there was a 93% rate of R0 resection. A complete response was seen in four

patients, and the median survival was 29 mo, with 34% 5-year survival[99]. In the French FREGAT study, palliative gastrectomy was performed because of solid organ metastases (5.6%), localized PC (4.6%), diffuse PC (2.3%) or incomplete tumor resection (12.8%)[100]. Median survival of patients with resection was better than in the non-resection group (11.9 mo *vs* 8.5 mo, $P < 0.001$). Multivariate analysis proved that factors associated with survival were: ASA score Ⅱ-Ⅳ, localized PC, diffuse PC, and signet ring histology. Patients with ASA Ⅰ/Ⅱ and incomplete resection without metastasis or PC, one-site solid organ metastasis without PC, or localized PC without signet ring cell histology, showed the highest benefit from surgery. This subgroup of patients had median survival from 12 to 18.3 mo. Analyzing surgical treatment in the case of distant metastases, we must also mention treatment of liver metastases from GC. No trials have been performed in this field, and a recent review by Grimes *et al*[101] was based on 17 retrospective studies. The solitary disease patients had better OS than those with metachronous disease, and patients with metachronous disease had better prognosis than those with synchronous disease. Hepatectomy in these patients is a safe procedure with about 2% perioperative mortality, and morbidity from 17% to 60%. The authors state that metachronous metastatic disease limited only to the liver, with the possibility of surgical resection, should be consider in a clinical trial.

In the latest GIRCG study on synchronous hepatic metastases in cases of GC, it was clear that clinical criteria could be used to select candidates for curative surgery. The surgical approach has an impact on survival especially when adjuvant chemotherapy is added[102].

Multivisceral resection of advanced forms

The role of multivisceral resection, in the setting of locally advanced GC, has been evaluated in several studies. Most of them reported a higher risk for perioperative morbidity and mortality, with limited objective benefit in terms of survival, but a potential advantage of extended resection for some subgroups[103]. In a recent GIRCG study, 206 patients with a clinical T4b carcinoma were evaluated[104].

One hundred and twelve patients underwent combined resection of the adjacent organs for clinical T4b stage disease. Postoperative mortality and complication rates were acceptable, and overall 5-year survival rate was 27.2%. The completeness of resection and lymph node invasion were independent prognostic parameters at multivariate analysis. At present, even if a chance of cure with an extended surgical approach could be obtained in subgroups of patients with invasion of adjacent organs, a multimodal approach should include neoadjuvant treatment, followed by extended surgery in responders. The addition of HIPEC should be considered.

CONCLUSION

Results of treatment in specialized western centers are good in early stage (Ⅰ/Ⅱ) GC, but are still unsatisfactory in more advanced stages (ⅢB and higher), when compared with eastern studies. Treatment options have changed in recent years from a standard to a tailored approach. Different individualized procedures can range from endoscopic resection, D2 with open or minimally invasive approach, to neoadjuvant therapy followed by extended surgery (Figure 5). In more advanced stages, a combined approach with the inclusion of HIPEC may represent a new frontier for multimodal treatment of resectable GC. It should be also emphasized that tailored treatment of GC involves appropriate pretreatment clinical staging of the disease. Clinicians should expect to face, in the future, fewer GC cases, but with higher biological aggressiveness, due to the relative increase of proximal and DT tumors. The high propensity of DT to lymph node metastasis and peritoneal dissemination makes multimodal treatment, in particular including NC and HIPEC, a modern and necessary approach to this still fatal disease.

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EDITORIAL

Management of ampullary neoplasms: A tailored approach between endoscopy and surgery

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Abstract

Ampullary neoplasms, although rare, present distinctive clinical and pathological features from other neoplastic lesions of the periampullary region. No specific guidelines about their management are available, and they are often assimilated either to biliary tract or to pancreatic carcinomas. Due to their location, they tend to become symptomatic at an earlier stage compared to pancreatic malignancies. This behaviour results in a higher resectability rate at diagnosis. From a pathological point of view they arise in a zone of transition between two different epithelia, and, according to their origin, may be divided into pancreatobiliary or intestinal type. This classification has a substantial impact on prognosis. In most cases, pancreaticoduodenectomy represents the treatment of choice when there is an overt or highly suspicious malignant behaviour. The rate of potentially curative resection is as high as 90% and in high-volume centres an acceptable rate of complications is reported. In selected situations less invasive approaches, such as ampullectomy, have been advocated, although there are some concerns mainly because of a higher recurrence rate associated with limited resections for invasive carcinomas. Importantly, these methods have the drawback of not including an appropriate lymphadenectomy, while nodal involvement has been shown to be frequently present also in apparently low-risk carcinomas. Endoscopic ampullectomy is now the procedure of choice in case of low up to high-grade dysplasia providing a proper assessment of the T status by endoscopic ultrasound. In the present paper the evidence currently available is reviewed, with the aim of offering an updated framework for diagnosis and management of this specific type of disease.

Key words: Ampulla of Vater; Cancer of the ampulla of Vater; Pancreaticoduodenectomy; Ampullectomy; Prognosis; Ampullary neoplasm; Lymphadenectomy;

Recurrence

Core tip: In this paper we review current evidence regarding ampullary neoplasm, with a particular focus on diagnosis and treatment. We are providing a framework for management of these neoplasms that, although rare, display distinctive clinical features. Current evidence about optimal management is reviewed, outlining the role of surgery as compared to newer endoscopic techniques: indeed, while surgery is mandatory for invasive carcinomas due to possible nodal involvement, endoscopy should be considered for non-invasive lesions.

Panzeri F, Crippa S, Castelli P, Aleotti F, Pucci A, Partelli S, Zamboni G, Falconi M. Management of ampullary neoplasms: A tailored approach between endoscopy and surgery. *World J Gastroenterol* 2015; 21(26): 7970-7987 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i26/7970.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i26.7970

INTRODUCTION

Neoplasms of the ampulla of Vater account for only 0.5% of all gastrointestinal malignancies[1]. Although ampullary carcinomas are rare neoplasms, they occur more frequently in the ampullary region than elsewhere in the small intestine[2]. The papilla is a nipple-like structure on the medial aspect of the second portion of the duodenum, best visualized with a side-viewing endoscope. Ampullary carcinomas are defined as gland-forming malignant epithelial neoplasms, which originate in the ampullary complex, distal to the bifurcation of the common bile duct and the pancreatic duct[3].

One of the possible causes of developing neoplasms in this area is that the ampullary region contains a transition from pancreatobiliary to intestinal epithelium, and such areas of transition are inherently unstable. As observed by Cattell and Pyrtek in 1949, the ampullary region is "an area of epithelium transition which is constantly being irritated chemically and mechanically"[4].

The appropriate diagnosis of ampullary neoplasms can be challenging and nowadays different diagnostic modalities can be considered including high-resolution imaging techniques, endoscopy and endoscopic ultrasound (EUS)[5].

As a matter of fact, there are no specific guidelines for the diagnosis of these neoplasms. Usually ampullary neoplasms are incorporated into the guidelines of biliary tract[6] or pancreatic carcinomas[7].

Regarding treatment, the first local resection of an ampullary lesion was reported in 1898 and the first radical resection (pancreaticoduodenectomy - PD) in 1912. The latter approach resulted in a reduced risk of recurrence, even if it maintains still nowadays high morbidity rates. In 1993 Binmoeller[8] reported the first endoscopic resection of the ampulla with a curative intent. This procedure is technically demanding, but in the last 20 years advances in endoscopic procedures with ablative techniques (such as mono, bi and argon plasma coagulation) as well as pancreatic and biliary stenting led to a low morbidity and mortality risk. Indeed endoscopic ampullectomy is the procedure of choice for ampullary adenomas and can be chosen as an alternative procedure in patients not eligible for surgery. The aim of this paper is to provide a review of the modern diagnostic tools and different treatments for ampullary neoplasms, including both endoscopic and surgical approaches.

ANATOMY

The vaterian system is located in the wall of the second part of the duodenum, at the confluence of the common bile duct and the major pancreatic duct. It includes the duodenal papilla (a mucosal elevation into duodenal lumen), Oddi's sphincter muscle, a fibrous covering and the ampulla of Vater. A true ampulla, defined as a dilated reservoir into which the ducts empty, is an infrequent finding (3%): indeed there are several anatomical variations in the connection between the two ducts.

In consideration of the anatomical characteristics we reported above, it is very difficult to localize the precise origin of tumors once they have invaded adjacent tissue[2].

EPIDEMIOLOGY

The cancer of the ampulla is a rare disease with an incidence of less than one per 100000; in autopsy series, ampullary neoplasms are seen in 0.06%-0.21% of the general population[3].

In a large series of 5625 patients with cancer of the ampulla, 10% of cases had a previously reported primary cancer in another anatomic site, while in 90% of patients the ampullary lesion was the initial primary neoplasm[9].

In the same study, women were found to be less frequently affected (0.36/100000) than men (0.56/100000, $P < 0.05$). The disease is also more common in Caucasians than in Afro-Americans.

In the study by Albores-Saavedra *et al*[9] an increase of ampullary cancer incidence from 1973 to 2005 has been reported, with an annual percentage rate of 0.9%.

The rates of incidence of the various histological subtypes of ampullary cancer have been approximately the same across all ages group, suggesting similar or overlapping carcinogenic pathways. In all of the histological types surveyed, cancer was found

predominantly in the older age groups. According to the age-specific rates, the incidence of cancers of the ampulla began to increase after age 30, but increased more rapidly after age 50 in both men and women; average age at diagnosis is between 60 and 70 for sporadic forms.

Although ampullary cancers are generally sporadic, some hereditary syndromes are associated with a higher risk for this type of cancer. The strongest predisposition for ampullary neoplastic disease is represented by the familiar adenomatous polyposis (FAP) syndrome. FAP patients frequently develop duodenal adenomas and their risk of periampullary cancer is 100%-200% higher compared to the general population; this results in a prevalence of ampullary cancer of 3%-12%[10]. Compared to sporadic cases, familiar cases of ampullary cancer also tend to present at a younger age.

CLINICAL FEATURES

Obstructive jaundice is the most common presenting symptom of ampullary cancer (85%)[11-13], caused by compression of the distal bile duct by the tumor. In contrast to biliary obstruction due to passage of calculi, in ampullary neoplasms jaundice is usually persistent rather than intermittent and may be accompanied by a distended, palpable gallbladder (Courvoisier's sign), that is however an uncommon finding (only 15% of cases). Gallstones are present in one third of patients, which may lead to misdiagnosis[14]. Presence of jaundice is associated with advanced stage of disease and increased risk of tumor recurrence after resection[15-20]. Other common symptoms include weight loss, fatigue and abdominal pain which are present in more than half of patients[21]. Acute pancreatitis is less frequent, but ampullary cancer should be ruled out in this case[22]. Up to one-third of patients have chronic, frequently occult, gastrointestinal blood loss but occasionally frank bleeding may occur[23]. Rarely, large lesions may produce gastric outlet obstruction.

Serum CA 19-9 is elevated in 86.4% of ampullary carcinoma patients[24].

PATHOLOGY

Because of their location, at the time of diagnosis ampullary carcinomas are often small[25] (at presentation 17% are less than 1 cm[26], 23% are less than 2 cm and 75% are less than 4 cm[27]) Despite their small size, the common bile duct is almost always dilated and the pancreatic duct is dilated as well in half of the patients[2]. As a collateral remark, this mismatch between tumor size and biliary obstruction explains why, compared to pancreatic cancers, resectability at presentation is significantly higher (70%-80% *vs* 10%-25%)[28,29].

Several classifications of ampullary carcinomas have been developed according to their gross appearance based on duodenal aspect or extension of neoplasm. Three distinct categories of carcinomas are recognized, after the correlation of gross and microscopic features: (1) intra-ampullary neoplasms, characterized by a prominent intraluminal growth of the pre-invasive neoplasms, which frequently protrude into the duodenal lumen from a patulous orifice of the papilla of Vater; (2) peri-ampullary, with prominent exophytic, ulcerous-vegetating components on the duodenal surface of the ampulla. The ulcerating part frequently corresponds to the invasive component, whereas the vegetating part represents the pre-invasive component; and (3) mixed exophytic and mixed ulcerated lesions[2,30,31]. The Presence of ulcerations is associated with poor survival rate[32].

Microscopy

The complex histological structure of the papilla of Vater gives rise to a heterogeneous group of neoplasms with different histologic types, classified according to the predominant component.

Kimura *et al*[33] were the first to demonstrate that adenocarcinomas originating in the ampulla of Vater may be divided in two subsets according to their type of differentiation, which can be either "intestinal" or "pancreatobiliary".

The intestinal type, the most common invasive subtype, is characterized by tubular or cribriform glands similar to those of colon-rectal adenocarcinomas. Incidence of this subtype is reported with a wide variability in different case studies (25%-78%)[2,33-36]. Most cases are associated with areas of residual adenoma, within the ampulla and in the surrounding duodenal mucosa. The adenocarcinomas arising in an adenoma (adenocarcinoma in villous adenoma, in tubulo-villous adenoma, in adenomatous polyp, and villous adenocarcinoma), are usually smaller and show a better prognosis. They show intestinal type immunophenotype, with the expression of keratin 20, MUC2 and CDX2[37,38] (Figure 1).

The pancreatobiliary type adenocarcinomas closely resemble primary tumors of the pancreas or extra hepatic bile ducts and represent 22%-74% of ampullary adenocarcinomas[2,33-36]. They are composed of glands associated with an abundant desmoplastic stroma, and stain positively for MUC1, MUC 5a and CK7[37,38] (Figure 2). Pancreatobiliary carcinomas have a worse prognosis, being frequently associated with unfavourable histopathologic features, such as lymph node invasion, perineural infiltration or areas of poor differentiation[25,28,33,39-42].

Some ampullary adenocarcinomas may exhibit mixed features of both intestinal and pancreatobiliary type; the distinction between the two patterns may be difficult in less differentiated cases.

Adenocarcinoma variants

Although closely related to the conventional type,

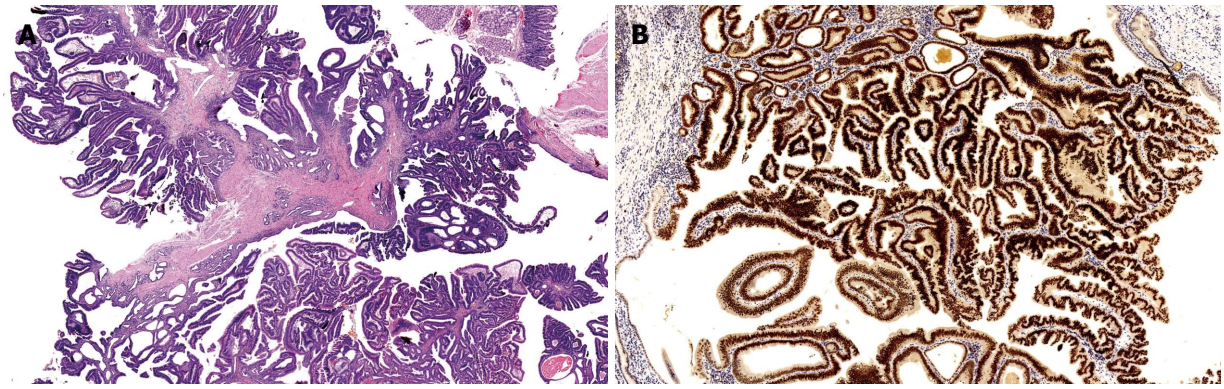

Figure 1 Intestinal type adenocarcinoma HE (A) and CDX2 (B).

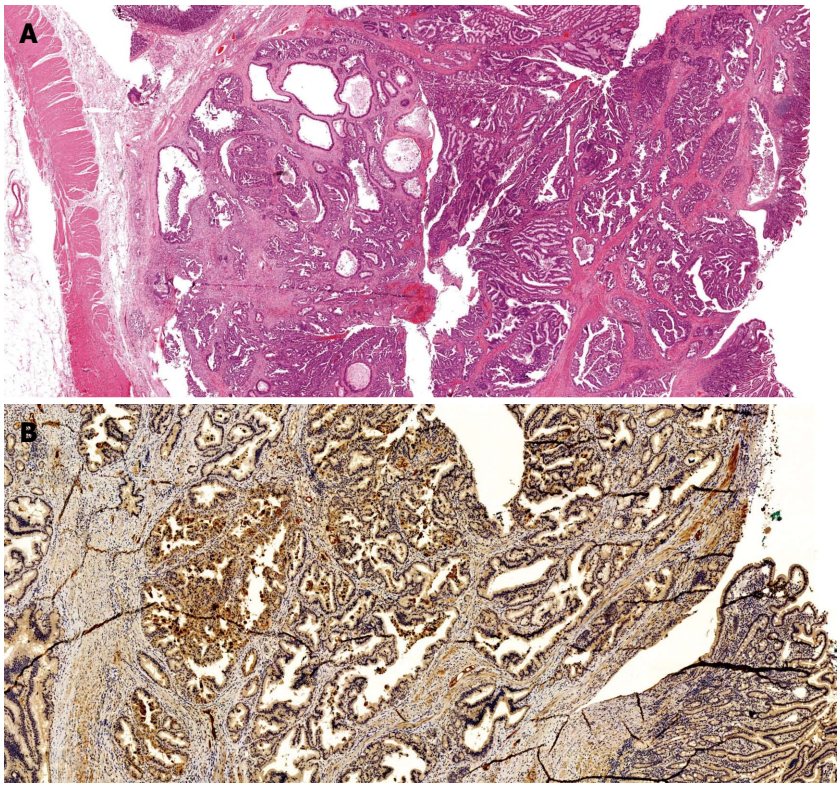

Figure 2 Pancreatobiliary type adenocarcinoma HE (A) and MUC1 (B).

distinct variants of adenocarcinomas include[43]: (1) adeno-squamous carcinoma: a malignant neoplasm composed of a mixture (> 30%) of two neoplastic components, a glandular and a squamous cell component; (2) colloid carcinoma[44] characterized by the presence of mucin-producing neoplastic cells (that should comprise at least 80% of the lesion) floating in large pools of extracellular mucin. Since the vast majority of colloid carcinomas of the ampulla express the intestinal markers CDX2 and MUC2, these tumours are regarded as variants of intestinal-type adenocarcinomas; (3) signet-ring cell carcinoma[9]: highly malignant neoplasm predominantly composed of infiltrating non-cohesive cells with intra-cytoplasmic mucin, which displaces the nucleus towards the periphery, creating the signet ring cell appearance. Cells may be associated with extracellular mucin but the large pools seen in colloid carcinoma are lacking. Since this entity is very rare, metastases from other more common signet cell carcinomas, mammary or gastric, should be ruled out; (4) undifferentiated carcinoma: highly aggressive neoplasm without a definite direction of differentiation. They can occur *ex-novo* or be associated with other ampullary neoplasms. They are usually large and widely invasive. The spectrum of morphology varies from highly cellular, pleomorphic epithelioid mononuclear cells with abundant cytoplasm, often admixed with bizarre multinucleated giant cells to relatively monomorphic epithelioid and spindle; (5) papillary adenocarcinoma[45]: they may show a non-invasive papillary component and an invasive carcinoma. The

Table 1 Staging classification of the ampullary neoplasms according to the American Joint Committee on Cancer TNM classification, 7th edition[47]

T = Primary Tumor			
TX	Primary tumor cannot be assessed		
T0	No evidence of primary tumor		
Tis	Carcinoma in situ		
T1	Tumor limited to ampulla of Vater or sphincter of Oddi		
T2	Tumor invades duodenal wall		
T3	Tumor invades pancreas		
T4	Tumor invades peripancreatic soft tissue or other adjacent organs or structures other than pancreas		
N = Regional Lymph Nodes			
NX	Regional lymph nodes cannot be assessed		
N0	No regional lymph node metastasis		
N1	Regional lymph node metastasis		
M = Distant Metastasis			
MX	Distant metastasis cannot be assessed		
M0	No distant metastasis		
M1	Distant metastasis		
G = Histologic Grade			
GX	Grade cannot be assessed		
G1	Well differentiated		
G2	Moderate differentiated		
G3	Poorly differentiated		
G4	Undifferentiated		
Stage	T	N	M
0	Tis	N0	M0
ⅠA	T1	N0	M0
ⅠB	T2	N0	M0
ⅡA	T3	N0	M0
ⅡB	T1	N1	M0
	T2	N1	M0
	T3	N1	M0
Ⅲ	T4	Any N	M0
Ⅳ	Any T	Any N	M1

invasive carcinomas show either pancreatobiliary or intestinal phenotype; and (6) neuroendocrine carcinoma[46]: characterized by either small or large neuroendocrine-cells, grade 3 (G3). Their histological features and prognosis resemble those of their pulmonary counterparts.

According to the Surveillance Epidemiology and End Results (SEER) program[9], well and moderately differentiated carcinomas (grade Ⅰ-Ⅱ) predominated over high-grade carcinomas (grade Ⅲ-Ⅳ), with a frequency of 15.6% and 33.6% for grade Ⅰ and Ⅱ respectively, as opposed to 20.8% and 1.4% for grade Ⅲ and Ⅳ, respectively.

STAGING

The 2010 AJCC staging system is reported in Table 1[47]. The T classification depends on the extension of the primary neoplasm: local spread begins from within the ampulla of Vater and the sphincter of Oddi (T1), then extends into the duodenal wall (T2) or beyond, into the head of the pancreas (T3) or contiguous soft tissue or organs (T4)[48].

Regional lymph nodes include the peripancreatic lymph nodes (superior and inferior pancreatic head nodes; anterior and posterior pancreatico-duodenal nodes) and the lymph nodes along the hepatic artery, proximal mesenteric artery, celiac axis and pyloric regions. Optimal histological examination of pancreaticoduodenectomy specimen should include analysis of a minimum of 12 lymph nodes[47].

Metastatic lymph nodes are found in 28% to 60% (Table 2) of resected ampullary carcinomas[49]. Tumors that invaded the duodenal submucosa showed regional lymph node involvement in 42% of cases, while metastatic disease was almost never found with tumors limited to the mucosa or to the sphincter of Oddi[48]. Cannon *et al* reported an incidence of node metastasis respectively of 0%, 46%, 50% and 100% in T1, T2, T3 and T4. While it has been consistently confirmed that T stage is an important predictor of nodal status, many other Authors have instead observed nodal metastases also in T1 tumors, with a frequency ranging from 10% to 50%[16,50-53]. This observation is particularly important for surgical management of these lesions, as we will discuss in the section about treatment.

In particular, lymph node involvement was significantly more common for pancreatico-biliary type tumors (55% *vs* 18% for intestinal type)[36], neuroendocrine carcinomas (57%)[54] and poorly differentiated carcinomas[2]. Metastasis to lymph nodes outside the regional groups described above, such as nodes of the pancreas tail or para-aortic ones, is considered as metastatic disease (M1).

Metastases (< 10% at presentation)[55] are commonly found in the liver and peritoneum and are less frequently seen in lungs and pleura.

In 35% to 80% of cases lymphatic and blood vessel involvement is encountered, while perineural invasion occurs less frequently[28,48].

Compared to the previous one, the new stage classification has been modified according to new prognostic information; nodal positivity is included in stage ⅡB, while stage Ⅲ comprises patients with extensive (T4) tumors, with or without nodal disease. Stage Ⅰ has now been divided into two subsets: IA, including tumors limited to the ampulla of Vater or sphincter of Oddi, and IB, indicating cancers that invade the duodenal wall. Similarly, stage Ⅱ has been split into ⅡA, indicating tumors that invade the pancreas (T3), and ⅡB, which include T1-3 tumors with nodal disease. Stage Ⅳ is represented by metastatic tumors[56].

Following surgical resection, recurrence may occur locally (involving the tumor bed or the para-aortic lymphatics)[57] or at a distant site. Peripancreatic lymph nodes are the most frequent site of nodal involvement and, compared to pancreatic carcinoma, disease is more likely to be limited to this region. The spreading of ampullary carcinoma generally follows a halsteadian progression: nodal involvement manifests first, followed by appearance of liver metastases and

Table 2 Survival in papillary tumors and predictors of poor prognosis

Ref.	*n*/resected	Operative mortality (%)	Positive nodes (%)	R0 (%)	Pancreatobil/ intestinal, *n* (%)	Survival-resected		Median survival - resected (mo)	Predictors of worse survival or recurrence
						5 yr	10 yr		
Albagli *et al*[128]	50/50	8.0	36.0	-	-	N0 52% N+ 39%	-	-	None
Allema *et al*[136]	67/67	1.5	52.0	75.0	-	50%	-		R+
Beger *et al*[50]	171/171	3.1	54.0	79.6	-	N+ 21% N0: 63%	-	-	N+[2], Pan+, R, Stage[2], G2-3
Bettschart *et al*[18]	88/70	0.0	40.0	97.2		37.9[1]		45.8	Bilirubin[2], Age > 70, G3, N+[2], Neu+, Pan+
Brown *et al*[91]	72/51	2.0	47.0	100.0	-	58%			N+[2], T, G
Carter *et al*[137]	157/145	-	33.0	86.0	53 (45)-54 (46)	-	-	-	Pancreatobil[2], Bilirubin, Stage[2], N+, T, Ly+[2], Neu+[2]
Chareton *et al*[35]	63/51	7.5	-	-	-	40%	-		Stage, Ampullectomy
Choi *et al*[129]	78/70	2.6	31.4	95.7	-	59.90% N0 63.5% N+ 50.8%	29%	70	Jaundice, Ulcerated, Pan+, G
Di Giorgio[1] *et al*[138]	94/64	8.6	28.0	100.0	-	64.4%		54	No resection , Size, G, Depth of infiltration[2]
Duffy *et al*[92]	55	0.0	41.8	98.2	-	67.70%	-	-	Neu+[2]
Falconi *et al*[19]	90/90	4.0	48.0			61% (disease free)			Stage, T, LNR[2]
Hornick *et al*[16]	157/150 106 Adenocarcinoma		59.8			Adenocarcinoma 45% Adenoma 82% N+ 47% N0 63%			Jaundice, Stage, N+
Howe[1] *et al*[28]	123/101	5.0	45.5	96.1	-	46%	-	58.8	No resection, N+[2], R+[2], G3
Iacono *et al*[139]	59/59	7.8	37.3	-	-	46%	33%	31	G, T[2], N+, Chromosome 17p[2], 18q[2], Micro satellite instab., Standard PD
Inoue *et al*[140]	34/34					52.6%			N+[2], Pan+, V+[2], Neu+[2], SMA[2]
Kawaguchi *et al*[141]	28/28					56.4%		37	Duo+, Ly+, V+[2]
Kim *et al*[34]	104/104		39.4		62 (60)-42 (40)	60.1%	57.6%		T[2], CEA, N+[2], G3[2], Pancreatobil[2], Neu+, Ly+
Kimura *et al*[36]	51	-	45.0	-	38 (74)-13 (25)				Pancreatobil
Klempnauer *et al*[142]	94/94	9.6	38.0	97.0	-	34.5%	28.6%	-	Size[2], G3, N+, Stage
Lazaryan *et al*[20]	72/72	-	34.0	96.0	-	61%	-	-	Neu+[2], Ulcerated[2], N+[2], Stage, Weight loss
Norero *et al*[143]	50/50					32%		28	N+[2]
Ohike *et al*[134]	244/244	-	38.0	95.0	162 (66) other 82 (34) intestinal	33% Hi budding 24% Low budding 68%	-	-	Budding[2], N+[2], T[2], R+[2]
Qiao *et al*[59]	127/127	9.7	35.0	98.4		43.3%	35.7%		N+[2], T[2], Stage, Pan+
Roder *et al*[144]	69/66	4.5	42.0	-	-	35%	-	41	N(nr)
Sakata *et al*[130]	71/71	0.0	48.0	97.2		64%	55%		G, Pan+, Ly+[2], V+[2], Neu+, R+, N+[2], LNR
Shinkawa *et al*[145]	23/23					52%	32%		Pan+, N+
Talamini[1] *et al*[51]	120/106	3.8	40.0	-	-	38% N+ 31% N0 43%	-	46	No resection, Transfusion[2], N+, G2-3
Todoroki *et al*[95]	66/59	0.0	60.0	93.2	-	52.6%			R+, CEA, Ulcerated, T[2], N+, Stage[2], Ly+[2], V+
Westgaard *et al*[25]	114/114 41 ampullary	3.5	57.0	65.0	67 (59)-47 (41.2)	-			Pancreatobil[2], N+[2], V+[2] Size[2]

Winter *et al*[127]	450/450 Adenoca: 347	2.0	54.5	96.1		Adenocarcinoma 45% Adenoma 86% N+ 35% N0 56%		N+ 23.4 N0 79	N+[2], Neu+[2], Transfusion[2]
Yokoyama *et al*[15]	74/59	-	53.5	100.0	-	61.0%	53%	-	Pan+[2], N+[2], Jaundice, V+, Ly+[2], Neu+, G
Zhao *et al*[146]	105/105	8.6	37.1			42.8%			Pan+, Size[2], T, TNM stage, N(nr)[2]

[1]Including not resected pts; [2]Independent predictor; duo+: Duodenal invasion; G: Histologic grade of differentiation; LNR : Lymph nodes ratio; Ly+: Lymphatic vessel invasion; N+: pOsitive lymph nodes; N(nr): Number of metastatic lymph nodes; Neu+: Perineural invasion; Pan+: Pancreatic invasion; Pancreatobil: Pancreatobiliary histological subtype; R+: Positive resection margins; No resection: Patients not resected *vs* those who underwent surgical resection; SMA: Lymphadenectomy around the superior mesenteric artery; V+: Blood vessel invasion.

subsequently distant dissemination.

Some data suggest a preferential lymphatic drainage flow from posterior pancreatico-duodenal lymph nodes to nodes located around the superior mesenteric artery, thereby underlining the importance of nodal dissection at least in these areas[32].

DIAGNOSIS

In the diagnostic evaluation of jaundiced patients with suspected malignant bile duct obstruction, benign tumors, inflammatory diseases and gallstones must be excluded first. Then the extent of tumor invasion and spread has to be established. During the diagnostic workup, another issue is to differentiate primary ampullary carcinoma from the more common periampullary tumors, including pancreatic, duodenum and bile duct carcinomas. In most cases, the distinction is not essential from a surgical point of view because, if a malignant lesion is suspected in that area, the patients will undergo the same operation (pancreaticoduodenectomy). However ampullary and periampullary tumors have substantially different oncologic implications and prognosis.

In the evaluation of patients with jaundice trans abdominal ultrasonography should be the first imaging study, since it can identify intra and extra hepatic bile duct dilatation, a distended gallbladder and gallstones; however the ampullary tumor may not be visualized[58]. As shown in Table 3, Ultrasound (US) sensitivity rate in tumor detection ranges from 5% to 15%[5,59-61], however indirect signs may be present in up to 70% of cases[60]. Due to low sensitivity of US, it is necessary not to underestimate indirect signs and to maintain a high level of suspicion. US evaluation in any case can give a clue to choosing the next most appropriate diagnostic exam: if a cystic mass is detected MRCP should be preferred. CT scan must be chosen if the mass is solid, whilst when no masses are identified (eco)endoscopy (EUS) must be performed.

Abdominal CT is more sensitive than US for evaluating the periampullary region, since it is not limited by the presence of bowel gas or obesity and is less operator-dependent. On the other hand, its diagnostic accuracy is not very high for small ampullary masses, especially if they are located within the duodenal wall or in the lumen. For the previous reasons, CT scan sensitivity is highly variable according to different reports ranging from 19% to 69%[5,60,62]. As shown in Table 3 CT specificity in tumor detection also varies widely, from 20% to 76%[60,61,63,64], while accuracy is only 28.6% as reported by Chen *et al*[5]. In order to maximize CT sensitivity, i.v. contrast medium should always be used in order to obtain arterial and venous phase imaging and water should be administered as oral contrast agent to distend the duodenum[2]. A bulging papilla can be encountered in healthy individuals as well as in patients with inflammatory diseases (papillitis from passage of biliary stones, parasites, infections or periampullary diverticula), benign or malignant tumors. Mural thickening and attenuation pattern of contrast medium may be of help for differentiating normal from pathological papilla[65]. CT spatial resolution is often inadequate to define the local tumor invasion into the surrounding structures[66]. As a final consideration, CT is however always necessary for staging any malignant disease, as it can identify distant metastatic involvement like regional lymph-nodes, liver, peritoneum, lung and bone. CT virtual endoscopy is a new non-invasive diagnostic tool, which still has to be more extensively evaluated and developed[67].

Endoscopy is particularly helpful in establishing the diagnosis. In about two thirds of cases, ampullary and duodenal neoplasm are visible endoscopically as exophytic or ulcerated masses[21]. Endoscopic retrograde cholangio-pancreatography (ERCP), is useful for distinguishing true ampullary malignancies from pancreatic and bile duct

Table 3 Accuracy of various methods in indenfying and staging papillary tumors

Ref.	*n*	Test	Tumor detection			T	N			Resectability		
			Sens	Spec	Acc	Acc	Sens	Spec	Acc	Sens	Spec	Acc
Artifon *et al*[147]	27	CT	-	-	-	51.8%	40.0%	65.0%	55.5%	-	-	-
		EUS	-	-	-	74.1%	70.0%	88.0%	81.4%	-	-	-
Buscail *et al*[148]	6	EUS	-	-	-	83.0%	-	-	100.0%	100.0%	100%	100%
Cannon *et al*[79]	50	CT	-	-	-	24.0%	-	-	59.0%	-	-	-
		MRI	-	-	-	46.0%	-	-	77.0%	-	-	-
		EUS	-	-	-	78.0%	-	-	68.0%	-	-	-
Chen *et al*[61]	19	US	5.0%	-	-	11.0%	7.0%	-	-	-	-	-
		CT	21.0%	-	-	22.0%	33.0%	-	-	-	-	-
		EUS	95.0%	-	-	72.0%	47.0%	-	-	-	-	-
Chen *et al*[5]	41	US	-	-	12.2%	-	-	-	-	-	-	-
		CT	-	-	28.6%	26.1%	0.0%	-	44.0%	-	-	-
		MRI	-	-	81.3%	53.8%	25.0%	-	77.0%	-	-	-
		EUS	-	-	97.6%	72.7%	47.0%	-	67.0%	-	-	-
Heinzow *et al*[84]	72	IDUS	87.5%	92.5%	90.2%	-	-	-	75.0%	-	-	-
		ETP	68.7%	100.0%	86.0%	-	-	-	-	-	-	-
		IDUS +ETP	97.0%	100.0%	94.5%	-	-	-	-	-	-	-
Howard *et al*[149]	21	CT	-	-	-	-	-	-	-	63.0%	100%	86%
		EUS	-	-	-	-	-	-	-	75.0%	77%	76%
Kubo *et al*[85]	35	EUS	-	-	-	74.0%	-	-	63.0%	-	-	-
Manta *et al*[82]	24	MRI	75.0%	-	-	-	-	-	-	-	-	-
		EUS	100.0%	-	-	-	-	-	-	-	-	-
Midwinter *et al*[63]	34	CT	76.0%	-	-	-	33.0%	86.0%	-	-	-	-
		EUS	97.0%	-	-	-	44.0%	93.0%	-	-	-	-
Mukai *et al*[83]	23	EUS	96.0%	-	-	78.0%	-	-	-	-	-	-
Qiao *et al*[59]	127	US	7.9%	-	-	-	-	-	-	-	-	-
		CT	19.0%	-	-	-	-	-	-	-	-	-
Rösch *et al*[64]	28	US	7.0%	-	-	-	-	-	-	-	-	-
		CT	29.0%	-	-	-	-	-	-	-	-	-
		EUS	93.0%	-	-	-	-	-	-	-	-	-
Skordilis *et al*[60]	20	US	15.0%	-	-	-	-	-	-	-	-	-
		CT	20.0%	-	-	-	-	-	-	-	-	-
		EUS	100.0%	-	-	82.0%	-	-	71.0%	-	-	-
Tio *et al*[150]	32	EUS	-	-	-	84.4%	68.8%	37.5%	53.1%	-	-	-

Acc: Accuracy; CT: Computerized tomography; ETP: Endoscopic transpapillary forceps biopsies; EUS: Endoscopic ultrasonography; IDUS: Transpapillary intraductal ultrasonography; MRI: Magnetic resonance imaging; Sens: sensitivity; Spec: Specificity.

tumors thanks to the visualization of the site and the extent of the stenosis[68]. Moreover ERCP allows the operator to perform a biopsy from the papilla and ampullary segment of the common bile duct (CBD) or pancreatic duct. In addition placement of a stent for biliary decompression if necessary is technically feasible. However it must be underlined that a biliary stent should be placed only once diagnosis is achieved, since it can interfere with all the radiologic exams (CT, RM, EUS) and create inflammatory reaction in the biliary duct[61].

Endoscopic signs suggesting the presence of carcinoma are ulceration, erosion, haemorrhage, necrosis and firm or friable consistency. In particular a malignancy is strongly suspected if the mass is ulcerated or over 3 cm in size[69,70]. Tumors contained within the ampulla appear as prominent submucosal bulge[71].

Biopsy of the lesion is mandatory, but since false negative rates of endoscopic biopsy can be as high as 50%, a negative result is insufficient to rule out the presence of a malignancy in an ampullary lesion[72,73]. Reported accuracy of biopsies range from 47% to 95%[74,75].

The overall accuracy rate with ERCP has been reported around 88% for the diagnosis and origin of the tumors in the ampullary region; attempts to enhance the accuracy include acquisition of tissue at least 48 h following sphincterotomy, multiple biopsies, the use of PCR (polymerase chain reaction) or immunohistochemical staining to detect p53 or k-ras gene mutations, but none of these methods are routinely used in clinical practice.

However, technical factors limit the ability to perform a satisfactory ERCP in 22% of patients with suspected ampullary carcinoma[68,76]. ERCP presents several limitations: intra-ampullary carcinomas are covered by intact duodenal mucosa and in 25%-50% of cases biopsy material discloses only adenoma when deeper portions of the lesion contain invasive carcinoma. In addition peri-vaterian diverticula (present in up to 20% of cases in endoscopic and autoptic series[77,78]) can obstacle technical feasibility of the endoscopic manoeuvres. Snare biopsies yield more

tissue than forceps and therefore are more sensitive in detecting adenocarcinoma[74].

MRCP is a non-invasive diagnostic tool with a well-established value in the evaluation of pancreatobiliary lesions[79,80] However, its accuracy for detection of ampullary tumors is limited by the small size of the ampulla and by the scarce amount of fluid due to tapering of the ducts: this area has been defined as a "blind spot" for MRI[81]. Compared to EUS, MRI can detect ampullary tumors in 3/4 of cases[82].

EUS, allowing close contact of the transducer to the duodenal wall, not only has an optimal sensitivity in lesion detection, approaching 100%, but also provides a precise definition of invasion of the surrounding tissue, with 63%-84% accuracy in T staging[5,60,64,74,82-85].

Nowadays EUS should be performed in all patients with suspected ampullary tumors, since the evaluation of the T code is of paramount importance for the choice of treatment (ampullectomy *vs* pancreaticoduodenectomy). In this setting EUS can give important data regarding the depth of wall infiltration.

More recently, intraductal ultrasounds (IDUS), by inserting the echo probe inside the ducts, has further increased diagnostic accuracy of endoscopic imaging modalities[74,84].

However, this technique still has a limited availability in daily clinical practice.

Although advanced endoscopic techniques can help to differentiate ampullary adenomas from carcinomas, it might be difficult to completely rule out a carcinoma without complete resection of the lesion. Endoscopic ampullectomy can therefore be also a useful diagnostic tool in case of a suspicious mass without definite malignant features.

TREATMENT

Pancreaticoduodenectomy

Once the diagnosis of ampullary carcinoma is made, provided that resectability is judged as feasible, pancreaticoduodenectomy (PD), either with conventional or pylorus-preserving approach (PPPD), is considered the standard of care. A recent meta-analysis of six randomized trials showed no significant differences in mortality and morbidity between the two procedures, although operating time and intraoperative blood loss are reduced in the PPPD group[86].

Resectability rate is high for ampullary neoplasm and, in current series, the rate of potentially curative resection has increased up to 90%[18,28,51,87-89] Long-term survival is possible after pancreaticoduodenectomy, even for patients with lymph node metastases or invasion beyond the duodenal wall (T3).

Pancreaticoduodenectomy is a demanding procedure, with significant morbidity. In recent reports from high-volume centers, perioperative mortality rate is consistently reported in less than 5% of cases (Table 2). However, significant complications occur in 20%-40% of patients, including pancreatic fistula, pneumonia, intra-abdominal infection, anastomotic leak and delayed gastric emptying[50,51,87,90-93]. In particular, compared to patients with pancreatic cancer, the incidence of postoperative pancreatic fistula is higher (28% *vs* 6%), perhaps since a normal, soft pancreatic tissue is less likely to hold a suture[94].

After curative resection, nodal status is one of the strongest predictors of survival: indeed, in one series pancreaticoduodenectomy in node-negative patients resulted curative in 80% of cases, while only 25% of patients with positive nodes were alive at 5 years[91]. Interestingly, in the same report no disease- related death occurred more than 3 years after the procedure, consistently with other data that indicate a median time to relapse of 11-13 mo[95,96]. However, cases of tumor recurrence beyond 5 years after resection have been reported[52], underlying the importance of performing accurate and long-lasting postoperative surveillance.

In contrast to pancreatic cancers, in case of ampullary cancer a "lymphatic pathway" has been identified, extending from posterior pancreaticoduodenal nodes around the mesenteric artery up to para-aortic lymph nodes[50,97]. As a result, even in advanced stages, compared to pancreatic carcinoma, nodal involvement is closer to the primary tumor and generally involves a single group of lymph nodes. However the clearance of the abovementioned nodal stations is of paramount importance during pancreaticoduodenectomy for ampullary carcinoma.

Ampullectomy: From surgical to endoscopic approach

Compared to standard PD, ampullectomy is a less invasive procedure. Due to technical improvements in endoscopic techniques over the last decade, local resection of the ampulla can be performed endoscopically and this approach reduces to minimum the procedural trauma. A general main limitation of ampullectomy is the lack of lymphadenectomy.

Endoscopic ampullectomy

Endoscopic ampullectomy is therefore a widely accepted therapy for benign ampullary lesions, provided that histological examination shows no signs of invasive carcinoma and that resection margins are negative. Its success rate ranges from 74% to 84%[98-103]. The incidence of periprocedural complications is reported from 10% to 21%. They include bleeding, papillary stenosis, cholangitis and acute pancreatitis, which is the most frequent complication, ranging from 8% to 19%[98,100-103]; this adverse event appears to be reduced by placing a pancreatic duct stent during the procedure[101,104,105].

One of the largest case series of benign adenomas has recently been reported by the retrospective analysis of Onkendi *et al*[106]; 180 patients were treated either with endoscopic (n = 130) or surgical resection

(*n* = 50, including local resection and PD). Endoscopic treatment was associated with fewer complications compared to surgery (29% *vs* 58%, *P* < 0.001). However, the recurrence rate was five-fold higher in this group (*P* = 0.006), and seven cases of recurrence presented with malignant behavior. Endoscopic ampullectomy was associated with an acceptable recurrence rate when complete resection could be achieved in one session, tumor size was < 3.6 cm and limited intraductal extension (< 5 mm) was found at EUS.

If histological examination shows severe dysplasia or carcinoma in situ, endoscopic ampullectomy can still be performed[50,107-109]; however the procedure should be converted to standard PD if an invasive cancer is detected. In this regard, endoscopic ampullectomy can be considered part of the diagnostic workup, when the lesion is small and no signs of malignancy are clearly evident at preoperative evaluation.

Surgical ampullectomy

The role of surgical local excision is nowadays less defined. First of all it should be remarked that, due to technical improvements in endoscopy over the time, there has been a progressive shift from surgical to endoscopic ampullectomy in reported case series. Therefore, literature about ampullectomy is quite inhomogeneous. Surgical ampullectomy allows to perform lymphadenectomy and has a mortality rate < 1%. However, lymphadenectomy in these cases does not include lymph node stations along the superior mesenteric artery. Apart from the same complications of endoscopic treatment, it carries the risk of duodenal dehiscence, intra-abdominal collections, wound infections and cardiopulmonary complications related to general anesthesia[50,110]. In a paper comparing adverse events after surgical and endoscopic ampullectomy, morbidity was significantly higher in the surgical arm (42% *vs* 18%, *P* = 0.006)[111]. On the other hand, in the paper by Onkendi, surgical ampullectomy, compared to endoscopic techniques, did not offer a significant benefit in preventing recurrences after adenoma resection[106]. Some operators however remark the fact that, compared to endoscopic approach, the feasibility of surgical ampullectomy is higher, particularly in case of duodenal diverticula; the success rate of surgical excision is reported to be > 95%[110]. However, in our opinion the morbidity advantage of surgical ampullectomy with lymph node dissection compared to standard PD is limited, provided that surgery is performed in a high-volume center.

Ampullectomy for malignant tumors

Some Authors have proposed that low risk carcinomas can be treated with ampullectomy[112]; this strategy is based on assumption that absence of muscularis propria involvement is associated with a low risk of lymphatic involvement. Klein *et al*[113] have retrospectively compared the results of 9 patients with ampullary carcinoma treated with surgical local excision (either because of unexpected malignancy or high surgical risk) with other 26 cases who underwent PD in the same period. They found reduced perioperative morbidity and mortality, no cases of local recurrence and comparable long term survival. Based upon these results, the Authors claimed that patients at low risk (defined as pT1, G1-2) could be routinely treated with local excision. However one of the patients who underwent PD was found to have positive nodes despite a pT1-G2 tumor, which would have remained undetected if a local resection had been carried out.

Accordingly, Beger *et al*[50] proposed surgical ampullectomy with local lymph node dissection in pT1, N0, G1-2 tumors. Among 10 patients who underwent this procedure for ampullary carcinoma, 6 had a R1 resection, but did not undergo PD because of major comorbidities and none of them survived at 3 years. Among the other 4 patients, 1 died at 6 years, but without tumor recurrence.

In our opinion, this approach - limited resection in low-risk patients - deserves some considerations. First of all, there are data suggesting that nodal involvement is not exceptional in T1 tumors[114]. As in recently presented data by Hornick *et al*[16] 45% of pathologically confirmed T1 tumors had nodal metastases; furthermore, 50% of T1 ampullary cancers have been found to have microscopic lymphatic invasion[52]. Moreover, complication rates with current surgical and anesthesiological techniques are much lower than previously reported and the benefit of a less invasive procedure appears questionable in a population with a standard surgical risk. In the series reported by Roggin *et al*[115], 7 out of 8 patients treated with ampullectomy experienced recurrence of disease (mostly at a loco regional level) and had a substantially higher mortality compared with the PD group. The data supporting the use of ampullectomy in ampullary cancer, conversely, are scarce and based on a limited number of patients.

Due to these considerations, patients presenting with an invasive ampullary carcinoma should be routinely treated with PD, even for "low risk" cases. Ampullectomy for malignant lesions should be reserved only for patients with a high surgical risk; if complete resection cannot be achieved conventional PD should be strongly considered because of the high recurrence rate.

An algorithm approach for ampullary lesions

Our approach is synthesized in Figure 3: if preoperatively the lesion appears as a mass at CT scan, then PD is advisable. In case of a small lesion, if biopsies exclude the presence of an infiltrating neoplasm and EUS shows that the lesion is confined within the mucosa, endoscopic ampullectomy should be

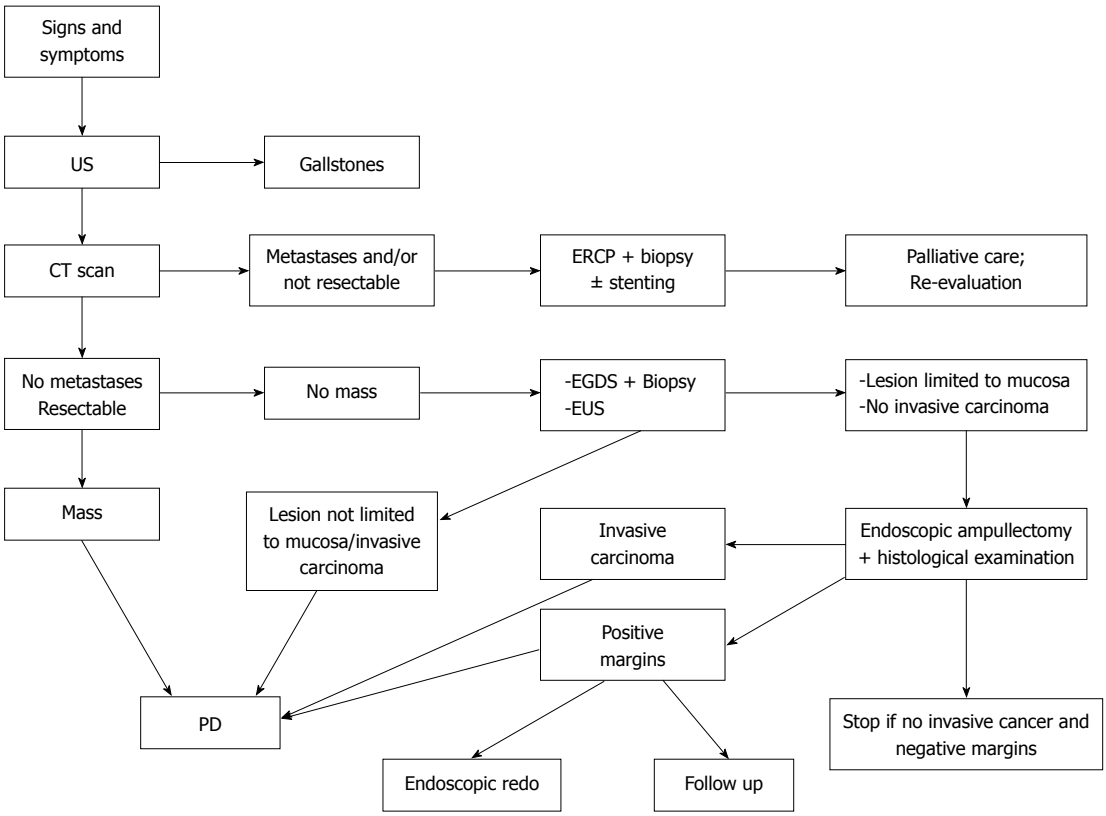

Figure 3 Diagnostic and therapeutic algorithm for suspected papillary lesions. Once alternative diagnoses have been excluded by ultrasound (US), computerized tomography scan is typically performed in order to assess the stage of the lesion, in particular the presence of metastases and resectability. After diagnostic work-up, if the lesion appears as an infiltrating neoplasia or has a significant mass, then a pancreaticoduonectomy (PD) should be performed. If the lesion is small, then an EGDS with biopsies and endoscopic ultrasound (EUS) staging must be performed. If (1) biopsies exclude the presence of an infiltrating neoplasm; (2) an aggressive histotype and (3) EUS shows that the lesion is confined within the mucosa, endoscopic ampullectomy is the appropriate therapeutic option. If the subsequent histological exam shows the presence of infiltrating cancer, then the intervention should be converted to a PD. If histology shows no evidence of infiltrating cancer but the resection margins are positive, various options are available, including endoscopic redo, close endoscopic follow-up or PD.

performed. If the subsequent histological exam shows the presence of infiltrating cancer, then the intervention should be converted to PD. In case of positive resection margins, with no evidence of infiltrating cancer, the management is more controversial: various options are available, including endoscopic second look, close endoscopic follow-up or PD. Age, comorbidities and patient's preferences also should be taken into account in order to select the best approach in each case; in most situations, our default strategy is to perform endoscopic redo or a close follow up, in order to avoid PD for a benign pathology.

OTHER THERAPEUTIC OPTIONS

Another matter of debate regarding surgical treatment of ampullary cancer is the opportunity to perform preoperative biliary drainage. This procedure appears to be reasonable if the surgical operation has to be delayed and there are high bilirubin levels (> 15 mg/dL). This policy seems to be suggested by a reduced incidence of wound infection in patients who underwent preoperative biliary drainage, with no differences in other outcomes[116]. On the other hand, biliary drainage can induce inflammation of the biliary tract and surrounding tissues, making assessment of resection margins more difficult.

Laser ablation and photodynamic therapy (which consists of intravenous administration of a photo-sensitizing agent, that is mainly retained in malignant tissue, followed by endoscopic irradiation with light) is a minimally invasive technique that might be applied for ampullary lesion treatment. However, given its limited efficacy, lack of histological data and risk of tumor recurrence, it is currently regarded only as a palliative procedure[117,118]. In a small series, photodynamic therapy appeared safe and useful as adjuvant treatment for biliary tract cancer (including one case of ampullary carcinoma)[119], but data from larger studies are lacking.

Despite good resectability rates and relatively better prognosis compared to other periampullary malignancies, recurrence of the disease still represents a substantial issue, particularly in patients with nodal involvement[91,95,96].

Currently, no clear indications exist regarding the role of adjuvant therapy after successful pancreatic resection. Some data have suggested a potential benefit of adjuvant chemo-radiotherapy with 5-fluorouracil, particularly in node-positive patients[120,121], but

randomized trials failed to confirm such a survival advantage[122,123].

Also the role of adjuvant chemotherapy alone is not clearly established. A recent randomized trial assigned 428 patients with a periampullary cancer (297 with papillary tumors) either to observation or adjuvant chemotherapy, with either gemcitabine or fluorouracil. Even if the difference was not significant at univariate analysis, after adjusting for some confounder factors, adjuvant chemotherapy was associated with a significant survival benefit[124].

The role of neo-adjuvant therapy for advanced ampullary cancer is not defined, as most studies have included ampullary neoplasms with other malignancies due to their rare occurrence. Most probably, chemotherapy should be tailored according to specific histological subtype, *i.e.* pancreatobiliary *vs* intestinal.

However, guidelines from both the National Comprehensive Cancer Network (NCCN)[125] and the European Society for Medical Oncology (ESMO)[126] do not provide any recommendation about treatment of ampullary cancer.

Similarly, the optimal post-treatment surveillance is poorly defined. Most clinicians perform follow up with history, clinical examination and serum tumor markers (CEA and CA 19-9) every six months for five years and annually thereafter. The use of abdomen CT scan is less defined in this setting.

SURVIVAL AND PROGNOSTIC FACTORS

Overall survival rate at 5 years is widely variable between different reports, ranging from 32% to 67.7%. Table 2 reports several studies. Only recent studies (publication from 1995) were considered in order to avoid diagnostic, histological or surgical biases. In fact more recent series report a better prognosis due to both diagnostic and surgical improvement[39].

In patients who undergo a potentially curative resection, the presence of nodal metastases, poorly differentiated histology, and tumor invasion into the pancreas are associated with a less favourable outcome. The great majority of data show positive nodes as a predictor of poor prognosis with worse survival or recurrence. In 17 out of 25 studies nodal metastases are demonstrated as an independent poor prognostic factor. In patients with metastatic lymph nodes 5-year survival varies from 21% to 50.8%, compared to 43%-63.5% in nodes negative patients (Table 2)[50,51,127-129].

In consideration of such a strong evidence, a few recent works better investigated lymph nodes role in ampulla tumors using lymph nodes ratio - a recently established prognostic factor in several gastrointestinal malignancies, that means ratio between metastatic and resected/examined lymph nodes (LNR) - and number of positive lymph nodes in relation to prognosis. In these studies the number of affected lymph nodes resulted as an independent poor prognostic factor; lymph nodes ratio results as a predictor of poor prognosis[19,130,131], but only in one study did it retain significance at multivariate analysis[19]. The issue of how many lymph nodes should be harvested during PD for ampullary cancer was addressed by Partelli *et al*[132] who found that removal of > 12 nodes was associated with an improved prognosis both in pN0 and pN1 patients, providing evidence that an adequate lymph node dissection plays an important role in better staging (pN0 cohort) and in more effective resection (in pN1 patients).

Poor differentiation (G3) was also found as a predictor of adverse prognosis in 12 studies but as an independent factor in only one study. An advanced T classification and AJCC stage - also poor prognosis predictors - were found as independent factors in 30% and 50% of considered studies, respectively. Pancreatic invasion and tumor size also result as predictors of poor prognosis but they can be easily correlated to T classification and AJCC stage by definition.

Although not included in the TNM classification, pancreatobiliary subtype, perineural, lymphatic and vessels invasion, macroscopic ulceration are other adverse prognostic factors[20,25]. As shown in Table 2 several studies proved that perineural, lymphatic and vessel invasion are predictors of poor prognosis, often also at multivariate analysis. The same consideration is true for pancreatobiliary compared to intestinal subtype, but there are very few studies that deal with this issue because this histological sub classification is relatively recent. In order to better investigate the importance of this predictor Westgaard *et al*[25] analysed 114 resected periampullary adenocarcinomas (including neoplasm originated from ampullary, duodenal, biliary and ductal pancreatic epithelium), dividing them into pancreatobiliary or intestinal type of differentiation, and compared their survival with a historical control group. The authors found that at multivariable analysis histologic subtype of differentiation was an independent predictor of survival, while tumor origin was not. Pancreatobiliary type was found to have a worse prognosis in the whole periampullary group and also in the subgroup of ampullary carcinomas, with a 5 years survival rate around 25%[33,54] and a more than 3-fold increase in mortality risk compared to intestinal type[25].

Tumor involvement of resection margins has also consistently been demonstrated to be an adverse prognostic factor in comparison with negative margin resections (median survival 11.3 mo *vs* 59.5 mo, respectively)[28] and warrants the use of the residual tumor classification on specimen assessment (R0: grossly and microscopically negative margins, R1: grossly negative but microscopically positive margins and R2: grossly and microscopically positive margins)[133].

A recent study has also shown the importance

of tumor budding. Tumor budding is an already established predictor of survival in colorectal, oesophageal and anal squamous cell carcinomas. It is defined as presence of isolated or small clusters of tumor cells that detached from the neoplastic epithelium and migrate a short distance into the neoplastic stroma. High-budding resulted to be a strong independent predictor of survival also in ampullary cancers (5-years survival of 24% compared to 68% for low-budding tumors)[134].

Moreover in a recent preliminary study Park *et al*[135] investigated the role of angiogenesis on survival in node negative ampullary tumors. Using a specific staining (Chalkley assay) for angiogenesis quantification they found that increased angiogenesis is associated with disease recurrence in patients with node negative tumors. Investigation of the mechanism of angiogenesis in cancer of the ampulla of Vater may provide further prognostic information and help to rationalize therapy.

It must however be remarked that currently, despite the large number of studies regarding prognosis of these neoplasm, our understanding of the argument is limited by the small cohorts of patients analyzed and by the presence of a lot of confounders, such as stage, pathological subtypes, surgical resection, co-morbidities or adjuvant treatments.

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EDITORIAL

Current status and progress of pancreatic cancer in China

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Abstract

Cancer is currently one of the most important public health problems in the world. Pancreatic cancer is a fatal disease with poor prognosis. As in most other countries, the health burden of pancreatic cancer in China is increasing, with annual mortality rates almost equal to incidence rates. The increasing trend of pancreatic cancer incidence is more significant in the rural areas than in the urban areas. Annual diagnoses and deaths of pancreatic cancer in China are now beyond the number of cases in the United States. GLOBOCAN 2012 estimates that cases in China account for 19.45% (65727/337872) of all newly diagnosed pancreatic cancer and 19.27% (63662/330391) of all deaths from pancreatic cancer worldwide. The population's growing socioeconomic status contributes to the rapid increase of China's proportional contribution to global rates. Here, we present an overview of control programs for pancreatic cancer in China focusing on prevention, early diagnosis and treatment. In addition, we describe key epidemiological, demographic, and socioeconomic differences between China and developed countries. Facts including no nationwide screening program for pancreatic cancer, delay in early detection resulting in a late stage at presentation, lack of awareness of pancreatic cancer in the Chinese population, and low investment compared with other cancer types by government have led to backwardness in China's pancreatic cancer diagnosis and treatment. Finally, we suggest measures to improve health outcomes of pancreatic cancer patients in China.

Key words: Pancreatic cancer; Incidence; Diagnosis; Treatment

Core tip: The health burden of pancreatic cancer in China is increasing, with annual mortality rates almost equal to incidence rates. Cases in China account for 19.45% of all newly diagnosed pancreatic cancer and 19.27% of all deaths from pancreatic cancer worldwide.

Facts including no nationwide screening program for pancreatic cancer, delay in early detection resulting in a late stage at presentation, lack of awareness of pancreatic cancer in the Chinese population, and low investment compared with other cancer types by government have led to backwardness of China's pancreatic cancer diagnosis and treatment.

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WORLDWIDE EPIDEMIOLOGY OF PANCREATIC CANCER

Pancreatic cancer, one of the most frequent cancers in the world, is a devastating malignant disease with a median survival of 3-6 mo and a 5-year survival rate of less than 5%[1-4]. Despite improvements in surgical techniques and adjuvant medical therapy, these figures have not changed in over four decades, with the mortality approaching the incidence. According to the latest global estimation, GLOBOCAN 2012, the age standardized rate (ASR) of pancreatic cancer incidence data is 4.9 per 100000 in men, and 3.6 per 100000 in women. ASR mortality rate is 4.7 per 100000 in men, and 3.4 per 100000 in women. Worldwide, the age-standardized rate (ASR-W) for the incidence and mortality of pancreatic cancer is 4.2% (Figure 1) and 4.0% (Figure 2), respectively[5]. In the United States, the ASR incidence and mortality of pancreatic cancer is 7.5% and 7.0%, respectively. The recent data showed that 48960 people were estimated to be diagnosed with pancreatic cancer in 2015, and 40560 people would die from pancreatic cancer in the United States[6].

Epidemiology in China

China is the largest developing country with nearly a fifth of the global population. As a result of rapid urbanization, more and more Chinese people live in urban areas. Combined with other factors such as aging and environmental pollution, the disease spectrum in China has shifted from infectious to non-infectious diseases. Among the non-communicable diseases, the health burden of cancer is increasing. Although China has a lower incidence of pancreatic cancer than western countries, the incidence of this disease in China has increased as fast as that worldwide recently. In 2010, 34509 men and 23226 women died from pancreatic cancer in China, with the number of deaths exceeding that in the United States[5,7,8].

Significant improvements of pancreatic cancer diagnosis and treatment have been achieved by China over the past 30 years. Here, we review the status of pancreatic cancer in China, and describe important epidemiological, risk factors, screening methods, diagnosis and therapy of pancreatic cancer. In addition, we discuss the challenges and trends of pancreatic cancer in China, and explore development of a multicenter cooperative research system to improve its clinical outcome.

Incidence and mortality

Population-based cancer registries collect data on annual cancer incidence and mortality to provide accurate and up-to-date information that is vital for cancer prevention, control, and research[7]. Since 2006, data contained in the Cancer Registry Annual Report released by the National Central Cancer Registry (NCCR) indicate that the incidence and mortality of pancreatic cancer in China has gradually risen[9]. Although the number of cancer registries in China is increasing, the available data for incidence and mortality of pancreatic cancer covers only about 13% of the nation's population, while nearly 100% of the population in the United States is covered[6,10]. Currently, the true burden of pancreatic cancer in China cannot be estimated by using data from the Cancer Registry Annual Report alone, due to the above limitation. Thus, expansion of cancer registries covering more population would improve accuracy of estimates of cancer burden.

Incidence

GLOBOCAN 2012 estimated that pancreatic cancer was one of the most frequent malignancies in China, with an ASR-W incidence of 3.6 per 100000 for both sexes[5]. According to the Chinese Cancer Registry Annual Report 2012, the incidence of pancreatic cancer fluctuated according to sex, region and age. The crude incidence rate of pancreatic cancer in registration areas was 7.28 per 100000, with 8.24 per 100000 for men and 6.29 per 100000 for women in 2009. The ASR was 3.35 per 100000 with 4.01 and 2.72 per 100000 for men and women, respectively[10].

The crude incidence rate of pancreatic cancer in Chinese urban populations was 8.19 per 100000 (9.36 per 100000 for men and 7.00 per 100000 for women), which was 51.39% higher than that in rural areas (5.41 per 100000 overall; 5.97 per 100000 for men and 4.83 per 100000 for women). The data remained 27.76% higher after age standardization[10]. Standardized by the age structures worldwide, the ASR incidence in urban areas was 4.96 per 100000, which was also higher than that in rural areas (3.83 per 100000). Among the urban cancer registration areas, Shanghai had the highest crude incidence rate of 15.19 per 100000 (Figure 3).

The age-specific incidence rates of pancreatic cancer dramatically increased after 40 years old in

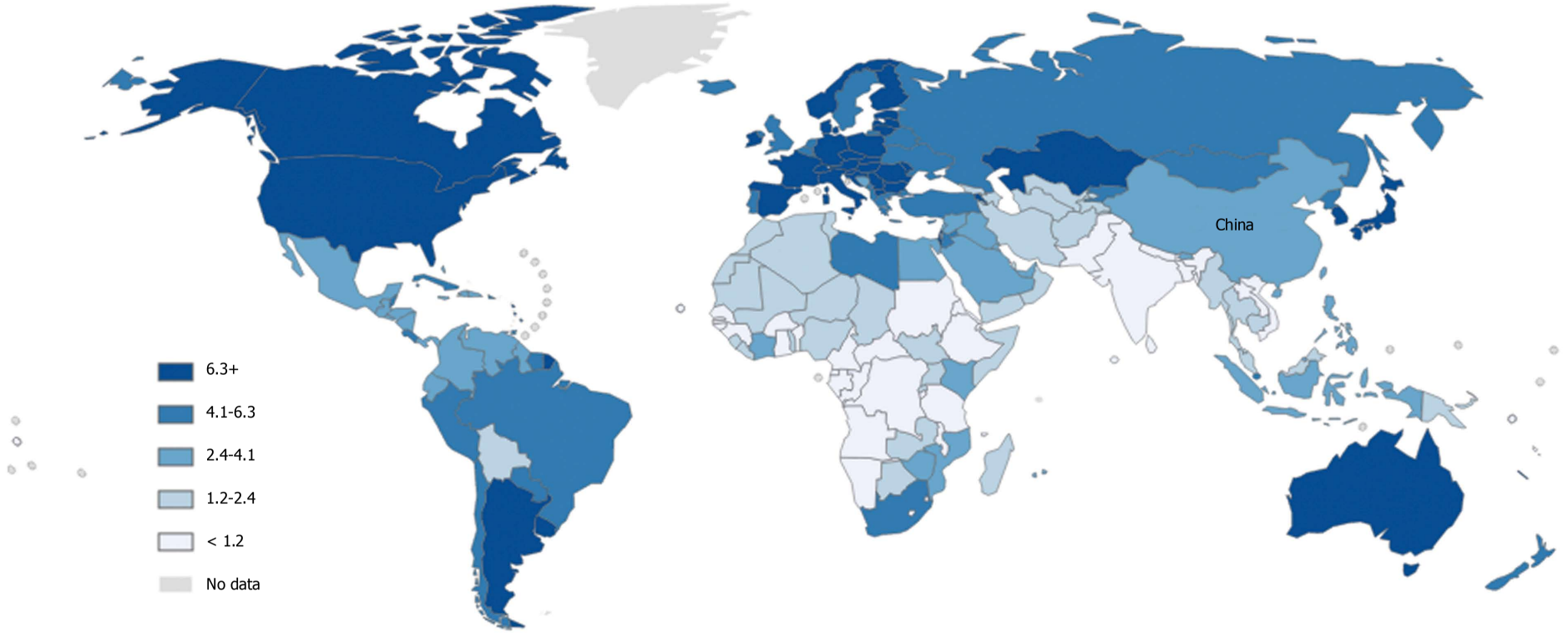

Figure 1 Map of estimated global pancreatic cancer incidence rates according to GLOBOCAN 2012.

China, with the incidence peak at about 80 years old (Figure 4). The trends in urban and rural areas were similar to those in the entire country, with the age-specific incidence rate in urban areas higher than that in rural areas after age 50 years. The cumulative incidence rate for patients aged 0-64 years was 0.21%, while for those aged 0-74 years it was 0.54%[10]. Comparison of age distribution among some major countries and continents showed that more patients younger than 65 years were diagnosed with pancreatic cancer in China, which means that age at diagnosis of pancreatic cancer in China is less than that in western countries (Figure 5). In China, 61.2% of patients with pancreatic cancer were aged 65 years or older, compared with 80.1% of patients in Japan.

Although there were fluctuations according to sex and region, the upward trend in the incidence rate of pancreatic cancer is real in China. According to the Chinese NCCR, the incidence rate increased from 6.26 per 100000 in 2003 to 8.37 per 100000 in 2009[11]. During this 7-year period, the incidence rate increased from 6.83 to 9.48 per 100000 in men and from 5.67 to 7.24 per 100000 in women. The increasing trend of pancreatic cancer incidence was more significant in rural than in urban areas. The incidence rate increased 1.27 times from 2003 to 2009 in urban areas, while the rate was 1.61 times higher in 2009 than in 2003 in rural areas.

Mortality

Pancreatic cancer is a rapidly disastrous malignancy with dismal prognosis. Recent mortality rates of pancreatic cancer in developed countries such as Japan have

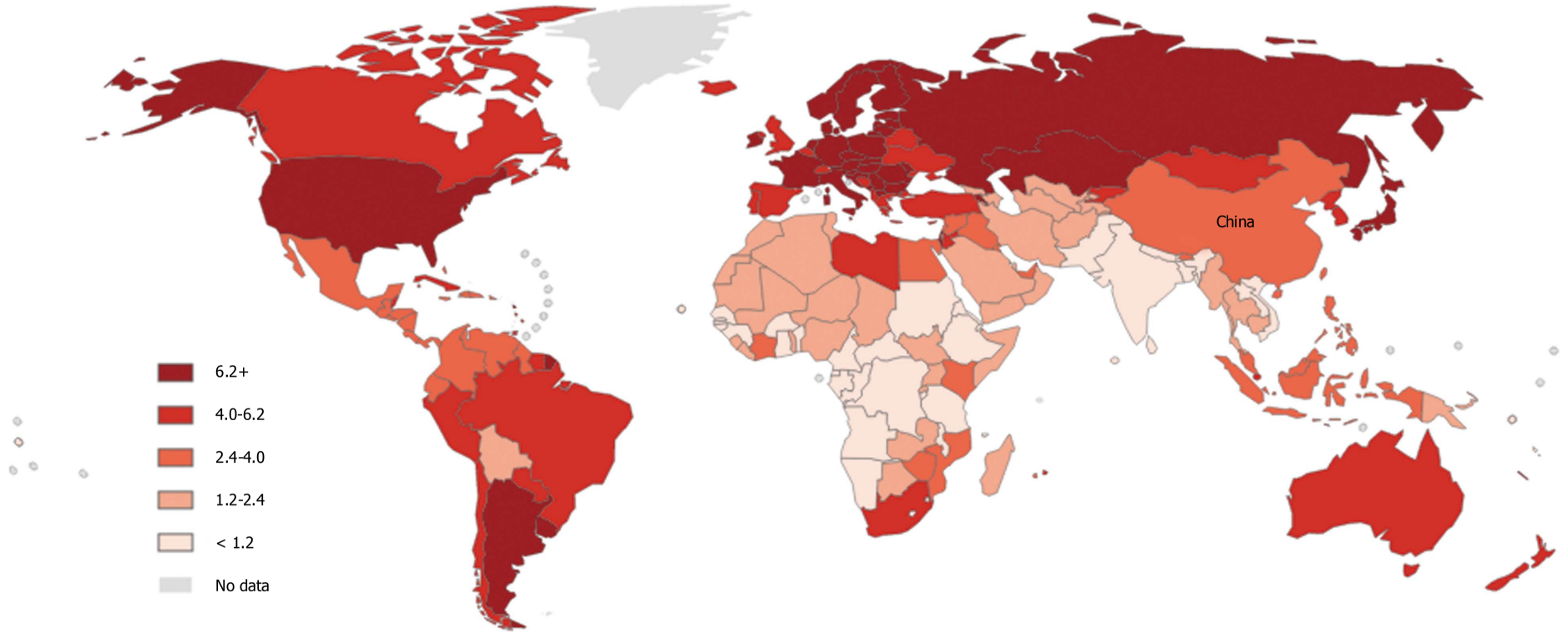

Figure 2 Map of estimated global pancreatic cancer mortality rates according to GLOBOCAN 2012.

stabilized after an increase[12], while in China mortality due to pancreatic cancer is rising. Despite that, the prevalence, incidence and mortality of pancreatic cancer in China are relatively lower than those in developed countries such as Europe, the United States and Japan, and comparison of mortality-to-prevalence ratios showed that survival outcome for pancreatic cancer is worse in China than in most other countries. The mortality-to-prevalence ratio amounts to 0.85 in China, compared with 0.7 in the United States, 0.56 in South Korea, 0.55 in Germany, and 0.4 in Japan (Figure 6). The phenomenon may be due to lack of improvement in the treatment of pancreatic cancer, especially in China[13].

According to the Cancer Registry Annual Report 2012, pancreatic cancer ranks as the seventh highest cause of cancer death in China, with a crude mortality of 6.61 per 100000. The mortality rate in urban areas (7.42 per 100000) was 50.20% higher than that in rural areas (4.94 per 100000). The data remained 27.09% higher after age standardization. The trend might be due to disparities in socioeconomic circumstance, and lifestyle between urban and rural areas. The mortality reached the peak at around 80 years old in both urban and rural areas (Figure 7), which was similar to the incidence. From 2003 to 2009, the mortality rate of pancreatic cancer increased from 5.63 to 7.78 per 100000[11]. The upward trend in mortality concerns both men and women, and both urban and rural areas.

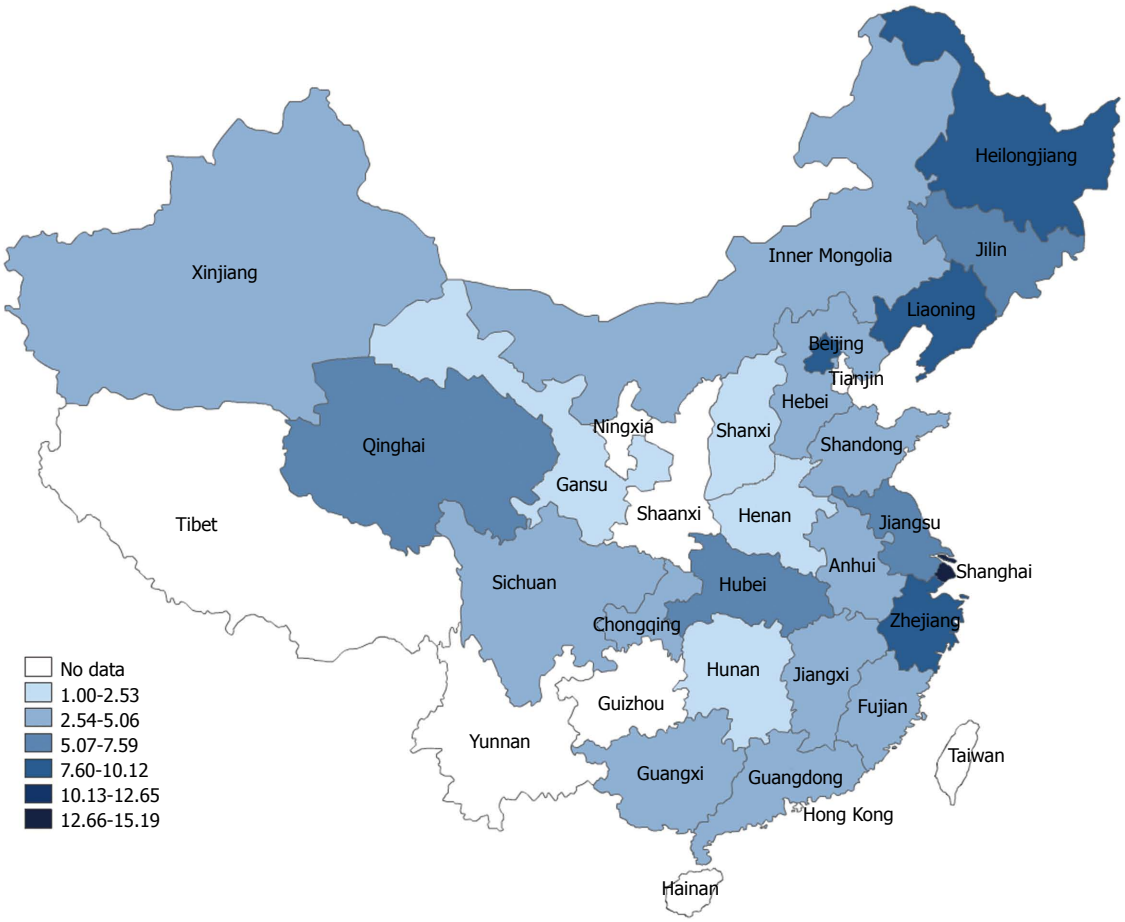

Figure 3 Crude rate (1/10^5) of pancreatic cancer in China. Figure based on data from the Chinese Cancer Registry annual report (2012).

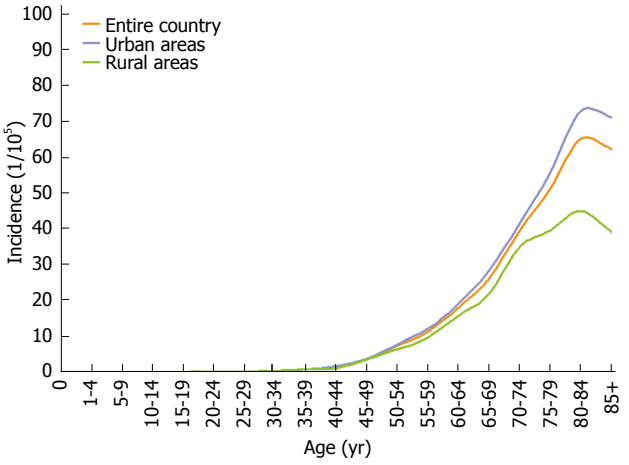

Figure 4 Age-specific incidence of pancreatic cancer in China, 2009. Figure based on data from the Chinese Cancer Registry annual report.

RISK FACTORS OF PANCREATIC CANCER

Pancreatic cancer is considered a malignancy correlated with industrialization as suggested by the fact that the majority of deaths occurred in developed countries. Despite the fact that it is unclear what factors cause pancreatic cancer, several risk factors and established genetic syndromes are associated with pancreatic cancer. Although identification of country-specific trends for risk of pancreatic cancer is valuable, risk factors in China are similar to those worldwide. Recent substantial increases in the prevalence of cigarette smoking, obesity, and diabetes mellitus in China may be related to the increasing incidence of pancreatic cancer. Also, studies show that severe deterioration of the environment in China and problems with food contamination may contribute to the increasing occurrence of cancer[14,15].

Tobacco use

Tobacco use is one of the most important risk factors of pancreatic cancer, and a dose- and duration-related pattern has been demonstrated for earlier age of onset[16,17]. Around the world, 9% of all cancer deaths are related to smoking among male smokers, and male smokers have a 74% higher risk of pancreatic cancer compared with non-smokers[18]. A recent hospital-based case-control study by Wang *et al*[19] showed that current smokers had a significantly increased risk of pancreatic cancer (OR = 1.71, 95%CI: 1.25-2.35) with a decreasing trend in risk correlated with years of smoking cessation. Another case-control study

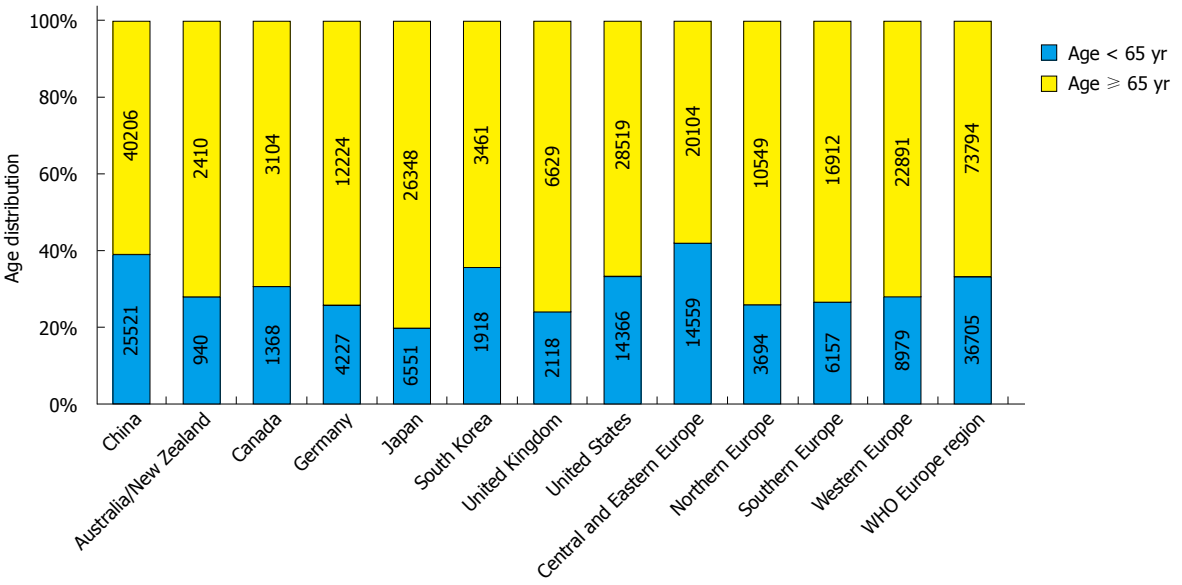

Figure 5 Comparison of age distribution of patients with pancreatic cancer between China and some major countries and continents.

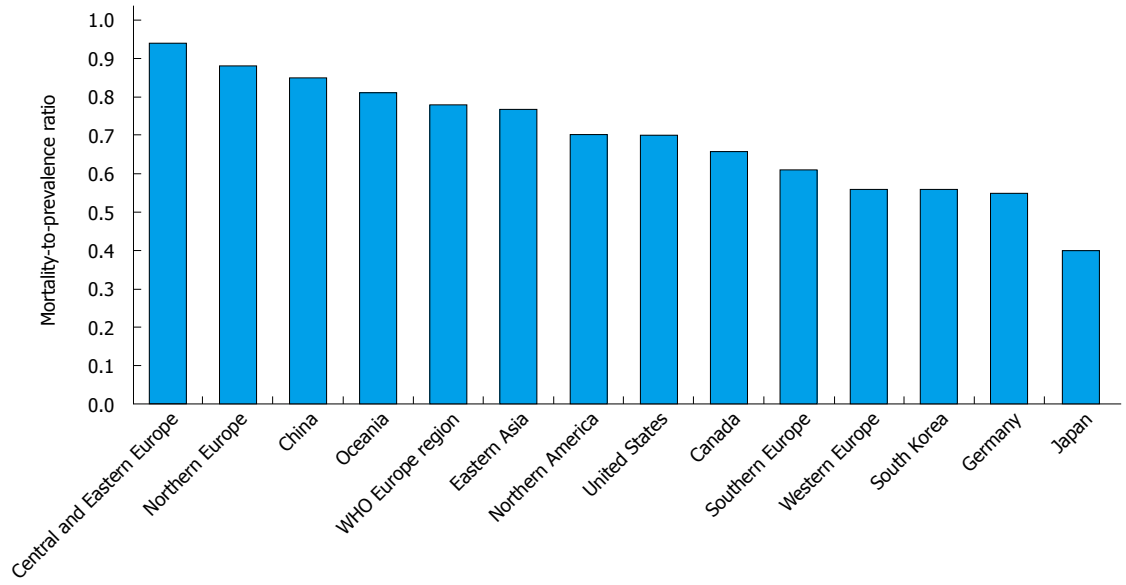

Figure 6 Comparison of mortality-to-prevalence ratio of pancreatic cancer between China and some major countries and continents.

by Yin *et al*[20] revealed that smokers had an OR for pancreatic cancer of 3.53 (95%CI: 3.0-9.6) compared with non-smokers, and smoking in the morning was an increased risk factor for pancreatic cancer (OR = 5.50, 95%CI: 1.22-24.81). In addition, secondhand smoke exposure may increase the risk of pancreatic cancer by 50%, and children exposed passively to tobacco smoke have a double risk of pancreatic cancer as adults[21,22].

Obesity and dietary factors

Obesity, which is associated with increased risk of diabetes mellitus, is also a risk factor for the development of pancreatic cancer[23]. A case-control study including 841 patients with pancreatic adenocarcinoma and 754 healthy individuals showed that being overweight [body mass index (BMI) of 25-29.9] or obese (BMI ⩾ 30) during early adulthood was associated with an increased risk of pancreatic cancer. A younger age of disease onset and obesity at an older age reduced overall survival in patients with pancreatic cancer, regardless of disease stage or tumor resection status[24]. Studies have shown that the relative risk of pancreatic cancer was 1.16 in men and 1.10 in women per 5-point increase in BMI[25,26].

Dietary factors are also related to pancreatic cancer. Although there is evidence that folate and folate-containing foods exert a protective effect against pancreatic cancer[27], this was not confirmed in a recent study[28]. A multicenter case-control study by Chinese researchers[29] showed that reduced vegetable consumption was significantly associated with pan-

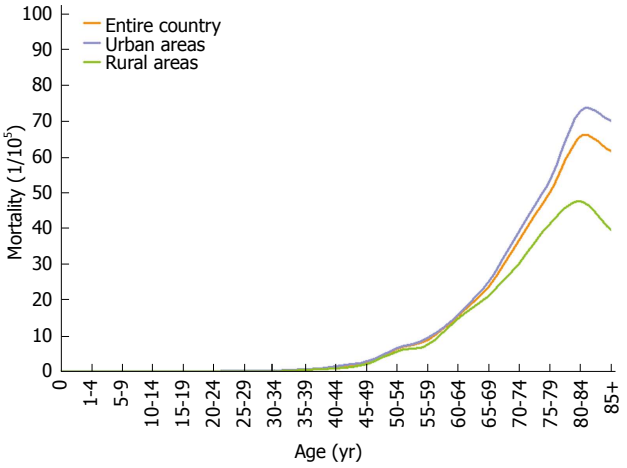

Figure 7 Age-specific mortality of pancreatic cancer in China, 2009. Figure based on data from the Chinese Cancer Registry annual report.

creatic cancer (*P* trend 0.04), and meat and fruit consumption was not significantly related to the risk of pancreatic cancer. A protective effect was discovered for fruit (OR = 1.73 for consumption of 1 or 2 times per week *vs* > 3 times per week; 95%CI: 1.05-2.86). A population-based case-control study from an urban area of Shanghai[30] reported that dietary energy density (defined as the amount of energy theoretically able to be metabolized per unit weight of food) had an OR for pancreatic cancer of 1.16 per unit increase (95%CI: 1.07-1.27), and dietary energy density was positively related to risk of pancreatic cancer. The molecular mechanisms to explain these results are not well investigated, but chronic inflammation mediated by secreted molecules from adipose tissue and hormonal factors is likely involved[24,31]. Supporting this hypothesis, a study by Zhang *et al*[32] showed that dietary and other lifestyle factors that influenced insulin resistance were also associated with the risk of pancreatic cancer.

Diabetes

Whether diabetes mellitus is a risk factor or a result of pancreatic cancer is still unclear. However, diabetes mellitus improves following pancreatectomy, suggesting that diabetes mellitus may be caused by pancreatic cancer. Studies have shown that 25% of patients diagnosed with pancreatic cancer had diabetes mellitus, among whom 40% were pre-diabetic[33,34]. The association between diabetes mellitus and pancreatic cancer has been summarized in several meta-analyses[35-37]. A study by Huxley *et al*[36] reported that the overall risk was 1.82 (95%CI: 1.66-1.89) for developing pancreatic cancer in patients with diabetes mellitus, relative to patients without diabetes mellitus. The risk of pancreatic cancer declines with increased duration of diabetes mellitus. Patients with diabetes mellitus within 4 years had a 50% increased risk of pancreatic cancer compared with patients with diabetes mellitus for 5 years or longer. Patients whose diabetes mellitus had lasted 5 years or longer had a 50% greater relative risk than those without diabetes mellitus[36]. This is not surprising because pancreatic-cancer-associated diabetes mellitus is predominantly new onset[38]. In a Chinese retrospective cohort study, male and female patients with type 2 diabetes mellitus were 2.97 and 2.68 times more likely, respectively, to develop pancreatic cancer compared with the general population[39]. Similar results were obtained by Kuang and coworkers[40], who showed that the incidence of diabetes mellitus was higher in pancreatic cancer patients than in controls.

Genetic risk factors

Although the occurrence of pancreatic cancer seems to be sporadic, it has been reported that 5%-10% of pancreatic cancer patients have hereditary factors[41]. Cases of inherited predisposition to pancreatic cancer fall roughly into three categories[23]. The first consists of hereditary cancer syndromes such as Lynch syndrome[42], familial adenomatous polyposis, Peutz-Jeghers syndrome, and familial atypical multiple mole melanoma syndrome, which are characterized by specific germ-line gene mutations and associated with increased risks of pancreatic cancer. The second category comprises conditions such as hereditary pancreatitis and cystic fibrosis, in which there is an inherited predisposition to the development of pancreatic cancer. The third category is familial pancreatic cancer, defined as two or more first-degree relatives with pancreatic cancer that does not fulfill the criteria of other hereditary cancer syndromes with increased risks of pancreatic cancer[43].

Other risk factors

Additional risk factors including male sex, low income, advanced age, alcohol use[40], chronic pancreatitis[44], a history of cholecystectomy or partial gastrectomy[45,46], and chronic infections have also been shown associated with pancreatic cancer[47]. Moreover, it has been reported that some pancreatic cystic lesions such as intraductal papillary mucinous neoplasm and mucinous cystic neoplasm have the potential to progress to invasive pancreatic cancer[48], and patients with these lesions belong to groups at high risk of pancreatic cancer. In addition to the above risk factors, other factors such as environmental pollution and food contamination, which are becoming serious issues affecting public health in China[49], may be associated with the increased trend of pancreatic cancer. Although so far these associations lack solid epidemiological evidence, a recent cohort study demonstrated that airborne particulate matter of diameter < 10 μm from the incinerator was associated with pancreatic cancer mortality[50].

SCREENING FOR EARLY PANCREATIC CANCER

Recent studies suggest that pancreatic cancer develops over a long time with an average of nearly 17 years from cancer-initiating cells to metastatic cancer subclones, followed by death after approximately 2.7 years[51,52]. Patients with small tumors detected at early stages have reportedly better outcomes[53-55]. Thus, a screening program for high-risk individuals has the benefit of better outcomes in patients with pancreatic cancer.

There is no nationwide screening program for pancreatic cancer in China at present. Obstacles to implementation of a population-based screening program include insufficient convincing accuracy and cost-effectiveness data; insufficient equipment; and inadequate insurance coverage for such a screening program. There has been no consensus on the definition of high-risk individuals for pancreatic cancer until now. Patients in China who are at high risk of pancreatic cancer may be characterized by any of the following[56,57]: (1) older than 40 years and presenting with nonspecific abdominal symptoms; (2) a family history of pancreatic cancer; (3) new-onset diabetes mellitus, especially in those older than 60 years with either atypical diabetes mellitus or rapidly developing insulin resistance, without family history or obesity; (4) chronic pancreatitis, especially when accompanied by precancerous lesions; (5) intraductal papillary mucinous neoplasms; (6) familial adenomatous polyposis; (7) distal subtotal gastrectomy for benign disease, especially 20 years after resection; and (8) heavy tobacco or alcohol use or long-term contact with hazardous chemical substances.

When encountering these populations, non-invasive screening methods such as ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), and magnetic resonance cholangiopancreatography (MRCP), combined with pancreatic-cancer-related biomarker examination are recommended. Among these high-risk groups, patients with new-onset diabetes mellitus may be an attractive screening target for early pancreatic cancer. The potential clinical benefit of screening for early pancreatic cancer in high-risk groups appears to exceed that for breast cancer[58].

Imaging

The screening modalities applied for detection of pancreatic cancer mainly include ultrasonography, CT, MRI, MRCP, and endoscopic ultrasound (EUS). Ultrasonography, a noninvasive and cost-effective modality, is frequently the first-line screening tool for patients with suspected pancreatic lesions, although it is not a reliable method, and highly dependent on the operator's experience and body habitus of patients[59]. Contrast-enhanced CT is now the worldwide imaging modality of choice for evaluation of pancreatic disease, and may be the best modality to assess resectability of pancreatic cancer[60]. Nevertheless, radiation exposure and the suboptimal detection rate limit its use as a routine screening tool for asymptomatic high-risk individuals[61]. Studies revealed that MRI and EUS may be better than CT for early diagnosis of pancreatic neoplasms[62]. Thus, it has been proposed that initial screening should include EUS with or without MRI or MRCP, but not CT or endoscopic retrograde cholangiopancreatography (ERCP)[61]. However, the high cost and limited availability of MRI generally mean that it is utilized only after ultrasonography or CT. While EUS has been used as a principal imaging modality for screening pancreatic cancer in multiple international programs[58], a study by Long *et al*[63] showed that in Shanghai only 5.7% of patients underwent EUS to detect pancreatic cancer. Evaluation of the results of EUS is dependent on the doctor's experience[64]. It is not widely used in China, and is only regularly performed in a few large medical centers[65].

Molecular markers

A limitation of screening for early pancreatic cancer is the absence of sensitive and specific markers. Compared with unwarranted imaging or more invasive testing, serological markers are always preferred due to the ease of collection, a relatively noninvasive trait. Carbohydrate antigen (CA)19-9, the only currently predictive biomarker for therapeutic outcome of pancreatic cancer, is commonly used to screen the disease. However, given the low incidence of pancreatic cancer, a blood-based marker with high specificity (99%) and sensitivity (100%) will lead to 83 false-positive for every true-positive case based on the incidence rate of 12.1 per 100000 in the United States[66]. Therefore, poor-to-moderate sensitivity and specificity in detecting pancreatic cancer limit its use in screening. For example, according to a study from China, the sensitivity of CA19-9 was only 57%, with an accuracy of 67.7%. These results indicated the limited value of CA19-9 in detecting early pancreatic cancer[67].

DIAGNOSIS

Cancer stage at diagnosis is an important factor influencing survival of pancreatic cancer patients. A multicenter nationwide study in China showed that 18.4% of pancreatic cancer patients received diagnoses at stages Ⅰ or Ⅱ, and 81.6% at stages Ⅲ or Ⅳ[68]. A recent study that included 11672 cases recorded in the Shanghai Cancer Registry 2004-2009[69] reported that nearly 42.9% of patients had regional or distant metastasis at diagnosis, whereas 49.2% had localized disease. The median survival was 3.9 mo (95%CI: 3.8-4.0 mo) and the overall 5-year survival rate was 4.1%. Reasons for poor survival of the cohort in that study included delayed diagnosis of pancreatic cancer. In contrast, the United States is better than

China at early detection of pancreatic cancer. In a recent study, Yu *et al*[70] analyzed 13131 patients with pancreatic ductal adenocarcinoma between 2004 and 2011 in the National Cancer Institute Surveillance, Epidemiology and End Results (SEER) database, which was considered representative of the United States population. They found that 62.9% of pancreatic cancer patients received diagnosis at stages Ⅰ or Ⅱ, and 37.1% at stages Ⅲ or Ⅳ. Since pancreatic cancer has no distinctive symptoms, it is usually diagnosed when a patient has symptoms of abdominal pain or jaundice. Most patients diagnosed with pancreatic cancer lack the chance of radical surgery due to late stage. It is important to determine the resectability of pancreatic cancer because the rate of postoperative complications after pancreatic surgery is high even in high-volume medical centers[71-73]. Radiological imaging is an important method for initial detection, staging, and evaluation of pancreatic cancer resectability, which includes identification of the primary tumor, local lesion resectability, and distant metastasis[59].

Multidetector row computed tomography (MDCT) is a worldwide imaging modality for evaluation of pancreatic cancer. More than 75% of patients who received a diagnosis of pancreatic cancer in China had undergone MDCT[63]. MDCT has good spatial and temporal resolution with anatomical coverage[74]. MDCT can assess both local tumor resectability and distant metastasis. It is also the best imaging modality for assessment of vascular involvement, which is crucial for prediction of tumor resectability[75,76]. MRI is also currently used for patients with pancreatic diseases[77]. Compared with CT, MRI is not only an outstanding tool for characterizing pancreatic mass, but also a successful technique for noninvasively delineating the pancreatic ductal system, as an alternative to ERCP. Positron emission tomography (PET)/CT is useful for detecting pancreatic cancer, especially metastases throughout the body[78,79]. However, widespread application of PET/CT is limited in China by its high rate of false-positive results, low spatial resolution, and high cost[80,81].

Expert for MDCT, other imaging modalities such as MRI and PET/CT are not widely used in Chinese patients[63]. In western countries, EUS has been widely used for detection of pancreatic cancer in recent years[82], especially for high-risk groups and patients with small tumors[58]. Furthermore, EUS-guided fine-needle aspiration (FNA) has the unique ability to acquire specimens for histopathological diagnosis of this devastating disease, especially in unresectable patients. A most recent study by Ngamruengphong *et al*[82], using the SEER-Medicare data including 2034 patients with pancreatic cancer, showed that preoperative EUS-FNA did not impair survival of pancreatic cancer. However, EUS-FNA is not widely performed in China, although it is now the standard of care in western countries. Less than 40% of cases diagnosed with pancreatic cancer in China had histological verification, mostly *via* surgery with pathological diagnosis[63]. Limited application of EUS in China may explain why so many patients diagnosed with unresectable pancreatic cancer lack histological confirmation.

THERAPY

Fewer than 20% of patients are eligible for curative resection as pancreatic cancer is usually detected at a late stage. Surgical resection is the cornerstone of treatment, which offers the only chance to cure patients with pancreatic cancer. Many patients have disease recurrence even after radical surgery. Adjuvant chemotherapy, radiotherapy, targeted therapy, and traditional Chinese medicine have been commonly used to improve quality of life.

Surgery

Surgical resection is the only potentially curative therapy for pancreatic cancer. Assessment of the involvement of local vessels is the key to determine tumor resectability. With improvements in safety of pancreatic surgery in recent decades, in the hope of improving long-term survival, surgeons have continued to explore the role of more extensive surgery. Whether patients should receive extended lymphadenectomy or not is controversial. A meta-analysis comparing standard lymphadenectomy with extended lymphadenectomy during pancreaticoduodenectomy for pancreatic cancer revealed that the extended procedure did not benefit overall survival, and might even cause a trend towards increased morbidity[83]. Due to no benefit in long-term survival being demonstrated, standard pancreaticoduodenectomy continues to be the choice for pancreatic head cancer.

A noteworthy change in pancreatic surgery in China may be towards a minimally invasive approach, particularly distal pancreatectomy, which could gain wide acceptance for benign and low-malignancy tumors[84,85]. A recent meta-analysis reported that laparoscopic distal pancreatectomy resulted in less loss of blood and time in hospital, and lower rates of overall complications and infections, but did not lower rates of postoperative pancreatic fistula or mortality[86]. Application of robotic surgery has advantages over laparoscopy, including the rate of R0 resections (*i.e.*, complete resection with no tumor within 1 mm of the resection margins), and greater lymph node yield[87]. A more recent study from Mayo Clinic showed that total laparoscopic pancreaticoduodenectomy (TLPD, n = 108) was feasible in the setting of pancreatic ductal adenocarcinoma, and had advantages over open pancreaticoduodenectomy (OPD), including shorter hospitalization and faster recovery, allowing patients to recover in a timelier manner and pursue adjuvant therapy. There was also a significantly longer progression-free survival in the TLPD group than in the OPD group (n = 214), while the overall survival rates of the two groups were similar[88]. Despite this, robotic

pancreatectomy is now not a common procedure in China, mainly due to cost pressures. Nevertheless, the effects and benefits need to be confirmed by more data in China.

Chemotherapy and targeted therapy

Chemotherapy for patients presenting with advanced pancreatic cancer is widespread in China. Gemcitabine is the gold standard for pancreatic cancer treatment and achieves a modest improvement in overall survival. Gemcitabine-based combination chemotherapy has been first-line for advanced pancreatic cancer for more than one decade and shows a superior clinical response and survival. In a study by Long *et al*[63], more than 30% of 846 pancreatic cancer patients recruited in a population-based study underwent chemotherapy, and 9.6% of patients received combination therapy. Regional intra-arterial infusion chemotherapy for pancreatic cancer has been safely used to suppress tumor growth and was more effective in reducing incidence of liver metastasis[89]. In a study by Jin *et al*[89], 50 patients who underwent regional intra-arterial infusion chemotherapy had disease-free and median survival times of 15.5 and 18 mo, respectively. However, the necessity for regional arterial chemotherapy remains controversial due to its invasive nature[90].

Gemcitabine plus nab-paclitaxel and FOLFIRINOX (oxaliplatin, irinotecan, fluorouracil, and leucovorin) have been recently reported to improve survival for metastatic pancreatic cancer significantly[91,92]. However, these two regimens are currently not popular in China due to increased toxicity and high cost not covered by insurance. The situation is similar for S-1, which is not inferior to gemcitabine for survival rate, with acceptable tolerance for locally advanced and metastatic pancreatic cancer[93]. Only small clinical studies have been conducted to evaluate these regimens for Chinese patients with advanced pancreatic cancer[94,95].

Targeted therapy may be promising to improve survival in advanced pancreatic cancer[96]. However, targeted therapy in several phase Ⅲ trials was not superior to standard chemotherapy[97-99]. Additionally, drug reimbursement policies strongly affect the availability of systemic therapy in China. Most targeted agents are not covered by insurance, leading to prohibitively high cost for patients and limitation of options for advanced pancreatic cancer.

Radiotherapy

The use of radiotherapy for pancreatic cancer is not popular in China. For instance, in Shanghai, only 3.5% of patients received radiotherapy as part of their primary treatment[63]. In a study by Chen *et al*[67] of pancreatic ductal adenocarcinoma, among 565 patients, only 14 underwent postoperative radiotherapy. The proportion of pancreatic cancer patients receiving radiotherapy in China is less than that in the United States. In a study by Mellon *et al*[100] using the SEER database including 2966 patients between 2004 and 2008, 62.1% of patients with pancreatic cancer received postoperative radiotherapy and had an associated survival benefit. However, similar to the incidence of new-onset pancreatic cancer in China, use of radiotherapy is also increasing. Given that patients who underwent concurrent chemoradiotherapy had better long-term survival compared with radiotherapy alone or chemotherapy alone[101], the efficacy of combination of S-1 with gemcitabine followed by concurrent radiotherapy has been investigated and appeared promising in Chinese patients with locally advanced pancreatic cancer[102]. Among the 32 patients who completed the scheduled course of chemotherapy, 30 received chemoradiotherapy. The median overall and progression-free survival was 15.2 and 9.3 mo, respectively. The 1-year and 2-year survival rates were 75% and 34.4%, respectively.

Traditional Chinese medicine

Traditional Chinese medicine, which mainly uses combinations of herbs, represents about 40% of the pharmaceutical market in China. Moreover, about 90% of oncologists prescribe herbs and 80% of patients with cancer have taken traditional Chinese medicine[103]. The widespread application of traditional Chinese medicine may be related to the belief that it can improve immune function and quality of life in cancer patients[104]. Huachansu injection, which is a water-soluble preparation made from skin of *Bufo gargarizans*, has been used in China for pancreatic cancer treatment. However, a recent randomized phase Ⅱ clinical trial showed that huachansu plus gemcitabine did not improve survival of patients with locally advanced or metastatic pancreatic cancer[105]. Turmeric root has been used medicinally for thousands of years in China. The active component is thought to be curcumin, which is commonly available worldwide and has been shown to exert activity against various malignancies, including pancreatic cancer[106]. In a phase Ⅱ study including 25 patients, curcumin showed clinical biological activity in some patients with advanced pancreatic cancer, without toxicity[107]. Another phase Ⅰ/Ⅱ study of 21 patients showed that patients with gemcitabine-resistant pancreatic cancer who received combination therapy using 8 g/d oral curcumin with gemcitabine-based chemotherapy had a median survival of 161 d (95%CI: 109-223 d) and the 1-year survival rate was 19%[108]. However, poor absorption limits the clinical activity of oral curcumin. To enhance curcumin absorption, nanotechnology and liposome-encapsulated curcumin have been studied *in vitro* and *in vivo* for pancreatic cancer[109,110].

PANCREATIC CANCER RESEARCH IN CHINA

Scientific publications related to pancreatic cancer

Table 1 Number of pancreatic cancer related publications and clinical trials by country

	China	Japan	South Korea	United Kingdom	United States	Germany
Total No. of publications[1]	3444	8126	1080	2431	16859	3650
Clinical trials published in PubMed[1]	84	400	69	153	825	227
Clinical trials ongoing[2]						
Clinicaltrials.gov	45	40	63	82	1105	122
ISRCTN	0	0	0	18	0	14
ICTRP	30	224	29	56	519	92
UMIN-CTR	0	237	0	0	0	0

[1]Retrieved from PubMed (Oct 26, 2014) with medical subject heading "pancreatic cancer" in "all fields" and "country" in "all fields" not in "text word". [2]Retrieved from Clinicaltrials.gov, ISRCTN, International Clinical Trials Registry Platform (ICTRP), and UMIN Clinical Trials Registry (UMIN-CTR), which are all approved by the International Committee of Medical Journal Editors (Oct 26, 2014) with search term "pancreatic cancer" and "country".

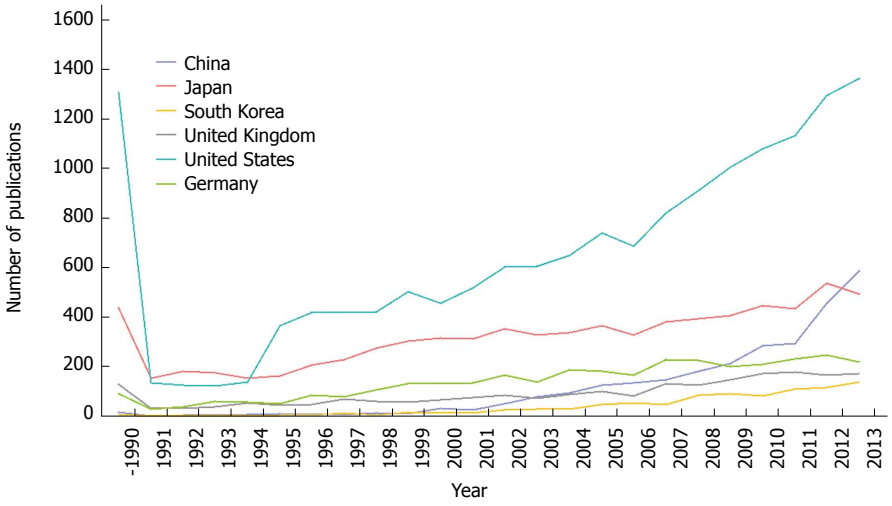

Figure 8 Publications by country in progress for pancreatic cancer over time.

from China have rapidly grown since 2007, regardless of the fact that the total number of publications in China is far less than that in the United States (Table 1). The number of publications from China in 2013 exceeded that from Japan, and was second only to the United States (Figure 8). Such great development of pancreatic cancer research in China may benefit from a steady increase in funding from the Chinese government, among which the National Nature Science Foundation of China (NSFC) is one of the most important research funds. Funding for pancreatic-cancer-related research from NSFC has steadily increased. However, investment in different cancer types remains uneven. Funding for research related to pancreatic cancer is less than for other cancer types, such as liver, breast and gastric cancers. The proportion of investment in research funding for pancreatic cancer has not been growing in the field of oncology in the past two decades (Figure 9). Nevertheless, concurrent with the rapid growth in scientific papers published from China, there is a rising concern that many of them are of inferior quality.

High-quality clinical trials are imperative for clinical decision making for care of pancreatic cancer patients. As a consequence of research funding shortage, there are only 84 clinical trials published in Pubmed and currently 75 clinical trials for pancreatic cancer registered in China (Table 1), with the numbers far less than those in developed countries. This may explain the inadequacy of high-quality pancreatic cancer research in China. Therefore, China needs to develop a multicenter cooperative research system for pancreatic cancer, take part in international multicenter clinical trials, or join an international cooperative group for pancreatic cancer.

CHALLENGES AND FUTURE DIRECTION

Each year more than 330000 patients are diagnosed with pancreatic cancer worldwide, with survival changing little in the last four decades. The incidence and mortality of pancreatic cancer in China are both increasing. According to the recent prediction from GLOBOCAN 2012, about 77497 men and 52868 women in China will be diagnosed with pancreatic cancer in 2035. Nearly 130000 patients will die from pancreatic cancer annually until then (Figure 10). The prediction means that the incidence and mortality of pancreatic cancer in China may increase faster in the next few years, and China will face a huge pancreatic cancer burden.

Unlike some cancers, pancreatic cancer is difficult

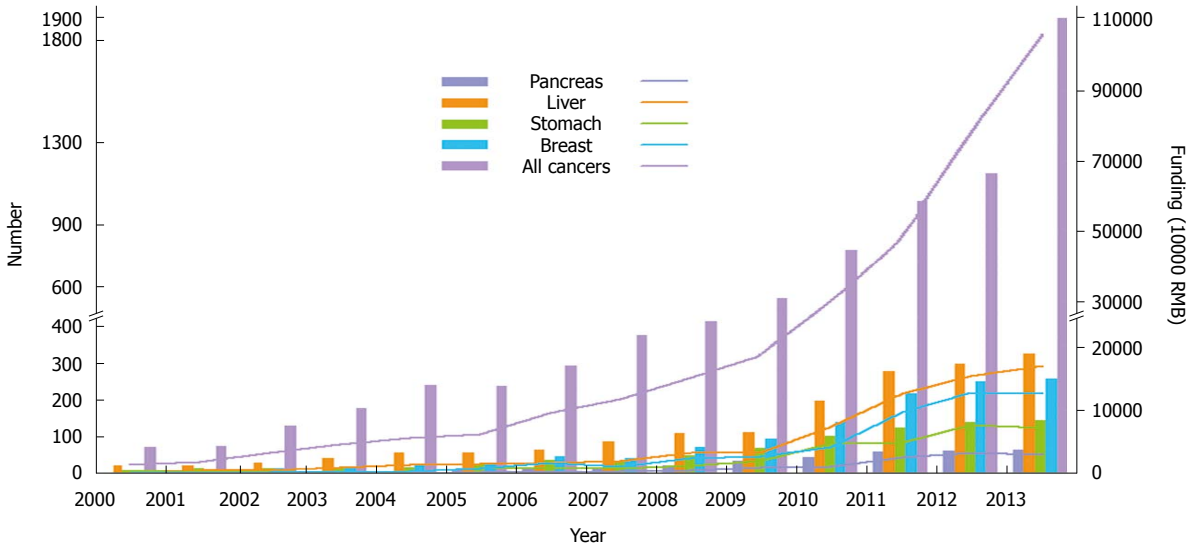

Figure 9 Numbers and research funding of National Natural Science Foundation of China about pancreatic cancer and other major malignancies over time.

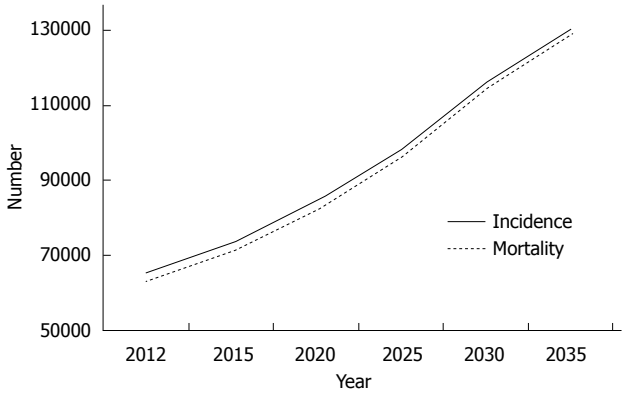

Figure 10 Estimated pancreatic cancer incidence and mortality in the next 20 years in China according to Globocan 2012.

to detect early, and usually presents at a late stage. With no established screening programs, resources might be allocated for earlier stage detection by offering a comprehensive strategy of imaging and genetics to identify curable cancers in high-risk individuals[111]. Some patients are overtreated with aggressive surgery or adjuvant therapy, while some are undertreated. Multidisciplinary care, which provides comprehensive evaluation and treatment, is the most effective approach to manage cancer patients[112]. However, multidisciplinary teams specialized in pancreatic cancer are currently rarely established in most centers in China, even in high-volume centers. Thus, multidisciplinary management of pancreatic cancer is an urgent need. Although more and more pancreatic cancer centers appear in China, rare multicenter collaborative studies have been conducted. The Chinese government should recognize shortfalls in research funding in areas of pancreatic cancer. More research supported by government funding can help us understand the biological behavior of pancreatic cancer and achieve successful treatment. Increased investment in education and healthcare are needed in order to upgrade the quality of care and to reduce the morbidity and mortality of pancreatic cancer. Last but not least, to improve national pancreatic cancer incidence and mortality statistics, the number of cancer registries still needs to be expanded, covering as much of the population as possible.

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Perioperative thrombotic complications in liver transplantation

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Abstract

Although the perioperative bleeding complications and the major side effects of blood transfusion have always been the primary concern in liver transplantation (OLT), the possible cohesion of an underestimated intrinsic hypercoagulative state during and after the transplant procedure may pose a major threat to both patient and graft survival. Thromboembolism during OLT is characterized not only by a complex aetiology, but also by unpredictable onset and evolution of the disease. The initiation of a procoagulant process may be triggered by various factors, such as inflammation, venous stasis, ischemia-reperfusion injury, vascular clamping, anatomical and technical abnormalities, genetic factors, deficiency of profibrinolytic activity, and platelet activation. The involvement of the arterial system, intracardiac thrombosis, pulmonary emboli, portal vein thrombosis, and deep vein thrombosis, are among the most serious thrombotic events in the perioperative period. The rapid detection of occlusive vascular events is of paramount importance as it heavily influences the prognosis, particularly when these events occur intraoperatively or early after OLT. Regardless of the lack of studies and guidelines on anticoagulant prophylaxis in this setting, many institutions recommend such an approach especially in the subset of patients at high risk. However, the decision of when, how and in what doses to use the various chemical anticoagulants is still a difficult task, since there is no common consensus, even for high-risk cases. The risk of postoperative thromboembolism causing severe hemodynamic events, or even loss of graft function, must be weighed and compared with the risk of an important bleeding. In this article we briefly review the risk factors and the possible predictors of major thrombotic complications occurring

in the perioperative period, as well as their incidence and clinical features. Moreover, the indications to pharmacological prophylaxis and the current treatment strategies are also summarized.

Key words: Vascular complications; Thromboembolic phenomena; Liver transplantation; Hepatic artery occlusion; Postoperative complications; Pulmonary emboli

Core tip: Data from many transplant centers demonstrate an underlying hypercoagulative state during liver transplantation. A dysfunctional hypercoagulable condition may persist for a variable time period after the transplant procedure. Occlusive vascular events deserve special attention because they pose a major threat to both patient and graft survival. Regardless of the lack of definitive guidelines on postoperative preventive antithrombotic treatment, many institutions recommend prophylactic anticoagulation in liver recipients at high risk of vascular thrombotic events. The present paper reviews the characteristics and risk factors of thrombotic complications after liver transplantation; in addition it presents valuable information on the relevance of pharmacologic thromboprophylaxis.

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INTRODUCTION

Liver transplantation (OLT) has become a particularly successful therapy for patients with end stage liver disease. Innovations in surgical techniques, anaesthesiological approaches and post-operative care, along with improvements in immunosuppressive regimens, have led to 80%-90% one-year survival rates for transplant recipients and satisfactory long-term overall outcomes[1].

Perioperative bleeding complications in liver transplantation and the major side effects of blood transfusion have always been the primary concern accompanying this procedure, as surgery includes native liver dissection and removal under conditions of deranged coagulation, portal hypertension, and diffuse collateral venous flow. However, improved surgical skill during hepatic dissection, the introduction of modern devices for the prompt haemostasis of the small vascular structures, a dedicated, protocol-guided, anaesthesia care team, and intraoperative continuous monitoring and treatment of coagulative abnormalities have contributed to an important decline in blood losses. Currently, not only is the number of liver recipients who undergo massive transfusions steadily decreasing but clinical evidence has also emerged to support the opposite trend, attesting to a possible cohesion of an underestimated intrinsic hypercoagulative state during and after the transplant procedure. The better definition and understanding of the so called re-balanced haemostatic system in end-stage liver disease has in fact underlined the substantial risk of developing perioperative thrombotic complications that is present in certain liver recipients[2].

Thrombotic events, although much more less frequently encountered than bleeding complications, deserve special attention because they pose a major threat to both patient and graft survival.

Fortunately, in the majority of institutions, the strict and continuous point-of-care, perioperative monitoring of haemostasis has led to the abandonment of traditional attempts to prophylactically reverse perioperative abnormal coagulation tests. An on-demand strategy of coagulation factor transfusion is, in fact, safe and allows for a restrictive transfusion policy.

It has to be underlined that surgical factors and abilities make critical contributions to the minimisation of blood loss. Operative approaches aiming at preventing splanchnic organ stasis, careful handling of perihepatic inflammatory adhesions, and the maintenance of meticulous haemostasis of damaged collateral vessels originating from portal and splanchnic hypertension may result in a substantial proportion of patients who receive transplants without any need for coagulative blood products. Furthermore, surgical technique and skill are among the central determinants of the intraoperative release of tissue factor, which, in association with possible related technical defects, undoubtedly contributes to OLT-related vascular thrombosis[3].

RISK OF THROMBOEMBOLISM IN OLT

Thromboembolism during OLT is complex in aetiology and unpredictable in onset and evolution. The initiation of a procoagulative process may be triggered by abnormal thrombin generation and platelet function, along with a lack of efficacy of the profibrinolytic system. Secondary to cirrhosis, pro- and anti-coagulants make up an unstable balance, and several factors inherent to the procedure of transplantation can promote a hypercoagulable state[2].

Liver-related vascular thrombosis, such as portal vein thrombosis and hepatic artery thrombosis, systemic thromboembolic processes, and the formation of thrombi in the central venous and pulmonary circulation, are among the reported causes of either graft failure or life-threatening adverse events (Table 1).

Various transplantation-related triggers, such as surgical damage, the release of activators from the donor liver, blood stasis, and systemic inflammatory responses, can activate coagulation or induce platelet

Table 1 Perioperative thromboembolic events in liver transplantation

Arterial complications
Intraoperative hepatic artery thrombosis
Early postoperative hepatic artery thrombosis
Coronary artery thrombosis
Peripheral arterial occlusion
Intracardiac thrombosis
Pulmonary emboli
Paradoxical air/thromboembolism
Early portal vein thrombosis/re-thrombosis
Post-orthotopic liver transplantion deep vein thrombosis and inferior vena cava thrombosis

Table 2 Major risk factors for perioperative thromboembolic events in liver transplant

Abnormal thrombin generation
Defective profibrinolitic system due to elevated plasminogen activator inhibitor 1 endothelial type levels
Platelet activation from venous stasis or inflammation
Intestinal ischaemia and liberation of infectious mediators
Tissue thromboplastin release
Vascular clamping/unclamping
Platelet aggregates and microthrombosis around catheters
Severe ischaemia-reperfusion phenomena
Veno-venous by-pass
High pulmonary artery pressure
Anatomical and/or technical causes

activation. Other factors capable of increasing the thrombotic risk include clamping (total or partial) of the vena cava or portal vein, ischemic insults to the intestine, liberation of splanchnic infectious mediators, injury to a large capillary bed, release of tissue thromboplastin and use of venovenous bypass (Table 2).

A causative role of venovenous bypass in the development of thrombosis has been suggested, as the exposure of blood to the foreign surface of the tubing system is a well-known trigger activating coagulation, despite any heparin coating. However, some authors have suggested that, by reducing the stasis of blood in the inferior vena cava and splanchnic region during the anhepatic phase, venovenous bypass can reduce the risk of thrombus formation[3].

Perioperative thromboembolic complications can also occur after the migration of thrombi produced around pulmonary artery catheter or venous catheters or originating from a transjugular intrahepatic portosystemic shunt (TIPS)[3,4]. Additional factors include the excessive use of haemostatic agents (*i.e.*, fresh-frozen plasma, anti-Vitamin K agents, platelets, recombinant factor Ⅶa and antifibrinolytic drugs). Microthrombosis of the pulmonary circulation has also been attributed to platelet aggregates that are activated in the liver graft during reperfusion[4,5].

The impaired clearance of activated coagulation factors with gradual increases in the thrombin-antithrombin-Ⅲ complex, excessive activation of coagulation and consumptive coagulopathy may also be caused by a decreased hepatic blood flow or the poor recovery of a new graft. High pulmonary artery pressures, haemodynamic instability, and increased levels of serum lactate have also been recognised as intraoperative factors predisposing to thrombotic complications[6].

An enhanced haemostatic capacity not only related to an altered local flow dynamics but also to various acquired and genetic thrombotic risk factors may be observed in patients with Budd-Chiari-syndrome, pre-existing portal vein thrombosis, pelvic and deep leg vein thrombosis, Factor V-Leiden (APC resistance), protein C deficiency, primary biliary cirrhosis, primary sclerosing cholangitis, and malignant liver tumors[7].

The incidence, distribution, and severity of hypercoagulative state with associated complications in liver transplant recipients are still unknown. Krzanicki *et al*[8] by retrospectively reviewing the database of intraoperative thromboelastography (TEG) of 124 liver transplant recipients, observed a high rate of hypercoagulation in patients with cholestatic pathologies (42.9%) and with primary biliary cirrhosis (85.7%), in those with primary sclerosing cholangitis, and in those fulminant hepatic failure (50%) and non-alcoholic steatohepatitis (37.5%).

INTRACARDIAC THROMBOSIS AND PULMONARY EMBOLI DURING OLT

Although the estimated incidence of intracardiac thrombosis (ICT) and/or pulmonary emboli (PE) is low (reported ranges from 1.2% to 6.2%)[6], these serious complications are an often-overlooked cause of mortality during adult liver transplantation that can occur in any phase of the transplant. As reported by Lerner *et al*[9], approximately 30% of the cases were noted during the preanhepatic phase versus over 30% during the anhepatic and the reperfusion phase of the procedure.

In a review by Warnaar *et al*[10], the number of intraoperative reported episodes up to 2006 was 74, with PE alone in 32 patients (43%) and a combination of PE and ICT in 42 patients (57%). In the retrospective, single-centre review by Cherian *et al*[11], out of approximately 3000 OLTx performed from 1982 until 2007, only 36 patients were suspected of developing a PE (incidence rate of 0.37%).

A more recent series by Sakai *et al*[6] showed that the incidence of PE was 4.0% (20 cases).

Many case reports have been published with different onsets, treatment and outcomes. Most of the ITC and PE during OLT occurred within a few minutes after graft reperfusion[12].

The lower rate of ITC and PE observed in previous studies may be due to the absence or very infrequent use of intraoperative transoesophageal echocardiography (TEE), which could have helped to identify ITC and/or PE as a possible cause of sudden serious

haemodynamic derangement.

The suggested association between intraoperative PE and ICT and the use of antifibrinolytic drugs remain controversial and have not been completely demonstrated[11,13].

In the review by Cherian *et al*[11] of 74 patients with PE and/or ICT, 34 (46%) did not receive any antifibrinolytic drugs; in various other cases, antifibrinolytics were not implicated[9]. The case reports of patients who developed an intraoperative PE and/or ITC when aprotinin was given[14] have led to criticisms of the clinical safety of aprotinin. However, Warnaar *et al*[15] in a retrospective analysis of 1492 patients, demonstrated that intraoperative treatment with aprotinin was not associated with a significantly increased risk of postoperative thromboembolic events in comparison with controls.

Very rarely in liver recipients with porto-pulmonary syndrome or as a consequence of severe post-reperfusion pulmonary arteriolar severe vasoconstriction or pulmonary emboli, an abrupt increase in pulmonary artery pressure and right ventricular pressure can develop. In the presence of a significant increase in the right atrial pressure, an intra-atrial patent septum or foramen ovale may facilitate the passage of air bubbles from the right heart into the systemic circulation. Paradoxical air emboli and thromboemboli may thus be distributed to the terminal arteriolar bed of the brain and heart[16].

Serious ITC and PE manifest intraoperatively with systemic hypotension associated with increased pulmonary artery pressure or persistent haemodynamic instability not responding to supportive therapy. The combination of haemodynamic compromise and TEE imaging allows for a correct diagnosis. The identification of ITC with TEE is quite easy, but the identification of clots in the peripheral pulmonary arterial tree is almost impossible.

The overall mortality in the reported cases is high (from 45% to 68%), and better survival rates have been observed when aggressive cardioactive therapies and thrombolysis are immediately initiated. Thrombolytic therapy with recombinant tissue plasminogen activator (rTPA), despite the increased risk of significant haemorrhage, may reduce the mortality of patients with massive pulmonary embolism, generating superior survival (77%) over embolectomy (53%) or heparin[17]. Surgical pulmonary embolectomy, despite the potential serious risks facing a recipient with end-stage cirrhosis, has also been performed in such cases, generating more favourable results and complications than aggressive haemodynamic support alone[10].

INTRAOPERATIVE HEPATIC ARTERY THROMBOSIS

Acute thrombosis of the hepatic artery is a complication that develops more frequently in the paediatric population due to their smaller vessel diameters[18]. In adults, anatomic abnormalities of either native or graft hepatic arteries, multiple donor arteries requiring reconstruction, and thrombophilia have been implicated.

Mechanical factors, including internal flaps, prolonged clamping of the hepatic artery during the performance of the anastomosis, the kinking of a long artery, and intra-arterial haematoma predispose for the unexpected abrupt development of hepatic artery thrombosis[18]. Other attributable factors include poor arterial flow, increased sinusoidal resistance, preservation injury and an imbalance in the coagulation factors, predisposing to hypercoagulability[18,19]. The diagnosis is confirmed by hepatic artery palpation or intraoperative Doppler ultrasonography. Immediate surgical arterial thrombectomy and/reconstruction are fundamental to restoring graft function.

EARLY POSTOPERATIVE HEPATIC ARTERY THROMBOSIS

Thrombosis of the hepatic artery (HAT) can occur both early and/or months after the transplantation. The reported incidence of HAT ranges between 2.5% and 6% in adults and 15%-20% in children[18]. Early HAT may frequently occur when laboratory values indicate an incomplete recovery of graft function, suggesting the presence of a hypocoagulable state. Children are more susceptible to the development of HAT due to the small arterial vessels, split procedures, and involuntary development of a high haematocrit[19].

Although, in many cases of HAT, no anatomical or technical causes can be identified, the following conditions are among the prominent predisposing factors: graft oedema due to poor initial graft function, multiple recipient arteries, coeliac trunk stenosis, lienalis steal syndrome, injury of the intima of the donor hepatic artery, previous transarterial chemoembolisation, split and living related liver transplantation, aneurysm of the donor hepatic artery, aberrant arteries with fragile intima and long backtable arterial reconstruction before implantation[20,21]. Additional risk factors include increased blood product transfusion during transplant procedure, aortohepatic grafting, the need for infrarenal aorta vascular extension with respect to the use of supracoeliac aorta, and the presence of portal vein thrombosis before transplant[20-22].

A reduction in postanastomotic hepatic artery flow after revascularisation has been considered one of the most important predictors of early HAT after OLT[23].

A transient state of hypercoagulability, mainly postoperative hypercoagulability (*e.g.*, due to unnecessary excessive fresh frozen plasma or platelet administration), especially when associated with anatomical or technical abnormalities, can particularly act to precipitate HAT[24].

Postoperative cytomegalovirus infection, which leads to endothelial cell activation and increased platelet reactivity, has been associated with an increased risk of HAT[25].

Early HAT typically leads to the ischaemia/necrosis of the graft. Unexpected dramatic increases in transaminases associated with a decrease in the bile flow and lightening of the bile colour, fever and haemodynamic impairment (septic-like syndrome) are typical signs of diffuse hepatic cell necrosis from early HAT[21].

Biliary complications (intrahepatic biliomas and biliary stenosis) with the preservation of graft function, represent signs of late HAT. Ischemic insults can, in fact, induce direct injury to the cholangiocytes and/or damage to the arterioles of the peribiliary vascular plexus. These microcirculatory disturbances lead to the apoptosis and necrosis of the cholangiocytes, resulting in the formation of strictures, biliary apoptosis, necrosis, and cholangitis[20,21].

In liver recipients at risk for HAT, thromboprophylactic strategies with heparin followed by aspirin are beneficial, particularly in children. Sufficient anticoagulation is most significantly mandatory during the early postoperative period, despite its increased risk of postoperative haemorrhage. The risk of late HAT seems to be substantially decreased in patients receiving aspirin (3.6% *vs* 0.6%), suggesting a potential role of excessive platelet activation in the aetiology of this complication[26].

The early detection and prevention of HAT is of utmost importance, as these factors can heavily influence the prognosis of patients, particularly when found early after OLT. When not associated with "evident" graft failure, these features invariably involve the biliary strictures due to bile duct ischaemia.

Doppler ultrasounds of the hepatic artery and MRI angiography are effective for the diagnosis of early postoperative arterial stenosis and thrombosis.

Treatment of early HAT relies rarely on interventional radiological thrombolysis, on surgical thrombectomy, and sometimes on reconstruction as the definitive therapy. A late diagnosis of HAT or a failed surgical approach may require urgent re-transplantation[27].

Treatment of early HAT relies rarely on interventional radiological thrombolysis; surgical thrombectomy and sometimes anastomotic arterial reconstruction are the definitive therapy.

Portal vein thrombosis

Portal vein thrombosis (PVT) has been reported to occur in 4.9% to 10.6% of liver recipients, with the highest incidence occurring in the paediatric living donor population receiving left-side grafts[28,29].

Postoperative PVT may be partial, if there is some preservation of portal flow, or complete, if the occlusion involves the entire lumen. PVTs can emerge in the transplanted patients early after OLT, with marked clinical and biochemical signs, or at a variable time in the long-term course, with more subtle manifestations.

One important risk factor for the development of postoperative PVT is the presence of preoperative PVT, which is frequently associated with cirrhosis and liver cancer. However, this association is still controversial and has not consistently been demonstrated. In a report by Enestvedt *et al*[30], patients with preoperative PVT who did not have a higher rate of postoperative thrombotic events showed no adversely impacted patient survival after OLT.

Previous studies have shown that the incidence of early post-OLT rethrombosis in liver recipients with native PVT ranged from 5% to 21%[31]. In more recent data, the incidence of post-OLT portal vein rethrombosis after previous portal vein thrombectomy was found to be highly variable (6% to 40%)[32,33].

The preoperative extent of a thrombus within the portal venous bed is the main determinant of post-reperfusion portal flow, guiding both the risk and the speed of portal vein re-thrombosis. Patients with complete PVT with extension throughout the portal venous bed were once excluded from receiving an OLT[31]. Currently the decision to include among OLT candidates those with a diffuse preoperative PVT is based on both the centre's expertise and the surgeon's skill and decision.

Optimal perfusion of the portal vein is very important during the early postoperative period, and factors causing a steal phenomenon (*e.g.*, previous splenectomy, or large collaterals) may decrease the portal vein blood flow.

Other risk factors for post-transplant PVT include severe pre-transplant portal hypertension, other treatments for portal hypertension (*e.g.*, TIPS, portocaval shunt, splenectomy, and splenic vein embolisation), hypoplastic portal veins, mismatches in the size of the donor and the recipient portal vein, severe graft oedema, and large portosystemic collaterals[22]. Any injury to the portal vein wall (intimal injury) caused by a previous thrombectomy may also predispose to PVT, as well as any technical factors leading to redundancy, kinking, torsion, and stenosis at the site of the anastomosis[34].

Liver transplant recipients with significant preoperative portal vein occlusion, apart from their increased risk of rethrombosis, seem to be affected by a higher rate of serious postoperative complications and have a higher mortality[34]. Sharma *et al*[35] compared the incidence of postoperative complications in 78 recipients with PVT (study group) with a random sample of 78 contemporaneous recipients without PVT (control group). In the study group, the rate of primary non-functioning was significantly higher than in control group (9.0% and 1.3%, $P = 0.063$), with retransplantation rates of 17.9% and 7.7%, respectively ($P = 0.055$).

Early PVT causes severe graft ischaemia, ascites and increased portal vein pressure. Hepatic infarction

may develop, even in the absence of arterial compromise[36]. Irreversible graft loss mandates the need for emergency retransplantation (when possible). The clinical signs of early PVT parallel those of ingravescent allograft dysfunction, which can lead to haemorrhage, haemodynamic instability, intestinal ischaemia, and higher associated mortality[20]. A delayed onset of PVT is often not associated with severe graft failure but instead determines unavoidable portal hypertension. Frequent Doppler ultrasonography in the immediate postoperative period is essential to the early detection of PVT, especially in patients who have had a previous PVT. MRI-angiography and CT scans can confirm the diagnosis.

Percutaneous treatment of post-OLT PVT, with mechanical and/or pharmacologic thrombolysis, has been attempted, but the favourable results are mostly described in case reports.

Cherukuri *et al*[37] reported a successful transjugular approach on postoperative day 10 following OLT with an overnight infusion of urokinase, followed by a pushing of the residual clot into a competitive splenorenal shunt. Mechanical fragmentation of a portal vein thrombus with contemporaneous use of urokinase and subsequent stent positioning has also been described[38].

However, percutaneous interventions, thrombolysis, and systemic heparinisation are not always effective alternatives to surgery. Immediate reoperation with acute thrombectomy or surgical resection followed by direct anastomosis with/without a venous graft, is frequently the most successful option.

Early post-operative anticoagulation with a short course of heparin should be recommended in high risk liver recipients with an underlying prothrombotic state, and especially in those undergoing caval transposition and "non-anatomical" procedures[39]. Patients with the persistence of a prothrombotic state not reversed by transplantation should receive long term anticoagulation (*e.g.*, with antiVit K agents). The effect of low dose aspirin on the prevention of PVT or re-thrombosis is still not defined[40].

POST-OLT DEEP VEIN THROMBOSIS AND INFERIOR VENA CAVA THROMBOSIS

Deep vein thrombosis (DVT) is a rare post-transplant complication, with a reported incidence of < 3% in previous studies and between 3.5% and 8.6% in more recent series[41-43].

Major risk factors for post-OLT deep-vein thrombosis include venous stasis due to prolonged immobilisation, the development of a hypercoagulable state as a result of an aggressive surgery, the administration of excessive procoagulant factors, lupus anticoagulant, and the prolonged maintenance of indwelling femoral vein catheters[43].

An apparently well-functioning graft may also synthesise abnormal proteins that could increase the risk for thrombosis.

It is worth noting that some defects in the proteins involved in the regulation of anticoagulation (inherited antithrombin, protein C or protein S deficiency, the factor Ⅱ G20210A mutation) can also be transmitted through liver grafts from donors to recipients[44,45].

Recurrent deep-vein thromboses have been described in patients with "acquired" activated protein C resistance caused by a homozygous factor V Leiden gene mutation in a donor liver and in a liver recipient with a heterozygous protein C deficiency associated with dysfibrinogenaemia[45].

Liver-transplanted patients who receive a graft from a donor who was not screened for thrombophilic abnormalities are most likely not diagnosed as having a prothrombotic tendency until the first thrombotic episode occurs. In fact, screening for prothrombotic abnormalities is very rarely performed emergently before cadaveric transplantation. On the contrary, living-donor liver transplantation, which is a scheduled procedure, generally includes donor screening for genetic thrombophilic abnormalities.

In a retrospective review of 917 patients over 15 years, Salami *et al*[42] reported a 4.58% incidence of venous thromboembolism occurring up to 1 year after OLT. Twelve (1.31%) patients had pulmonary emboli and DVT originating from both the upper and lower extremities. Upper extremity DVT is infrequently or not reported in this setting, even though liver recipients who need large bore catheters into the subclavian or internal jugular veins for a long time could easily develop this complication. Upper extremity DVT seems to be a risk factor for PE comparable with that of the lower extremities DVT, and with a similar, or even higher, mortality[46,47].

Doppler ultrasound is the preferred method for detecting a venous thrombosis.

In the presence of either upper or lower extremity DVT, therapeutic doses of LMWH should be administered, followed by oral anticoagulant therapy. Liver recipients with major risk factors for bleeding are candidates for to inferior vena cava filter insertion. This device is also essential in those who require urgent surgery that precludes anticoagulation and in cases of combined DVT and PE.

RELEVANCE OF PHARMACOLOGIC THROMBOPROPHYLAXIS AFTER OLT

Owing to the risks of bleeding and coagulopathy, antithrombotic prophylaxis to prevent DVT, HAT, PVT or PE is not routinely used in the immediate postoperative period. Clinical practice and laboratory data demonstrate that the normalisation of a stable haemostatic system by a new liver may take some time; furthermore, thrombocytopenia may persist for weeks. However, it has been shown that end-stage

Table 3 Major indications for post-orthotopic liver transplantion thromboprophylaxis

Thrombophylic states
Excessive intraoperative administration of procoagulant factors
Split and living donor transplant
Small arteries or reconstructed arteries
Pre-transplant portal vein thrombosis
Complicated vascular anastomosis
Decreased portal vein blood flow
Hypoplastic portal vein
Caval transposition and "non-anatomical" procedures
Poor arterial flow
Multiple recipient arteries
Infrarenal aortohepatic grafting

cirrhotic patients may be at risk of DVT and PE, and some subset of liver recipients are at a very high risk of vascular thrombotic complications. For this reason, regardless of the lack of studies and guidelines on prophylactic anticoagulation in this setting, many institutions recommend such an approach in very particular circumstances[34]. The most difficult task is the decision of when, how and at which dose to use chemical anticoagulants, as no consensus exists, even in special high-risk situations. The risk of postoperative thromboembolism causing either serious haemodynamic events or loss of graft function needs to be weighed against the risk of bleeding. Bleeding complications may lead to intra-abdominal hematomas, graft dysfunction, infections, haemodynamic instability, renal dysfunction, or the need for the transfusion of blood products. Postoperative anticoagulation may preclude or delay urgent re-operations or other procedures, such as biopsy, endoscopy, and drainage insertion[48].

Patients who mostly benefit from routine thromboprophylaxis are those with recognised thrombophilia (as in Budd-Chiari syndrome) or those receiving massive amounts of fresh frozen plasma, platelet transfusions, or fibrinogen intraoperatively, especially if the recovery of graft function is rapid (Table 3).

Another group who should be considered for routine thromboprophylaxis consists of living donor transplants, paediatric transplants, pretransplant PVTs, recipients of grafts with reconstructed donor arteries, or those with complicated anastomosis at the portal site or difficult portal vein thrombectomy[49].

The value of a decreased PT, prolonged aPTT or increased INR as a predictor of hypocoagulability remains unclear after OLT, and some patients go on to develop a hypercoagulable state as a result of diminished anticoagulant and fibrinolytic activity. In addition to laboratory tests, the point-of-care monitoring of coagulation by ROTEM/TEG or other devices may help physicians to better identify "real-time" coagulation profiles.

In general, whether to administer prophylaxis depends on the clinical status of the patient, whereas the clinical presentation of any specific thrombotic event dictates the degree of anticoagulation required. A recent paper by Mukerji *et al*[48] advises not to begin anticoagulation until the post-transplant INR is above 1.5 to 2 and the platelet count is below 50000.

Paediatric patients or those with preoperative PVT, diabetes, obesity, hepatic cancer and a high cardiovascular risk may benefit from more intensive thromboprophylactic treatments.

These pharmacological strategies mainly rely on IV LMWH heparin and the combination of heparin plus aspirin. The risk/benefit of each approach must also be evaluated in relation to the distance from surgery, the protein synthetic function of the graft, and the possibility of strictly monitoring the pharmacologic effects of the drug. Assessing the efficacy of thromboprophylaxis is, in fact, difficult because the monitoring of antiXa activity may be unpractical; that of protein C, S, and antithrombin post-operatively produced, defective; and that of platelet function, unknown.

The administration of antithrombotic drugs in high-risk OLT recipients requires careful watching, as the anticoagulation efficacy in the presence of both graft and distant organ dysfunction may be unpredictable.

For example, a reduction in the dosage of LMWH is recommended because LMWH has demonstrated an increased anticoagulant potency in patients with cirrhosis[50]. Heparin, which enacts its anticoagulant activity by virtue of the enhancing effect provided by antithrombin, may display wide variations in activity depending on the graft synthesis of antithrombin. In addition, in the presence of renal dysfunction, heparin may accumulate, leading to the need for frequent dose adjustments.

In the study by Shay *et al*[51] post-transplant prophylaxis with 325 mg daily of aspirin was associated with a significantly lower incidence of early HAT and a non-significant lower incidence of PVT. As previously mentioned, the effectiveness of antiplatelet prophylaxis in reducing the incidence of Hat was also demonstrated by Vivarelli *et al*[26].

Combination therapy with i.v. heparin or LMWH plus aspirin has also been proposed to prevent early events in individuals at a high risk of PVT or HAT. The use of bivalirudin in a patient with Budd-Chiari syndrome who required post-OLT anticoagulation and with a history of heparin-induced thrombocytopenia was reported by Anderegg *et al*[52].

P2Y12 blockers (such as clopidogrel) and/or aIIbb3 blockers (such as abciximab), as well as thrombin inhibitors and inhibitors of activated factor X, such as dabigatran and rivaroxaban, have been proposed to prevent post-transplant arterial thrombosis. However, the lack of established reversal agents, their mechanism of clearance *via* the kidneys or liver, the risk of excessive anticoagulation in the immediate postoperative period, and unknown dose regimens have led to their very scarce consideration for the

purpose of thromboprophylaxis.

The use of an implantable pump into the portal vein with the purpose of preventing and treating any rethrombosis of the portal vein after OLT has been reported by Shi *et al*[53] Using a minitype implantable pump that was implanted through the right gastro-epiploic vein and had an extracorporeal tip on the skin surface slightly below the right costal arch, the authors delivered 250 U/kg of heparin every 24 h. The rate of rethrombosis in the portal vein was significantly lower than the rate of PVT among patients without an implantable pump. The implantable pump in their series significantly reduced the rate of relaparotomy or retransplantation and the in-hospital mortality rates.

CONCLUSION

Despite the traditional belief that a prolonged PT and APTT suggest a hypocoagulable state and a bleeding tendency, some liver recipients in the early postoperative period are prone to developing micro- and macro-thrombi within the splanchnic, systemic and pulmonary circulation. A dysfunctional coagulation system associated with hypercoagulability may persist for a variable time period, and some individuals may display a normal or even higher thrombin generation capacity[1].

It is worth noting that patients who are examined with conventional coagulation tests may not have their potentially reduced level of anticoagulant proteins be detected; more sophisticated investigations are often necessary. The delayed recovery of the anticoagulant proteins along with the normal activity of almost all of the procoagulant factors achieved from day 1 to 3 postoperatively was established several years ago[54].

Because conventional coagulation tests do not provide information about the quality or the dynamics of clot formation, the correction of postoperative coagulation parameters is unjustified unless clinical bleeding manifests.

An increasing awareness that hypercoagulability may potentially be exacerbated during transplant procedures should put physicians on alert for the rapid identification of possible predictors of vascular thrombosis. This awareness may help with the selection of those liver recipients most suitable for prophylaxis, a practice that continues to vary widely among centres.

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MINIREVIEWS

Helicobacter pylori-negative gastric mucosa-associated lymphoid tissue lymphomas: A review

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Abstract

Since Isaacson and Wright first reported on the extra-nodal marginal zone B-cell lymphoma of the stomach in 1983, following studies have clarified many aspects of this disease. We now know that the stomach is the most affected organ by this disease, and approximately 90% of gastric mucosa-associated lymphoid tissue (MALT) lymphomas are related to *Helicobacter pylori* (*H. pylori*) infection. This implies that approximately 10% of gastric MALT lymphomas occur independent of *H. pylori* infection. The pathogenesis of these *H. pylori*-negative gastric MALT lymphomas remains unclear. To date, there have been several speculations. One possibility is that genetic alterations result in nuclear factor-kappa B (NF-κB) activation. Among these alterations, t(11;18)(q21;q21) is more frequently observed in *H. pylori*-negative gastric MALT lymphomas, and such translocation results in the synthesis of fusion protein API2-MALT1, which causes canonical and noncanonical NF-κB activation. Another possibility is infection with bacteria other than *H. pylori*. This could explain why *H. pylori* eradication therapy can cure some proportions of *H. pylori*-negative gastric MALT lymphoma patients, although the bacteria responsible for MALT lymphomagenesis are yet to be defined. Recent advances in endoscopy suggest magnifying endoscopy with narrow band imaging as a useful tool for both detecting gastric MALT lymphoma lesions and judging the response to treatment. A certain proportion of *H. pylori*-negative gastric MALT lymphoma patients respond to eradication therapy; hence, *H. pylori* eradication therapy could be considered as a first-line treatment for gastric MALT lymphomas regardless of their *H. pylori* infection status.

Key words: *Helicobacter pylori*; Mucosa-associated lymphoid tissue lymphoma; API2-MALT1; Antibiotics; Endoscopy

Core tip: Although the majority of gastric mucosa-associated lymphoid tissue (MALT) lymphoma patients are infected with *Helicobacter pylori* (*H. pylori*), approximately 10% of patients do not have *H. pylori* infection. Recent studies have demonstrated that eradication

therapy for *H. pylori* is effective not only for *H. pylori*-positive but also for *H. pylori*-negative gastric MALT lymphoma patients.

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INTRODUCTION

Mucosa-associated lymphoid tissue (MALT) lymphoma is an extra-nodal B-cell lymphoma that arises in MALT, which was first reported by Isaacson and Wright in 1983[1]. The following studies have elucidated many aspects of this disease.

The stomach is one of the most affected organs by this disease, and approximately 90% of the affected stomachs are infected with *Helicobacter pylori* (*H. pylori*)[2].

This implies that approximately 10% of gastric MALT lymphomas occur independent of *H. pylori* infection. These gastric MALT lymphomas were considered to be resistant to *H. pylori* eradication. Although there were reports of *H. pylori*-negative gastric MALT lymphomas that were successfully treated with antibiotic therapy[3,4], some assumed that those were only false negative cases[5]. We now know that some proportions of gastric MALT lymphoma in *H. pylori*-uninfected stomachs can be successfully treated with antibiotic therapy[3]. However, there are not as many reports on *H. pylori*-uninfected MALT lymphomas compared with the infected ones. Therefore, in this review, we will summarize the current knowledge of *H. pylori*-negative gastric MALT lymphomas.

PATHOGENESIS

The pathogen in *H. pylori*-infected cases of gastric MALT lymphomas is clearly *H. pylori*. This statement is supported by the fact that approximately 75% of *H. pylori*-positive gastric MALT lymphomas achieve complete remission (CR) by eradication of this bacterium alone[6,7] (Table 1). Chronic *H. pylori* infection attracts lymphoid cells to the gastric MALT, where these cells are continuously stimulated by *H. pylori* and give rise to MALT lymphomas. Previous studies have demonstrated that not only B cells, but also T cells and macrophages play an important role in this lymphomagenesis[8-10].

Because *H. pylori*-negative gastric MALT lymphoma patients are not infected with *H. pylori*, this bacterium cannot be responsible for the lymphomagenesis. To date, there have been several opinions regarding the pathogenesis of *H. pylori*-negative gastric MALT lymphomas.

Several genetic alterations have been identified in gastric MALT lymphomas: t(11;18)(q21;q21), t(1;14)(p22;q32), t(14;18)(q32;q21) and t(3;14)(p13;q32)[11,12]. Among these genetic abnormalities, t(11;18)(q21;q21) is the most frequently detected translocation in gastric MALT lymphomas[11-14] and is more frequently observed in *H. pylori*-negative gastric MALT lymphomas. This translocation fuses the N-terminus of the *API2* gene to C-terminus of the *MALT1* gene, which results in the synthesis of an API2-MALT1 fusion protein[15]. *MALT1* was first reported in 1999 by two groups, and they both suggested that *API2-MALT1* is important to the pathogenesis of gastric MALT lymphomas[16,17]. The following studies revealed that API2-MALT1 fusion protein activates nuclear factor-kappa B (NF-κB) through noncanonical pathway by inducing the proteolytic cleavage of NF-κB-inducing kinase (NIK), resulting in deregulated NIK activity and noncanonical NF-κB activation[18] (Figure 1 left panel). Conversely, Zhou *et al*[19] reported that API2-MALT1 chimeric protein causes canonical NF-κB activation *via* deregulated ubiquitin ligase activity, which increases K63-polyubiquitination of NEMO, and Lucas *et al*[20] reported that heterotopic API2-MALT1 oligomerization and binding of TNF receptor associated factor 2 (TRAF2) are required for maximal NF-κB activation. Recent report detailed the role of RIP1 ubiquitination, resulting from TRAF2 recruitment to API2-MAT1, as necessary for full NF-κB activation[21] (Figure 1 right panel). This deregulated activation of NF-κB induces tumorigenesis[22]; therefore, this genetic alteration could be a cause of *H. pylori*-negative gastric MALT lymphoma.

Some groups suspected the involvement of a bacterium other than *H. pylori*. Indeed, there are several bacteria and viruses identified to have a correlation between marginal zone B cell lymphomas (*Campylobacter jejuni* and immunoproliferative small intestinal disease, *Borellia burgdorferi* and primary cutaneous B-cell lymphoma, *Chlamydophila psittaci* and ocular adnexal lymphoma, hepatitis C virus and splenic marginal zone lymphoma)[23], and it is possible that bacteria other than *H. pylori* are causing chronic inflammation in the stomachs of *H. pylori*-negative gastric MALT lymphoma patients. Morgner *et al*[24] reported 5 cases of *H. pylori*-negative but *H. heilmannii*-positive gastric MALT lymphomas. These patients were treated with 40 mg omeprazole and 750 mg amoxicillin 3 times per day for 14 d, which is a similar treatment to *H. pylori* eradication, and the eradication of this bacterium resulted in CR in all 5 patients. *H. heilmannii* infection causes gastric B-cell lymphomas in mice[25], hence gastric infection by this bacterium may be a cause of *H. pylori*-negative gastric MALT lymphomas. Nonetheless, bacteria that have not yet been identified may be involved in this MALT lymphomagenesis, and further studies are warranted to clarify the details.

Another possibility is the involvement of autoimmune diseases. Sjögren's syndrome is associated with an

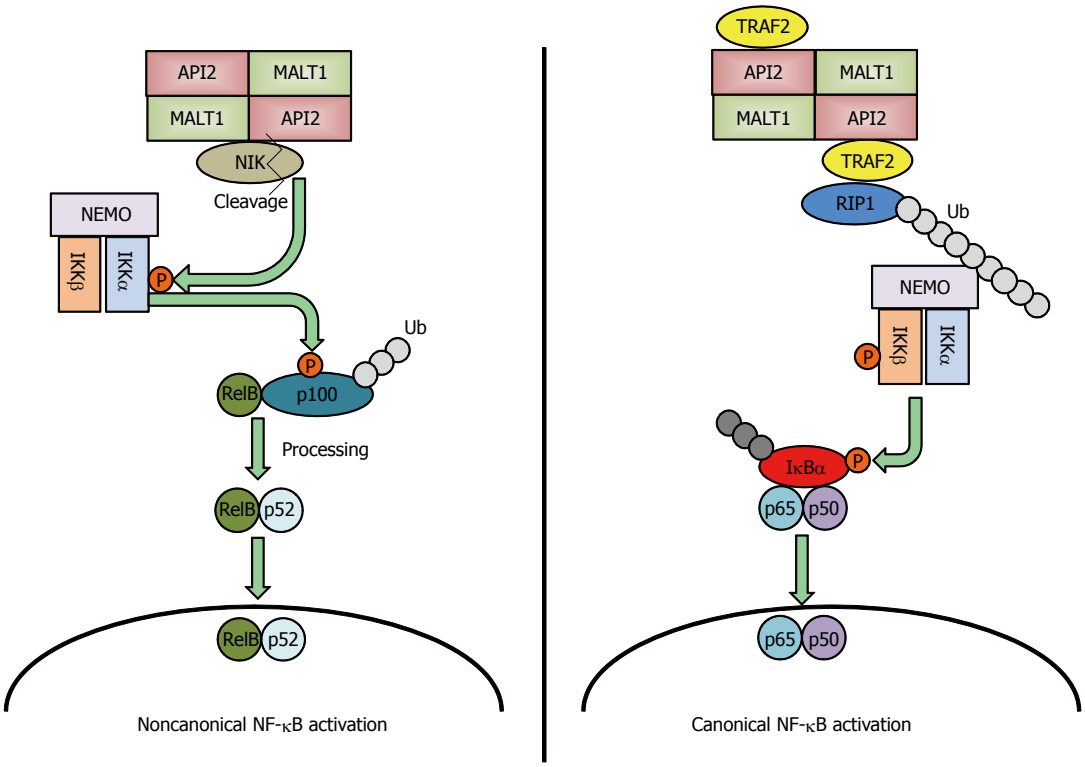

Figure 1 API2-MALT1 fusion protein induces noncanonical and canonical nuclear factor-kappa B activation.

Table 1 Comparison of *Helicobacter pylori*-positive and *Helicobacter pylori*-negative gastric mucosa-associated lymphoid tissue lymphomas

	H. pylori-positive	*H. pylori*-negative
Pathogenesis	*H. pylori* infection	Genetic alterations Other bacterial infection Autoimmune diseases
First-line therapy	Antibiotic therapy	Antibiotic therapy
Response rate for antibiotic therapy	75%	28%

H. pylori: *Helicobacter pylori*.

increased risk for parotid gland MALT lymphomas[26], and a meta-analysis reported that the odds ratio of MALT lymphoma development for Sjögren's syndrome was 18.8 (95%CI: 9.5-37.3)[27]. Hashimoto's thyroiditis is also a known risk factor for thyroid lymphoma[28]. Therefore, we cannot deny the possibility that autoimmune diseases are also involved in gastric MALT lymphomagenesis, and further investigations are warranted.

DIAGNOSIS

Because the clinical symptoms of gastric MALT lymphomas are usually non-specific[29], gastric MALT lymphoma lesions are traditionally detected by screening esophagogastroduodenoscopy. Gastric MALT lymphoma lesions of *H. pylori*-negative subjects are similar to those observed in *H. pylori*-positive subjects[30]. Their endoscopic findings are classified as the superficial-spreading type, mass-forming type, diffuse infiltrating type and unclassified[31,32]. Many gastric MALT lymphoma lesions have similar endoscopic findings to early gastric cancers, but the discrimination between them is critical because the treatment for these diseases is different. Among these, the superficial-spreading type is most sensitive to antibiotic therapy[6].

The depth of the affected lesion is evaluated by endoscopic ultrasonography. Depth evaluation is important because gastric MALT lymphomas with deep submucosal invasions respond less to eradication therapy[33].

A definitive diagnosis for gastric MALT lymphomas is made by histopathology. Histological scoring of lymphoid infiltrations in the stomach according to Wotherspoon *et al*[34] has been broadly used for histopathological evaluation. It is important to perform an adequate number of biopsies from the lesions both for making an accurate diagnosis and ruling out the possibility of diffuse large B cell lymphomas, and Fischbach proposed that at least ten biopsies are required[35]. In addition to histopathology, immunostaining for B-cell markers can aid in diagnosis[36].

Genetic alterations that are observed in MALT lymphomas can be helpful for diagnosing the disease and for predicting the response to antibiotic therapy as mentioned below. The presence or the absence of these alterations can be diagnosed by reverse transcription polymerase chain reaction or by fluorescence in situ

Table 2 Previous reports on *Helicobacter pylori*-negative gastric mucosa-associated lymphoid tissue lymphomas *n* (%)

Responding patients/treated patients (responding rate)	
Steinbach *et al*[45] (1999)	0/6 (0)
Ruskoné-Fourmestraux *et al*[30] (2001)	0/10 (0)
Ye *et al*[14] (2003)	0/5 (0)
Nakamura *et al*[3] (2006)	2/7 (29)
Raderer *et al*[4] (2006)	5/6 (83)
Akamatsu *et al*[46] (2006)	1/9 (11)
Terai *et al*[47] (2008)	1/4 (25)
Park *et al*[48] (2010)	3/4 (75)
Asano *et al*[49] (2012)	5/17 (29)
Choi *et al*[50] (2013)	2/5 (40)
Raderer *et al*[52] (2015)	5/13 (46)

hybridization[36].

Clinical staging of gastric MALT lymphomas is defined according to either the Lugano international conference classification[37] or the modified Ann Arbor staging system[36]. The former is mainly based on radiological findings, whereas the latter takes the depth of gastric wall infiltration into consideration. Because depth of the affected lesion correlates with antibiotic therapy responsiveness[33], the latter may reflect the prognosis better than the former, but validation by prospective studies are warranted.

There are a number of methods for the diagnosis of *H. pylori* infection[38]. It is always important to combine different methods together, usually combining a non-invasive and an invasive method (*e.g.*, urea breath test and histopathology), to exclude the possibility of a false-negative result. We must also be aware of the possibility of a pseudo-negative result in patients with extreme gastric mucosal atrophy and patients taking proton-pump inhibitors (PPI).

Recently, the usefulness of magnifying endoscopy in detecting gastrointestinal lesions has been given attention[39], and magnifying endoscopy with narrow-band imaging (MNBI) is proposed to be a useful technique to diagnose gastric MALT lymphomas[32,40,41]. Interestingly, Nonaka *et al*[41] reported that MNBI was useful in not only detecting gastric MALT lymphomas but also in evaluating their response to eradication therapy. This additional information is very helpful because it is often not easy to judge whether the patient should undergo second-line therapy after *H. pylori* eradication therapy in *H. pylori*-negative gastric MALT lymphomas.

TREATMENT

All of the gastric MALT lymphoma patients are considered to have an indication for antibiotic treatment regardless of their clinical stages[36,42]. Standard antibiotic therapy consists of a combination of amoxicillin, clarithromycin and PPI[43]. According to previous reports, approximately 75% of *H. pylori*-positive gastric MALT lymphomas are successfully treated by the eradication of this bacterium[6,7]. However, due to the increasing drug resistance of *H. pylori*, many alternative therapies are proposed, such as the therapy adopting metronidazole instead of clarithromycin[44].

The first-line treatment for *H. pylori*-negative gastric MALT lymphomas is also antibiotic therapy[36]. Even in the absence of *H. pylori* infection, several studies have reported that certain proportions of patients responded to this antibiotic therapy[3,4,14,30,45-52] (Table 2).

Raderer *et al*[4] reported that five out of six *H. pylori*-negative gastric MALT lymphoma patients responded to antibiotic therapy (one had partial remission and four had complete remission), which corresponds to an excellent response rate of 83%. This was the highest reported response rate among *H. pylori*-negative gastric MALT lymphomas. The response rate decreased to 46% in their later study[52], but this was still relatively high compared with other reports[3,46-50]. Contrary to these reports, earlier studies reported that *H. pylori*-negative gastric MALT lymphomas did not respond to antibiotic therapy[14,30,45]. One possible explanation for this discrepancy could be the insufficient time span between antibiotic therapy and the judgment of the treatment. For example, Ye *et al*[14] judged the antibiotic treatment as not effective at the median time of 7.5 mo (range: 4-12 mo). However, the median time span to reach complete remission after antibiotic therapy was 6 mo in our study, and we experienced a number of cases that took 24 mo or longer to reach remission[47]. Raderer *et al*[52] also noted that, although most of the patients responded to antibiotic therapy in 3-9 mo, they did experience a case that took 36 mo to achieve complete remission. Nonetheless, further studies are warranted to clarify the cause underlying this discrepancy.

Regarding the predictive factor for responsiveness, multiple lesions[49], lesions in both proximal and distal parts of the stomach[50], and the presence of t(11;18)(q21;q21)[50] were predictive factors for non-responsiveness to antibiotic therapy. We have previously reported that 5 out of 17 *H. pylori*-negative gastric MALT lymphoma patients treated with antibiotics and PPI responded to the therapy, and 3 out of 5 responders had single lesions, while all non-responders had multiple lesions[49]. Similarly, Choi *et al*[50] reported that non-responders had lesions in both proximal and distal parts of the stomach, although they did not distinguish patients' *H. pylori* infection status (2 out of 40 in responders, 4 out of 15 in non-responders). Regarding the t(11;18)(q21;q21) translocation, 2 out of 3 *H. pylori*-negative patients without this translocation were indicated to have responded to antibiotic therapy, while the remaining patient with this translocation did not[50]. Other studies also reported that gastric MALT lymphoma patients with t(11;18)(q21;q21) are more resistant to antibiotic therapy[6,12]. Because this translocation is more frequently observed in *H pylori*-negative gastric MALT lymphoma patients[12,14], this needs to be taken into consideration.

The reason why *H. pylori*-negative gastric MALT lymphomas respond to antibiotic therapy is not clear,

but there are several possible speculations.

One speculation, as mentioned above, is that these patients were infected with bacteria other than *H. pylori*, and the antibiotic eradication therapy for *H. pylori* was able to eradicate this non-*H. pylori* bacteria[24].

Another explanation is that clarithromycin (included in the eradication medication) affected the patients' immune system through its immunomodulatory effect[53,54]. Nevertheless, further studies are warranted to elucidate the mechanisms involved in this reaction.

Previously, when antibiotic therapy was considered ineffective, radiotherapy was used as the first-line therapy for *H. pylori*-negative gastric MALT lymphomas[55]. Gastric MALT lymphomas are sensitive to radiotherapy, and, according to Zullo *et al*[56], 97.8% of patients who were resistant to antibiotic therapy responded to radiotherapy. Chemotherapy efficiency for these patients has also been described[56], and a combination of anti-CD20 monoclonal antibodies with chemotherapy reportedly gives promising results[57,58].

However, currently there is no consensus for the patients who did not respond to antibiotic therapy. Because the disease is indolent by nature, and because complications are possible[59,60], a "watch-and-wait" strategy may be efficient for these patients if they do not have progressive disease[2,36,52].

CONCLUSION

Recent studies on *H. pylori*-negative gastric MALT lymphomas have suggested that *H. pylori* eradication therapy is effective in some proportion of patients with this disease and could be considered as a first-line treatment.

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ORIGINAL ARTICLE

Basic Study

Losartan activates sirtuin 1 in rat reduced-size orthotopic liver transplantation

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Author contributions: Pantazi E carried out the experimental work; Pantazi E, Bejaoui M and Folch-Puy E provided protocols and analyzed data; Zaouali MA, Bejaoui M and Panisello A established the animal experimental model and injury parameters; Rolo AP and Palmeira CM determinated NAD^+, NAMPT levels and contributed to critical analysis of the data and discussion; Pantazi E, Folch-Puy E and Roselló-Catafau J designed the study, coordinated the experiments and wrote the paper.

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Data sharing statement: Any further information related to technical appendix, statistical code and dataset are available from the corresponding author at jrcbam@iibb.csic.es. The authors gave informed consent for data sharing.

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Abstract

AIM: To investigate a possible association between losartan and sirtuin 1 (SIRT1) in reduced-size orthotopic liver transplantation (ROLT) in rats.

METHODS: Livers of male Sprague-Dawley rats (200-250 g) were preserved in University of Wisconsin preservation solution for 1 h at 4 ℃ prior to ROLT. In an additional group, an antagonist of angiotensin Ⅱ type 1 receptor (AT1R), losartan, was orally administered (5 mg/kg) 24 h and 1 h before the surgical procedure to both the donors and the recipients. Transaminase

(as an indicator of liver injury), SIRT1 activity, and nicotinamide adenine dinucleotide (NAD^+, a co-factor necessary for SIRT1 activity) levels were determined by biochemical methods. Protein expression of SIRT1, acetylated FoxO1 (ac-FoxO1), NAMPT (the precursor of NAD+), heat shock proteins (HSP70, HO-1) expression, endoplasmic reticulum stress (GRP78, IRE1α, p-eIF2) and apoptosis (caspase 12 and caspase 3) parameters were determined by Western blot. Possible alterations in protein expression of mitogen activated protein kinases (MAPK), such as p-p38 and p-ERK, were also evaluated. Furthermore, the SIRT3 protein expression and mRNA levels were examined.

RESULTS: The present study demonstrated that losartan administration led to diminished liver injury when compared to ROLT group, as evidenced by the significant decreases in alanine aminotransferase (358.3 ± 133.44 *vs* 206 ± 33.61, $P < 0.05$) and aspartate aminotransferase levels (893.57 ± 397.69 *vs* 500.85 ± 118.07, $P < 0.05$). The lessened hepatic injury in case of losartan was associated with enhanced SIRT1 protein expression and activity (5.27 ± 0.32 *vs* 6.08 ± 0.30, $P < 0.05$). This was concomitant with increased levels of NAD^+ (0.87 ± 0.22 *vs* 1.195 ± 0.144, $P < 0.05$) the co-factor necessary for SIRT1 activity, as well as with decreases in ac-FoxO1 expression. Losartan treatment also provoked significant attenuation of endoplasmic reticulum stress parameters (GRP78, IRE1α, p-eIF2) which was consistent with reduced levels of both caspase 12 and caspase 3. Furthermore, losartan administration stimulated HSP70 protein expression and attenuated HO-1 expression. However, no changes were observed in protein or mRNA expression of SIRT3. Finally, the protein expression pattern of p-ERK and p-p38 were not altered upon losartan administration.

CONCLUSION: The present study reports that losartan induces SIRT1 expression and activity, and that it reduces hepatic injury in a ROLT model.

Key words: Losartan; Sirtuin 1; Endoplasmic reticulum stress; Liver ischemia reperfusion injury; Angiotensin Ⅱ

Core tip: Losartan is an angiotensin Ⅱ type 1 receptor (AT1R) antagonist known to protect livers against ischemia-reperfusion injury (IRI). However, the mechanisms underlying this hepatoprotective effect are not fully understood, especially in case of reduced-size orthotopic liver transplantation (ROLT). SIRT1 has recently emerged as an important target to modulate for alleviating IRI. In our study, we describe that AT1R antagonism enhances SIRT1 activity and prevents endoplasmic reticulum stress and liver apoptosis in a rat model of ROLT. Consequently, losartan increases the resistance of ROLT grafts against IRI.

Pantazi E, Bejaoui M, Zaouali MA, Folch-Puy E, Pinto Rolo A, Panisello A, Palmeira CM, Roselló-Catafau J. Losartan activates sirtuin 1 in rat reduced-size orthotopic liver transplantation. *World J Gastroenterol* 2015; 21(26): 8021-8031 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i26/8021.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i26.8021

INTRODUCTION

Ischemia-reperfusion injury (IRI) is an important obstacle during liver transplantation, contributing to a significant loss of graft function. It is characterized by a cascade of deleterious cellular responses that lead to inflammation, cell death, and ultimately, organ failure[1]. These complications are increased in case of reduced-size liver grafts compared with standard liver transplant operations[2,3]. Thus, further investigation is required to explore new therapeutic strategies to counteract IRI.

Various reports have associated the renin-angiotensin system (RAS) with liver IRI[4,5]. The main efector of RAS is angiotensin Ⅱ, which is produced *via* angiotensin converting enzyme (ACE) from angiotensin Ⅰ. It exerts its biological actions through two receptor subtypes: angiotensin Ⅱ type Ⅰ receptor (AT1R) and angiotensin Ⅱ type Ⅱ receptor[6]. Angiotensin Ⅱ has been associated with increased inflammation and oxidative stress in liver IRI, and various studies have evidenced that AT1R antagonists, such as losartan, efficiently protected livers against IRI in both warm ischemia and transplantation models[7-10].

Sirtuins are deacetylases dependent on nicotinamide adenine dinucleotide $(NAD)^+$ that either activate or suppress various proteins. Thus, they are implicated in various cellular pathways, including metabolic processes, apoptosis and oxidative stress[11]. Sirtuin 1 (SIRT1) and the mitochondrial sirtuin 3 (SIRT3) are the most studied sirtuins and represent interesting targets for counteracting IRI in various organs[12,13]. SIRT1 has been shown to be involved in a wide range of cellular processes related to cell cycle and the cellular response to stresses, including the endoplasmic reticulum stress (ERS)[14-17].

IRI is known to promote ERS which finally induces cellular death[18]. In addition, we have previously shown that inhibiting ERS can be a useful strategy against IRI[19]. In a model of partial hepatectomy with ischemia-reperfusion in steatotic and non-steatotic rat livers, ERS inhibition ameliorated hepatic damage by reducing inflammation and apoptosis[19]. Therefore, we may hypothesize that preventing ERS might be useful for ameliorating the negative outcomes of reduced-size orthotopic liver transplantation (ROLT).

There is little evidence about a potential relationship between SIRT1 and angiotensin Ⅱ antagonists. Miyazaki *et al*[20] have reported that SIRT1 overex-

pression suppresses AT1R in cultured vascular smooth muscle cells. In addition, a recent study in primary cultures of adipocytes evidenced a mutual interaction between RAS and SIRT1, with an association with metabolic homeostasis[21]. Conversely, there are no reports concerning a relationship between SIRT1 and angiotensin Ⅱ antagonists in liver transplantation. Given that both are involved in common processes related to IRI, ERS, and apoptosis[22,23], we hypothesized that SIRT1 may be implicated in the protective effects of an AT1R antagonist against hepatic IRI following ROLT.

The present study therefore aimed to assess whether an AT1R antagonist, losartan, could be effective in protecting reduced-size liver grafts from IRI and to examine the possible underlying mechanisms involved. Furthermore, a potential relationship between losartan and SIRT1 was explored.

MATERIALS AND METHODS

Experimental animals

Male Sprague-Dawley rats (200-250 g) were used as donors and recipients. Animals were housed in conventional temperature- and humidity-controlled facilities with a 12-h light/dark cycle. All animals had free access to water and a standard laboratory diet. All procedures were performed under isoflurane inhalation anesthesia. Animal experiments were approved by the Ethics Committees for Animal Experimentation (CEEA, Directive 400/12), University of Barcelona and all procedures complied with European Union regulations for animal experiments (EU guideline 86/609/EEC). Rats were randomly distributed into groups as described below.

Experimental design

The following three experimental groups were created: (1) Sham (n = 6): Animals were subjected to transverse laparotomy and silk ligatures were located in the right suprarenal vein, diaphragmatic vein, and hepatic artery. After 24 h, animals were sacrificed and blood and liver samples were collected and stored at -20 ℃ and -80 ℃ respectively, for further investigation; (2) ROLT (n = 12, 6 transplants): ROLT was performed according to the Kamada's cuff technique, without hepatic artery reconstruction[24]. During the donor surgery, the right suprarenal vein, diaphragmatic vein, and hepatic artery were ligated and the bile duct was cannulated. Then, the reduction of the liver was carried out. Liver reduction was achieved by removing the left lateral lobe and the two caudate lobes just before harvesting the liver, resulting in a 40% reduction of the liver mass. The pedicle of the left lateral lobe was ligated with 5.0 silk ligature, and the lobe was removed. The two caudate lobes were removed separately with the ligation[25]. Then, the donor livers were flushed and preserved with cold (4 ℃) University of Wisconsin (UW) solution for 1 h and then implanted to the receptor. Receptors were killed 24 h after transplantation and blood and liver samples were collected and stored at -20 ℃ and -80 ℃ respectively for further investigation; and (3) Losartan + ROLT (n = 12, 6 transplants): We used the same protocol as for group 2, but an AT1R antagonist (losartan) was orally administered (5 mg/kg) at 24 h and 1 h before the donor and the recipient surgery[9].

Transaminase assay

Hepatic injury was assessed in terms of transaminase levels with commercial kits from RAL (Barcelona, Spain). Briefly, plasma extracts were collected before liver extraction and centrifuged at 4 ℃ for 10 min at 3000 rpm. Then, 200 μL of the supernatant were added to the substrate provided by the commercial kit. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined at 365 nm with an ultraviolet spectrometer and calculated according to the manufacturer's instructions[26].

NAD^+/NADH determination

Liver NAD^+/NADH levels were quantified with a commercially available kit (MAK037, Sigma Chemical, St. Louis, MO, United States) according to the manufacturer's instructions.

Western blot analysis

Liver tissue was homogenized in a HEPES ((N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer as previously described[27]. Then, 50 μg of proteins were separated on 8%-15% SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) gels and trans-blotted on PVDF (polyvinylidene difluoride) membranes (Bio-rad Laboratories). Membranes were then blocked for one hour with 5% (w/v) non-fat milk in T-TBS (tween-tris-buffered saline) and incubated overnight at 4 ℃ with the corresponding primary antibody: SIRT1 (07-131), purchased from Merck Millipore, Billerica, MA; ac-FoxO1 (D-19, sc-49437) and GRP78 (GRP78, H-129, sc-13968), both purchased from Santa Cruz Biotechnology Inc, CA, United States); SIRT3 (2627), cleaved caspase-3 (Asp175, 9664), phosphorylated-eukaryotic translation initiation factor 2 (p-eIF2a) (Ser51, 9721), inositol-requiring enzyme 1α (IRE1α) (3294), caspase-12 (2202), p-p38 Thr180/Tyr182, 9211), p-p44/42 (Erk1/2, Thr202/Tyr204, 9101) purchased from Cell Signaling, Danvers, MA; HSP70 (610607, Transduction Laboratories, Lexington, KY); Heme Oxygenase-1 (H4535), NAMPT (AP22021SU, Acris Antibodies GmbH, Germany); and b-actin (A5316, Sigma Chemical, St. Louis, MO, United States). Membranes were then incubated for 1 h at room temperature with the corresponding secondary antibody linked to horseradish peroxidase. Bound complexes were detected using WesternBright ECL-HRP substrate (Advansta, Barcelona, Spain) and quantified *via* the Quantity One software for image analysis.

Table 1 Effect of losartan administration in liver injury after orthotopic liver transplantation

	Sham	ROLT	Losartan + ROLT
ALT (U/L)	48.8 ± 2.58	358.3 ± 133.44[a]	206.00 ± 33.61[a,b]
AST (U/L)	88.2 ± 4.65	893.57 ± 397.69[a]	500.85 ± 118.07[a,b]

Alanine aminotransferase (ALT) levels and aspartate aminotransferase (AST) in plasma after 24 h of reperfusion. [a]$P < 0.05$ *vs* Sham, [b]$P < 0.05$ *vs* ROLT. Sham: liver harvested without transplantation; ROLT: Liver subjected to reduced-size orthotopic liver transplantation after 1 h of cold storage in University of Wisconsin solution; losartan + ROLT: Same as ROLT group, but with further administration of losartan 24 h and 1 h before the surgical procedure to both the donor and the recipient.

Results were expressed as the densitometric ratio between the protein of interest and the loading control (β-actin).

Real-time quantitative reverse-transcription polymerase chain reaction

Real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was performed. Total liver RNA was isolated using a TRIzol reagent (Invitrogen). Reverse transcription was realized on a 1 μg RNA sample using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories). The reaction included incubation at 25 ℃ (5 min), at 42 ℃ (30 min) and 85 ℃ (5 min) and then cDNA was stored at -80 ℃. Subsequent PCR amplification was conducted in an iCycler iQ Multi-Color Real-Time PCR device (Bio-Rad Laboratories) using SsoAdvanced™ Universal SYBR Green Supermix (Bio-rad Laboratories) and the following rat primers for SIRT3: forward, 5′-TAGTCCAGGGTGTGGAAAGG-3′ and reverse, 3′-CCGCAGGTGAAGAAGTAAGC-5′. Reactions were performed in duplicate and threshold cycle values were normalized to GAPDH gene expression. The ratio of SIRT3 relative expression to GAPDH was calculated by the ΔCt formula.

Statistical analysis

Data are expressed as mean ± SE. Statistical comparison was performed by variance analysis, followed by the Student-Newman-Keuls test, using the GraphPad Prism software. P value < 0.05 was considered statistically significant.

RESULTS

Hepatic injury

We first examined whether treatment with losartan affected hepatic injury in our experimental model. As shown in Table 1, increased ALT and AST levels were observed when rats were submitted to ROLT in comparison with the sham group. However, treatment with losartan significantly reduced the transaminase levels in the ROLT group.

Losartan-induced SIRT1 expression and activity

To investigate the possible interaction of SIRT1 with angiotensin Ⅱ, we investigated the activity and the protein expression pattern of SIRT1. Animals subjected to ROLT showed augmented SIRT1 protein expression levels, which were further enhanced when losartan was administered (Figure 1A). In addition, losartan administration prior to the ROLT procedure significantly increased SIRT1 activity compared with both the ROLT and sham groups (Figure 1B). However, no significant differences were observed between the sham and ROLT groups.

In addition, we examined the levels of NAD^+, the co-factor necessary for SIRT1 activity and nicotinamide phosphoribosyltransferase (NAMPT) protein expression, which is the major precursor for NAD^+ biosynthesis. Figure 1C demonstrates that NAD^+ levels were high in the sham group, but decreased in the ROLT and losartan + ROLT groups; however, losartan pre-treatment contributed to elevated NAD^+ levels compared with ROLT alone. NAMPT protein was significantly augmented in both the ROLT and losartan + ROLT group in comparison to sham (Figure 1D).

Further, the forkheadbox (FoxO) transcription factors subfamily have been shown to mediate some of the effects of sirtuins. Given that FoxO1 is a direct substrate of SIRT1, we therefore determined its acetylation (Figure 1E). Animals subjected to ROLT showed elevated ac-FoxO1 protein levels compared with the sham group. By contrast, the augmented SIRT1 activity found when losartan was administered was consistent with a decrease in the ac-FoxO1 protein levels.

Losartan acted independently of SIRT3 expression

Because SIRT1 appeared to be modulated, we explored the role of SIRT3. We observed that SIRT3 mRNA levels were significantly downregulated in both ROLT and losartan + ROLT groups when compared with the sham group (Figure 2A). The same pattern was observed for SIRT3 protein levels, with significant decreases in animals subjected to ROLT and losartan + ROLT (Figure 2B).

Angiotensin Ⅱ inhibition attenuated ERS

To identify other potential molecular mechanisms involved in the hepatoprotective effect of losartan against IRI, we examined different ERS parameters, including GRP78, IRE1α, and p-eIF2. As indicated in Figure 3, important increases of all ERS parameters occurred following ROLT but not the sham operation. Losartan pre-treatment also restored the ERS parameters.

Losartan affected heat shock protein expression

Because heat shock proteins are implicated in liver IRI, we determined the protein expression pattern of heme oxygenase 1 (HO-1) and of the heat shock protein 70

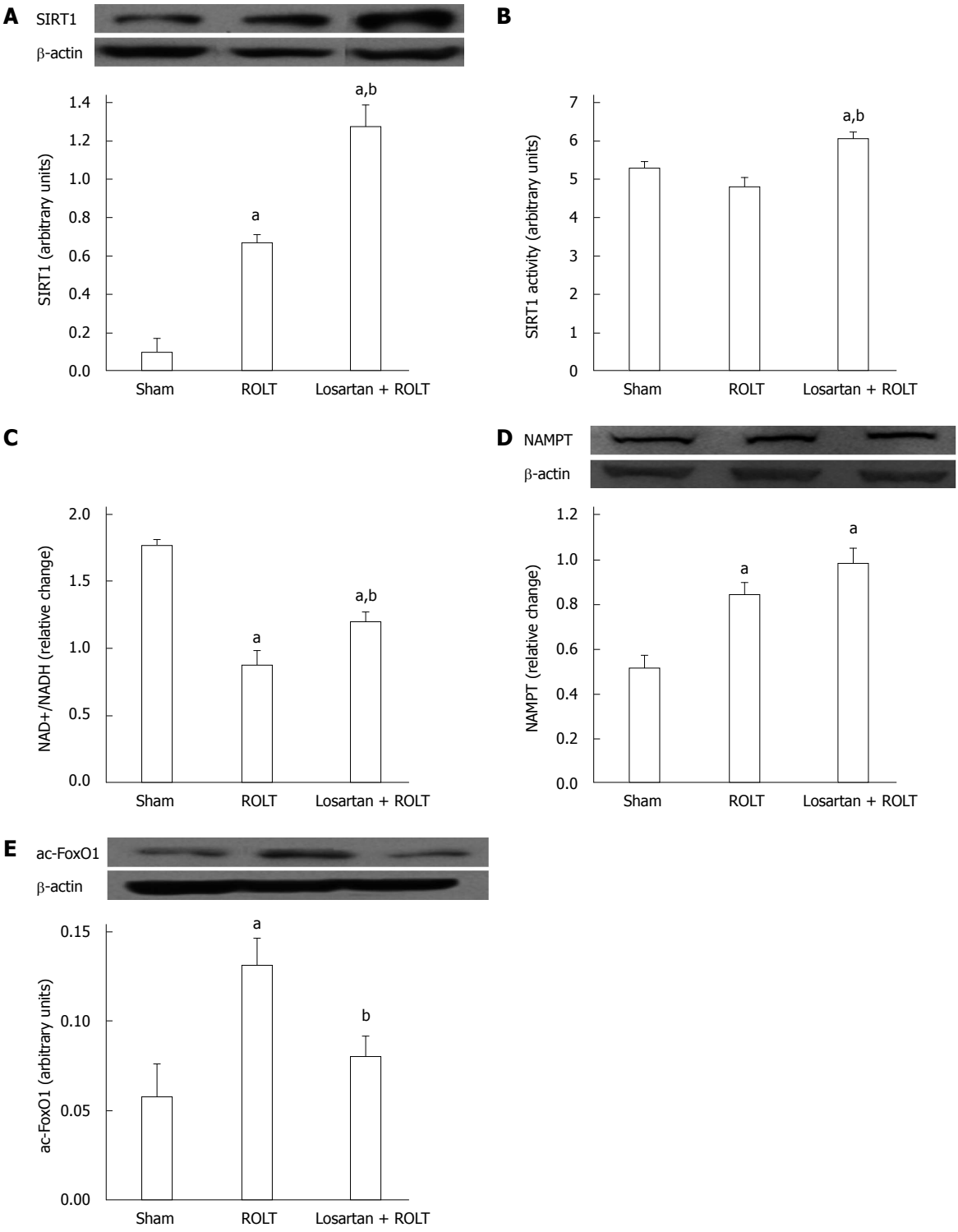

Figure 1 Effect of losartan treatment in sirtuin 1 protein expression and SIRT1 activity parameters. A: Sirtuin 1 (SIRT1) protein expression; B: SIRT1 activity; C: NAD+/NADH levels; D and E: NAMPT and ac-FoxO1 protein expression in livers after 24 h of reperfusion. [a]*P* < 0.05 *vs* Sham, [b]*P* < 0.05 *vs* ROLT. Sham: Liver harvested without transplantation; ROLT: Liver subjected to reduced-size orthotopic liver transplantation after 1 h of cold storage in University of Wisconsin solution; losartan + ROLT: same as ROLT group, but with further administration of losartan 24 h and 1 h before the surgical procedure to both the donor and the recipient.

(HSP70). As it is shown in Figure 4, enhanced HO-1 and HSP70 protein levels were found in animals subjected to ROLT. However, Losartan treatment decreased HO-1 protein levels and increased HSP70 protein levels.

Angiotensin Ⅱ inhibition reduced liver apoptosis

Liver IRI is characterized by increased hepatic apoptosis, so we determined the protein levels of caspase-12 and caspase-3, which are known to promote apoptosis.

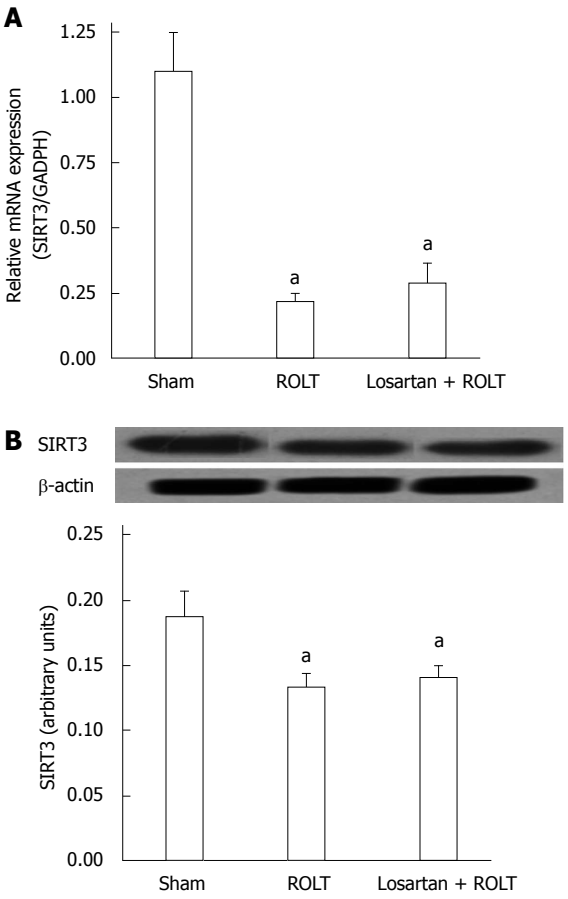

Figure 2 Implication of losartan administration in mRNA (A) and protein levels of sirtuin 3 (B). [a]*P* < 0.05 *vs* Sham. Sham: Liver harvested without transplantation; ROLT: Liver subjected to reduced-size orthotopic liver transplantation after 1 h of cold storage in University of Wisconsin solution; losartan + ROLT: Same as ROLT group, but with further administration of losartan 24 h and 1 h before the surgical procedure to both the donor and the recipient.

Figure 5 shows that increased levels of both proteins in animals undergoing ROLT were diminished by losartan pre-treatment.

MAPK regulation

The mitogen activated protein kinases (MAPKs) are serine/threonine protein kinases that mediate intracellular signal transduction events associated with IRI. Therefore, we determined the activation of extracellular signal-regulated kinase (ERK) and p38. Figure 6A shows that animals undergoing ROLT had increased levels of p-ERK, but that losartan pre-treatment did not enhance ERK activation compared with ROLT alone. Moreover, the content of p-p38 was decreased in both the ROLT and losartan + ROLT groups. Losartan pre-treatment did not alter p-p38 content when compared to ROLT alone (Figure 6B).

DISCUSSION

This study demonstrated that inhibition of AT1R lessens hepatic injury in ROLT. Specifically, we provide new insights into losartan-mediated hepatoprotection in rats undergoing ROLT, including the induction of SIRT1 and the attenuation of ERS.

The protective effects of losartan against IRI were

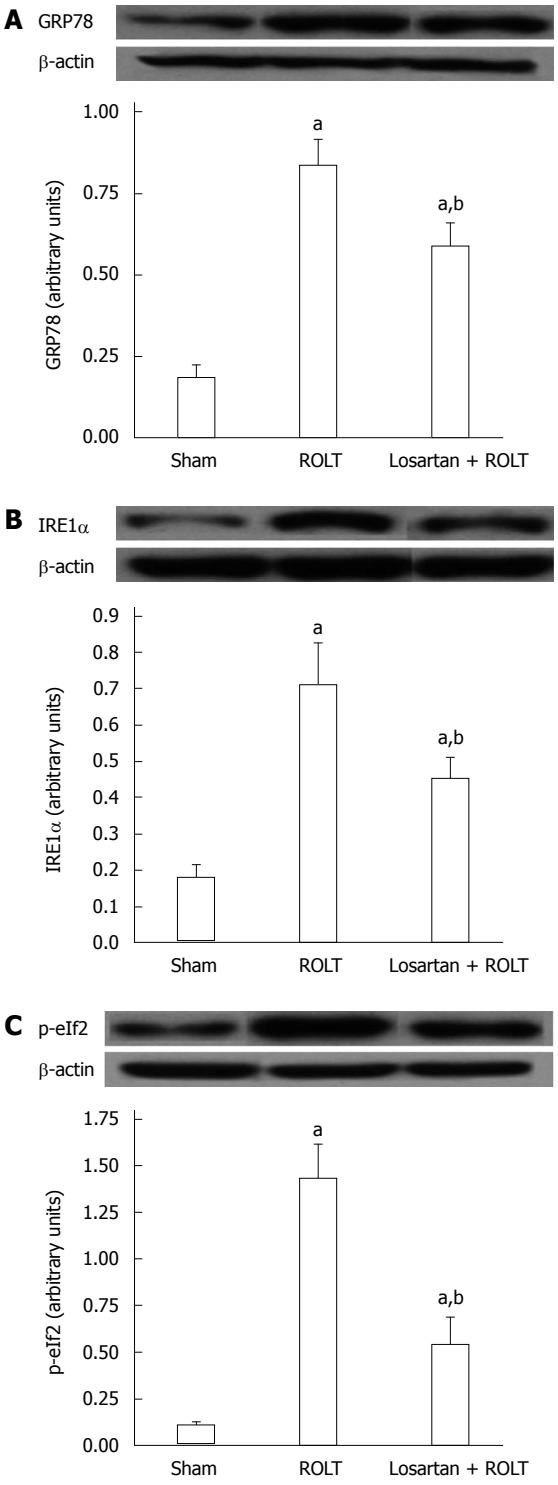

Figure 3 Role of losartan pretreatment in endoplasmic reticulum stress parameters. A: GRP78; B: IRE1α; C: p-eIF2 protein levels. [a]*P* < 0.05 *vs* Sham, [b]*P* < 0.05 *vs* ROLT. Sham: Liver harvested without transplantation; ROLT: Liver subjected to reduced-size orthotopic liver transplantation after 1 h of cold storage in University of Wisconsin solution; losartan + ROLT: Same as ROLT group, but with further administration of losartan 24 h and 1 h before the surgical procedure to both the donor and the recipient.

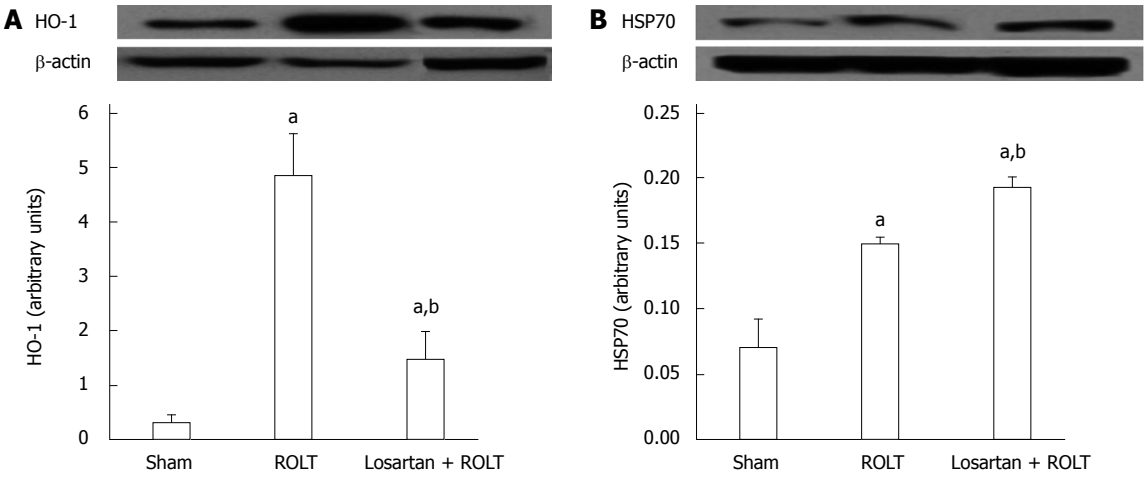

Figure 4 Losartan administration regulates heat shock proteins expression. HO-1 (A) and HSP70 protein levels (B). [a]*P* < 0.05 *vs* Sham, [b]*P* < 0.05 *vs* ROLT. Sham: Liver harvested without transplantation; ROLT: Liver subjected to reduced-size orthotopic liver transplantation after 1 h of cold storage in University of Wisconsin solution; losartan + ROLT: Same as ROLT group, but with further administration of losartan 24 h and 1 h before the surgical procedure to both the donor and the recipient.

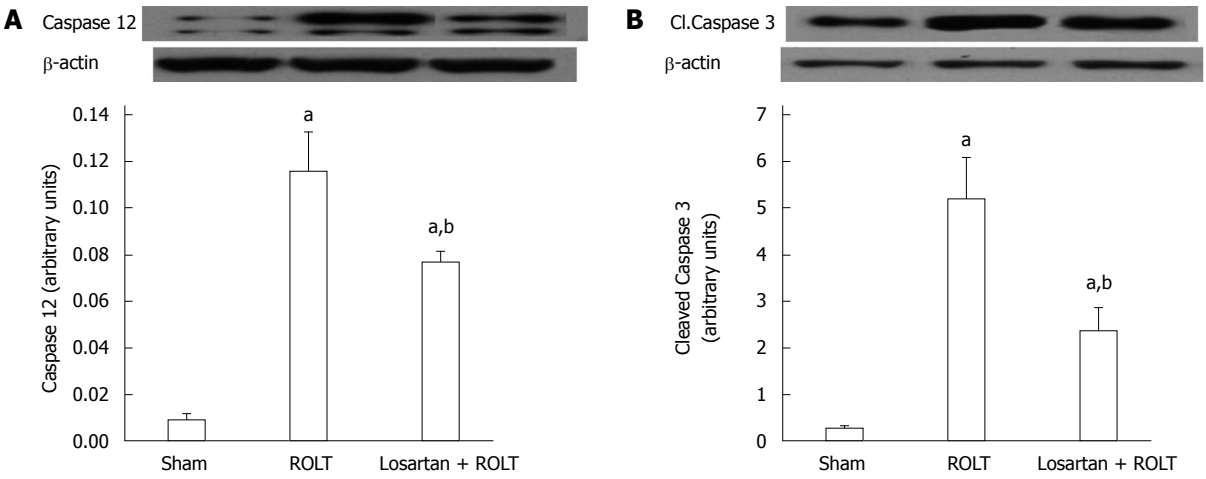

Figure 5 Liver apoptosis in reduced-size orthotopic liver transplantation after losartan treatment. Protein levels of Caspase 12 (A) and Cleaved Caspase 3 (B). [a]*P* < 0.05 *vs* Sham, [b]*P* < 0.05 *vs* ROLT. Sham: Liver harvested without transplantation; ROLT: Liver subjected to reduced-size orthotopic liver transplantation after 1h of cold storage in University of Wisconsin solution; losartan + ROLT: Same as ROLT group, but with further administration of losartan 24 h and 1 h before the surgical procedure to both the donor and the recipient.

associated with increased SIRT1 activity and protein expression. SIRT1 up-regulation and angiotensin Ⅱ blockade have been separately reported as therapeutic strategies against IRI in various organs[5,12,28,29]. Enhancement of SIRT1 has also been associated with decreased hepatic injury in rat orthotopic liver transplantation[30]. In our experimental rat ROLT model, SIRT1 protein expression was upregulated, but we observed no differences in its activity. Furthermore, FoxO1 deacetylation was inhibited in the ROLT group. SIRT1 overexpression and failure to augment its activity during IRI has also been reported in a recent work by our group[27]. In addition, losartan administration not only enhanced SIRT1 expression but also significantly increased both SIRT1 activity and FoxO1 deacetylation in comparison with the ROLT group. Further, losartan-induced increases in SIRT1 activity can be attributed to the enhanced NAD^+ levels, which are indispensable for sirtuin activity. In turn, the NAD^+ levels may be attributed to the NAMPT levels, which were slightly, but not significantly, increased after losartan treatment. Moreover, enhanced deacetylation of FoxO1 was related with NAMPT and NAD^+ increases in rat orthotopic liver transplantation[30]. The present data demonstrate the existence of an angiotensin Ⅱ/SIRT1 axis in liver transplantation, and that the benefits of angiotensin Ⅱ inhibition against liver IRI are mediated, at least in part, through SIRT1 activation. This is consistent with a recent study in rat skeletal muscle, in which angiotensin Ⅱ administration decreased SIRT1 expression[31].

Next, we speculated that SIRT3 might be affected by ROLT and losartan treatment. Real-time qRT-PCR and Western blot analysis revealed that SIRT3 mRNA and protein levels were significantly decreased in

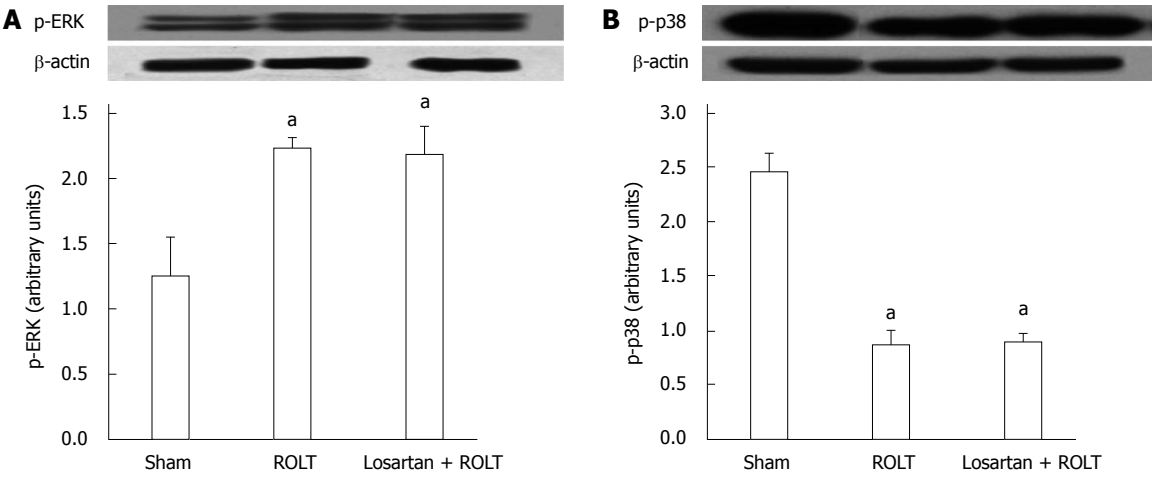

Figure 6 Mitogen activated protein kinases modulation by losartan administration. Effect of losartan in p-ERK (A) and p-p38 protein expression (B). $^{a}P < 0.05$ *vs* Sham. Sham: Liver harvested without transplantation; ROLT: Liver subjected to reduced-size orthotopic liver transplantation after 1 h of cold storage in University of Wisconsin solution; losartan + ROLT: Same as ROLT group, but with further administration of losartan 24 h and 1 h before the surgical procedure to both the donor and the recipient.

both the ROLT and losartan + ROLT groups compared with the sham group. This may be attributed to the mitochondrial disturbances that commonly take place during IRI[32]. SIRT3 is the major mitochondrial deacetylase implicated in metabolism, oxidative stress responses, and cardiac IRI[13,33-35]. The fact that SIRT3 mRNA and protein levels were comparable between the ROLT and losartan + ROLT groups suggests that the protective effect of losartan was independent of the SIRT3 pathway.

The endoplasmic reticulum is an organelle responsible for protein folding. Under stress conditions, the homeostasis of the endoplasmic reticulum is disturbed, leading to accumulation of unfolded proteins. In this case, an adaptive unfolded protein response (UPR) is activated to lessen the effects of ERS; however, when the insult is exaggerated in IRI, the ERS response can lead to cell death[36]. The UPR has three core branches: an IRE1α that induces the cleavage of the mRNA encoding X-box-binding protein 1 (XBP-1); a PKR-like endoplasmic reticulum kinase (PERK) that phosphorylates the eIF2a; and an activating transcription factor (ATF6). Under stress conditions, IRE1α, PERK, and ATF6 are released from their binding with the 78-kD glucose-regulated/binding immunoglobulin protein (GRP78) and become activated[37]. In a liver transplantation model, we have previously seen that activation of these UPR branches is associated with cell death and is a determinant factor of liver injury[18]. In this study, we observed that ROLT triggered the activation of GRP78 and the subsequent activation of the IRE1α and p-eIF2 pathways. Moreover, losartan pre-treatment abolished the activation of all ERS parameters. This is consistent with a recent study in human islets, which revealed that losartan exerted its protective effects against glucotoxicity by reducing ERS[38].

Losartan treatment was also accompanied by significant regulation of HSP70 and HO-1. The chaperone activity of HSP70 has been associated with cellular attempts to maintain proteins in an accurately folded state[36]. In our study, losartan pre-treatment induced HSP70 overexpression, which could have contributed to a decreased accumulation of unfolded proteins and therefore less ERS. Furthermore, because a direct relationship has previously been reported between SIRT1 and HSP70 in hepatic IRI, SIRT1 might contribute to HSP70 enhancement[27]. The increased ERS levels observed in the ROLT group were consistent with enhanced HO-1 protein expression that probably occurred due to an adaptive cell mechanism to prevent stress, as previously proposed by Liu *et al*[39]. In this sense, HO-1 expression was decreased when losartan pre-treatment diminished ERS.

Apoptosis is one of the most significant events in the pathophysiology of liver IRI. Aiming to mitigate the effects of ERS-mediated apoptosis could be an effective strategy for minimize IRI. It is known that IRE1α provokes caspase 12 cleavage, which in turn activates caspase 9 and then caspase 3 to stimulate apoptosis[40,41]. In our study, the induction of ERS in the ROLT group led to increased cell death, as reflected by the enhanced caspase 12 and caspase 3 protein levels. Further, the decrease in ERS in the losartan + ROLT group coincided with decreases in the levels of these caspases.

MAPKs are linked with cell cycle, liver regeneration, apoptosis, and oxidative stress pathways. The ERK cascade is closely connected with the regulation of cell growth and differentiation, whereas p38 is involved in cellular responses to environmental stress[42]. It has been reported that active p38 MAPK is present in the quiescent liver, and that it is dephosphorylated in the regenerating liver[43,44]. ERK phosphorylation is also involved in the signaling pathways of liver regeneration[45]. Therefore, the lowered p-p38 and

increased p-ERK levels observed in the ROLT and losartan + ROLT groups could be associated with enhanced liver regeneration. In a previous study, our group reported that losartan pre-treatment did not enhance liver regeneration after ROLT[46]. Thus, losartan pre-treatment did not provide an additional increase in liver regeneration, resulting in no differences in p-p38/ERK activation between the two ROLT groups. Consequently, we can assume that SIRT1 activation by losartan treatment is not associated with liver regeneration in a ROLT model. Losartan administration decreased significantly hepatic injury and affected signaling processes related to IRI, such ERS and apoptosis. However, it could not further enhance liver regeneration, an essential processes for the success of transplantation with reduced-size liver grafts. Further studies will be required to elucidate the mechanisms by which losartan improves hepatic injury after ROLT.

Furthermore, angiotensin Ⅱ is known to exert vasoconstrictor effects[47-49] and angiotensin Ⅱ blockers, such as losartan, have been reported to decrease arterial pressure and act as effective antihypertensive agents[50,51]. A potential hypotensive effect of losartan was out of the scope of the present study, whereas prolonged time treatments with losartan are usually applied in order to evaluate blood pressure changes[52].

In conclusion, the present results indicate that SIRT1 is implicated in the protective effects of AT1R inhibition by losartan against IRI following ROLT. Losartan pre-treatment markedly attenuates liver injury by regulating signaling pathways that are involved in the pathophysiology of IRI, including heat shock protein, ERS, and liver apoptosis pathways. Moreover, it is evidenced that SIRT1 is a downstream target of angiotensin Ⅱ in a rat ROLT model. Further studies are required to identify whether other angiotensin peptides (*i.e.*, 1-7) can also modulate SIRT1.

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COMMENTS

Background

Ischemia-reperfusion injury (IRI) is a complex pathophysiological process inherent to liver transplantation. Endoplasmic reticulum stress (ERS) and apoptosis are common features of liver IRI in this context. Angiotensin Ⅱ is a basic constituent of the renin-angiotensin system and has been shown to worsen IRI. Angiotensin Ⅱ acts by binding to angiotensin Ⅱ type Ⅰ receptors (AT1R) and angiotensin Ⅱ type Ⅱ receptors. Of note, antagonists of these receptors have been found to protect against liver IRI. In addition, sirtuin 1 (SIRT1) is a NAD^+-dependent deacetylase that modulates various cellular pathways associated to IRI, but its relationship with angiotensin Ⅱ in liver IRI has not been studied. In this study, the authors demonstrate that administration of losartan, an antagonist of AT1R, significantly reduced liver injury in a rat model of reduced-size orthotopic liver transplantation (ROLT) by activating SIRT1 and decreasing ERS and liver apoptosis.

Research frontiers

Angiotensin Ⅱ has been associated with inflammatory responses and oxidative stress in liver IRI. Inhibition of its action with AT1R antagonists, such as losartan, results in decreased hepatic injury by attenuating pro-inflammatory responses, activating HIF-1α and peroxisome proliferator-activated receptor gamma in various hepatic IRI models. The present study report that the hepatoprotective effects of losartan against IRI associated with ROLT are mediated through SIRT1 enhancement, HSP70 overexpression, and attenuation of ERS and liver apoptosis.

Innovations and breakthroughs

The role of SIRT1 in a ROLT model has not yet been determined, nor has the potential link between angiotensin Ⅱ and SIRT1 or ERS in liver IRI. The present study evaluated the potential role of losartan administration on SIRT1 expression and activity and on ERS activation in a rat ROLT model. The present study demonstrated that angiotensin Ⅱ inhibition led to SIRT1 up-regulation and a subsequent decrease in ERS that contributed to reduced hepatic injury following ROLT.

Applications

Pharmacological activation of SIRT1 by losartan might be a promising therapeutic tool for ameliorating the detrimental effects of IRI following ROLT in rat models.

Peer-review

The manuscript is well written and data presented are detailed as well as the figures.

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ORIGINAL ARTICLE

Basic Study

Changes in gene expression in liver tissue from patients with fulminant hepatitis E

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Author contributions: Naik A carried out the laboratory work and data analysis, and prepared the first draft of the manuscript; Goel A carried out initial screening and identification of patients and liver biopsies, and helped in drafting and critical revision of the manuscript; Agrawal V supervised the histological and immunohistochemical studies; Sarangi AN did the microarray data analysis and helped in drafting the related segments of the manuscript, and in critical revision of the manuscript; Chhavi N carried out initial screening and identification of patients and liver biopsies; Singh V did the laboratory work related to microarray analysis; Jameel S conceived the idea, participated in design of the study and interpretation of data, and helped revise the manuscript; Aggarwal R conceived the idea, participated in design of the study, clinical identification of patients, supervision of laboratory work, analysis of microarray data and overall supervision of the work, and helped in drafting and revision of the manuscript; all authors read and approved the final manuscript.

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Abstract

AIM: To study host gene expression and number

of immune cells in liver tissues from patients with fulminant hepatitis E (FH-E).

METHODS: Microarray-based expression profiling was done using Illumina Human WG-6_v3_BeadChip arrays on post-mortem liver tissue from 5 patients with FH-E, and compared with similar tissue from 6 patients with fulminant hepatitis B (FH-B; disease controls) and normal liver tissue from 6 persons. Differential expression was defined as ⩾ 2.0-fold change with Benjamini-Hochberg false discovery rate below 0.05 using t-test in liver tissue from FH-B and FH-E, than healthy liver tissue. For some genes that showed differential expression in FH-E, microarray data were validated using quantitative reverse transcription PCR. Differentially expressed gene lists were then subjected to "Gene Ontology" analysis for biological processes, and pathway analysis using BioCarta database on the DAVID server. In addition, tissue sections were stained for $CD4^+$, $CD8^+$ and $CD56^+$ cells using indirect immunohistochemistry; cells staining positive for each of these markers were counted and compared between groups.

RESULTS: Compared to normal livers, those from patients with FH-E and FH-B showed differential expression of 3377 entities (up-regulated 1703, downregulated 1674) and 2572 entities (up 1164, down 1408), respectively. This included 2142 (up 896, down 1246) entities that were common between the two sets; most of these belonged to metabolic, hemostatic and complement pathways, which are active in normal livers. Gene expression data from livers of patients with FH-E but not those of FH-B showed activation of several immune response pathways, particularly those involving cytotoxic T cells. The fold-change values of mRNA for selected genes in livers from FH-E than in normal liver tissue determined using quantitative reverse transcription PCR showed excellent concordance with microarray analysis. At immunohistochemistry, $CD8^+$ T cells showed an increase in liver biopsies from both FH-E [median 53.4 per arbitrary unit area (range 31.2-99.9)] and FH-B [median 49.3 (19.3-51.0); P = 0.005] compared to control liver tissue [median 6.9 (3.1-14.9)].

CONCLUSION: FH-E patients show $CD8^+$ T cell infiltration and increased gene expression of cytotoxic T cell pathways in liver, suggesting a possible pathogenetic role for these cells.

Key words: Cytotoxic T cells; Gene expression; Hepatitis E; Hepatitis E virus; Immune response; Liver biopsy; Microarray; Natural killer cells; Pathogenesis

Core tip: Data on pathogenesis of hepatitis E virus (HEV) infection, which is a common cause of acute hepatitis in several developing countries, are quite limited. This manuscript reports our data on microarray-based gene expression analysis and immunohistochemistry in liver tissue from patients with HEV infection, as compared to liver tissue from patients with hepatitis B virus infection and normal liver tissue. These data advance the current knowledge about the pathogenesis of HEV infection.

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INTRODUCTION

Hepatitis E virus (HEV), a member of genus *Hepevirus* in family *Hepeviridae*, consists of 32-34 nm diameter, icosahedral, non-enveloped virions[1]. It has a 7.2-kb single-stranded, positive-sense RNA genome with three open reading frames (ORFs) that code for a viral non-structural polyprotein (ORF1), the major capsid protein (ORF2) and a phosphoprotein with possible regulatory functions (ORF3)[1]. The virus has at least four genotypes, named 1 to 4[2]; however, these all belong to a single serotype.

HEV infection is common in developing countries of Asia and Africa, where it causes an acute disease, known as hepatitis E, either as water-borne outbreaks or as sporadic cases[3,4], caused by infection through the fecal-oral route. The disease occurs predominantly among young adults and resembles acute hepatitis caused by other hepatotropic viruses. It is usually self-limiting, with overall case fatality rate below 0.5%[3]. However, some patients, in particular pregnant women, develop severe liver injury that progresses to fulminant hepatic failure (FH), which is often fatal. In these areas, the virus belongs most often to genotype 1 and sometimes to genotype 2[3,4].

In areas with lower disease endemicity, such as Europe, North America, Japan, *etc.*, occasional cases with locally-acquired HEV infection have been reported[5]. These are mainly elderly men, often with other co-existing diseases, who are believed to acquire infection with genotype 3 or 4 HEV through ingestion of undercooked meat of HEV-infected animals[6]. In immunosuppressed persons, the infection may become persistent[6].

To develop therapeutic measures against hepatitis E, it is important to understand the pathogenesis of liver injury in this disease. In infection with other hepatotropic viruses, such as the hepatitis A, B and C viruses, tissue injury is mediated not by the infectious agent but is related to the host immune response[7-11]. We have shown that HEV infection is associated with activation of specific cellular immune responses in the

peripheral blood[12-16]. However, little data are available on the liver tissue from hepatitis E. Therefore, the mechanism of liver injury in this disease remains unclear.

We therefore studied gene expression profile in liver biopsies from patients with FH due to acute hepatitis E (FH-E), in comparison with those from healthy adults and patients with FH due to acute hepatitis B (FH-B; a disease control group).

MATERIALS AND METHODS

Patients and specimens

Post-mortem needle liver biopsies were obtained from patients dying of FH-E (n = 5) or of FH-B (n = 6). The biopsies were obtained within 30 min after death, and separate pieces were collected in RNA*Later* (Qiagen) and stored at -80 ℃ for gene expression analysis, and in formalin for histology and immunohistochemistry. In addition, a blood specimen was collected from each subject, for biochemical tests and serological markers of viral hepatitis.

Patients dying more than 2 wk after the onset of disease, and those with major sepsis or clinical evidence of pre-existing liver disease were excluded. Diagnosis of acute hepatitis E was based on detection of IgM anti-HEV (Genelabs, Singapore) in the absence of hepatitis B surface antigen (HBsAg), IgM anti-HBc antibody, anti-hepatitis C virus (HCV) antibody and IgM anti-hepatitis A virus (HAV) antibody (all from BioMerieux, Marcy l'Etoile, France), and that of acute hepatitis B on detection of HBsAg and IgM anti-HBc, in the absence of IgM anti-HEV, anti-HCV and IgM anti-HAV.

In addition, normal liver tissue was obtained from six persons undergoing partial hepatic resection for focal diseases, such as hydatid liver disease, gallbladder cancer without biliary obstruction.

The study was reviewed and approved by Sanjay Gandhi Postgraduate Institute of Medical Sciences institutional review board. Written, informed consent was obtained from all patients or their families, as appropriate.

RNA isolation

Total RNA was isolated from tissue biopsies using RNeasy Protect minikit (Qiagen, Carlsbad, CA). RNA concentration was measured using NanoDrop 1000 spectrophotometer (Nanodrop, Wilmington, DE). RNA integrity was assessed using Bioanalyzer 2100 (Agilent, Santa Clara, CA). Specimens with A260/A280 and A260/A230 ratios > 1.9, and RIN > 8.0 were processed further.

Microarray analysis

Gene expression profiling was done using Illumina Human WG-6_v3_BeadChip arrays (Illumina, San Diego, CA), each containing more than 46000 entities (gene probes or probesets) derived from NCBI, RefSeq and UniGene databases. Poly(A)-RNA was reverse transcribed to complementary DNA (cDNA), using an oligo(dT)-primer that contained a phage T7 RNA polymerase promoter sequence at its 5′-end, followed by conversion to double-stranded cDNA. The double-stranded cDNA was transcribed *in vitro* to yield large quantities of biotin-labelled anti-sense RNA, which was then hybridised to the bead arrays at 55 ℃ for 16-18 h, and scanned using an Illumina iScan reader.

Bioinformatic analysis

The array intensity data were initially analysed using Illumina Genome Studio Gene Expression Module (v1.1.1) (Illumina, Cambridge, United Kingdom) for visualisation and normalisation. After quantile normalisation and background correction using medians within the BeadStudio software, the data were exported for further analysis in GeneSpring GX software 11.5 (Agilent).

Differential expression between various subject groups was analyzed using Illumina Custom Algorithm. The normalized data were first subjected to quality check using principal component analysis. This was followed by analysis to determine genes showing differential expression (fold-change $\geqslant$ 2.0 and Benjamini-Hochberg false discovery rate < 0.05 using *t*-test) in liver tissue from patients with FH-B and FH-E, as compared to healthy liver tissue. Such gene lists were then subjected to "Gene Ontology" (GO) analysis for biological processes, and pathway analysis using BioCarta database on the DAVID server (http://david.abcc.ncifcrf.gov). Separate analyses were done for genes that were differentially expressed in both FH-E and FH-B, and those differentially expressed in only one of these.

Validation of microarray results

For some genes that showed differential expression in FH-E, microarray data were validated using quantitative reverse transcription PCR (qRT-PCR). In brief, cDNA was prepared using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) from 2 μg of RNA. This was followed by real-time PCR in 20-μL reactions comprising of 50 ng cDNA, primers (Appendix 1) and SYBR Green (Applied Biosystems).

The validation assay included liver tissue for all the subjects used for microarray analysis (a biopsy piece other than that used for microarray analysis) and four additional healthy liver tissues. All assays included RNA from peripheral blood mononuclear cells of a healthy person as a calibrator. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and 18S rRNA was used as housekeeping genes. Relative fold-change was determined for each specimen and gene from cycle threshold (Ct) values as follows, and compared to fold-change in expression in microarray data:

Table 1 Characteristics of study subjects in the three groups

Characteristic	Healthy controls (n = 6)	Fulminant hepatitis B (n = 6)	Fulminant hepatitis E (n = 5)
Age (yr)	52 (30-58)	24 (16-60)	28 (18-32)
Gender (Male:Female)	4:2	4:2	1:4
Duration of illness before death (d)	-	9 (6-14)	10 (7-13)
Maximum total serum bilirubin (mg/dL)	0.7 (0.4-1.4)	21 (13.1-41.4)	25 (5.4-32.0)
Serum ALT[a] (IU/L)	30 (21-58)	1939 (524-5795)	770 (240-1302)
Serum AST (IU/L)	28 (18-35)	859 (250-2345)	458 (332-1000)
Alkaline phosphatase (IU/L)	-	257 (131-427)	306 (164-561)
Serum albumin (g/dL)	-	2.8 (2.0-3.3)	2.8 (2.1-3.2)

Data are shown as median (range), except for gender distribution; patients with fulminant hepatitis B and fulminant hepatitis E were comparable in all variables except serum ALT levels ([a]P < 0.05, Mann-Whitney U test). ALT: Alanine aminotransferase.

$\Delta\Delta Ct = [(\Delta Ct_{\text{gene of interest}} - \Delta Ct_{GAPDH})_{\text{patient}} - [(\Delta Ct_{\text{gene of interest}} - \Delta Ct_{GAPDH})_{\text{calibrator sample}}]$ and Fold change = $2^{-\Delta\Delta Ct}$.

Immunohistochemistry on liver biopsies

Formalin-fixed, paraffin-embedded liver sections were stained with hematoxylin and eosin, and examined to confirm the presence of acute inflammation in patients with FH, and absence of disease in controls.

In addition, immunohistochemistry (IHC) was performed for specific T-cell subsets ($CD4^+$ and $CD8^+$) and NK cells ($CD56^+$). In brief, formalin-fixed, paraffin-embedded 3-μm liver sections on silanized glass slides (Dako) were fixed at 60 ℃ overnight. The slides were deparaffinised in xylene and rehydrated in graded alcohol. Antigen retrieval was done in 10 mmol/L EDTA (pH 9.0) at 98 ℃ for 30 min. After washing with distilled water and Tris-buffered saline (TBS; pH 7.4), endogenous peroxidase was blocked using 3% hydrogen peroxide in methanol for 30 min in dark. Sections were then incubated with monoclonal primary antibodies [prediluted mouse anti-human CD8 (clone C8/144B), CD4 (clone 4B12) or CD56 (clone 123C3) (Dako, Denmark)] for 1 h at room temperature, followed by peroxidase enzyme-labelled secondary antibody (Dako) for 30 min. Diaminobenzidine was used as chromogen to detect the bound antibodies. Slides were counterstained with Mayer's hematoxylin for 1 min, cleared and mounted with DPX (Sigma-Aldrich). Stained cells were counted in five fields, averaged and expressed as number in an area measuring 73000 μm^2.

Statistical analysis

Inter-group comparisons were done using t-test with Benjamini-Hochberg correction in case of gene expression data and Mann-Whitney U test for other quantitative data. Relationship of fold-change in the microarray and real-time PCR data was assessed using Pearson's correlation coefficient. The statistical methods of this study were reviewed by Dr. Rakesh Aggarwal from Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India.

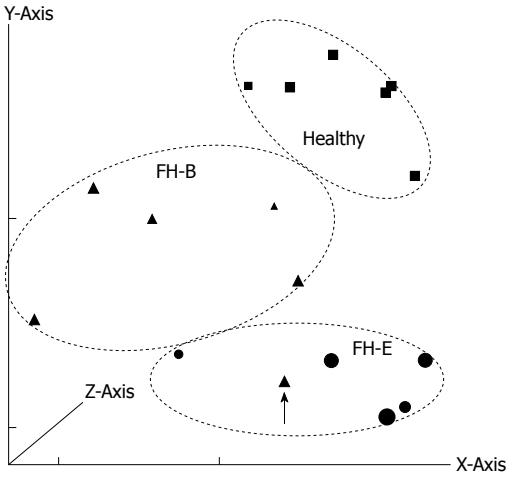

Figure 1 Principal component analysis of gene expression data from various specimens included in the study. The components along X, Y and Z axes were 62.54%, 21.17%, and 8.88%, respectively. Patients with fulminant hepatitis E (FH-E) are represented using circles, those with fulminant hepatitis B (FH-B) using triangles and healthy controls using square symbols. The size of markers varies according to their placement on the Z axis. Patients with FH-E and FH-B clustered separately, except for one patient with FH-B who was an outlier (arrow).

RESULTS

Demographic features and biochemical findings in the three subject groups are shown in Table 1. Alanine aminotransferase levels were higher in FH-B than in FH-E.

Principal component analysis

On principal component analysis of gene expression data (Figure 1), all specimens except one (with FH-B) showed clustering with other specimens in the same group.

Microarray gene expression

A total of 3377 entities, each representing a discrete gene, showed differential expression of ⩾ 2-fold with P-value of < 0.05 in liver tissue from patients with FH-E than that from healthy persons (Figure 2); this included 1703 entities with over-expression and 1674 showing reduced expression in FH-E. In liver tissue from FH-B, 2572 entities showed differential expression than in healthy livers (up-regulation 1164, down-regulation 1408).

Of the entities differentially expressed in FH-E or FH-B compared to normal liver, 2142 were common (up-regulation in both 896, down-regulation in both 1246) (Figure 2, Appendix 2); none of these entities showed discordance in the direction of differential expression between FH-E and FH-B. In contrast, 1235

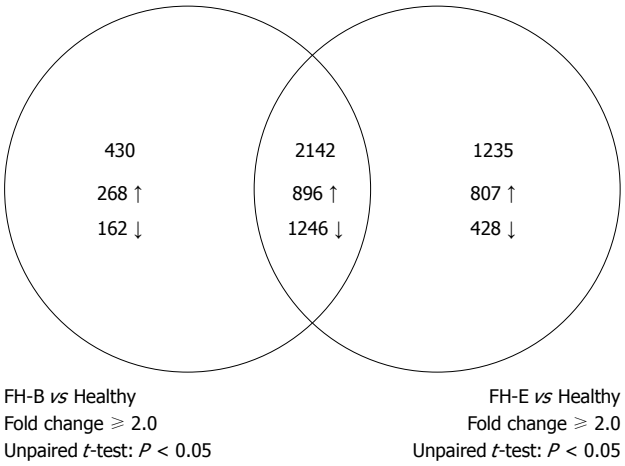

Figure 2 Venn-diagram showing comparison of number of entities differentially expressed in liver tissue from fulminant hepatitis B or E, as compared to healthy liver tissue. The intersection of two circles shows entities that were differentially expressed in both fulminant hepatitis E and fulminant hepatitis B.

entities (Appendix 3) were differentially expressed in FH-E but not in FH-B, and 430 entities (Appendix 4) were differentially expressed in FH-B but not in FH-E.

Gene ontology analysis

Genes differentially expressed in FH-E and FH-B livers: Table 2 lists the biological processes that showed differential regulation on GO analysis of the 2142 entities differentially expressed in both FH-E and FH-B. These included several metabolic processes in which the liver plays an active part, such as general body metabolism, or those whose proteins are mainly synthesized in the liver, such as coagulation pathways and complement system. Further, both the disease conditions showed differential regulation of innate immune responses and humoral immune responses mediated by B cells.

Genes differentially expressed in FH-E but not in FH-B: On GO analysis, the 1235 entities that showed differential expression in FH-E but not in FH-B were related mainly to the immune system pathways (Table 3); for each of these immune pathways, several genes showed upregulation.

Genes differentially expressed in FH-B but not in FH-E: GO analysis of these entities did not reveal significant changes in any specific pathway.

Pathway analysis

Genes differentially expressed in both FH-E and FH-B: Pathway analysis for the 2142 entities differentially expressed in both FH-E and FH-B using the BioCarta database showed downregulation of several pathways related to metabolic processes, hemostasis, and complement system (Table 4). Appendix 5 shows individual genes involved in each of these pathways.

Genes differentially expressed in FH-E but not in FH-B: BioCarta pathways whose genes were significantly over-represented among genes differentially expressed in liver tissue from FH-E compared to normal liver but not in FH-B were mostly related to cellular immune mechanisms (Table 4), *e.g.,* T cytotoxic cell surface molecules, CTL mediated immune response against target cells, T helper cell surface molecules, co-stimulatory signal during T-cell activation, T cell receptor signalling pathway, Lck and Fyn tyrosine kinases in initiation of TCR activation, IL-7 signal transduction, T cell receptor and CD3 complex, IL 17 signalling pathway. Several individual genes involved in these pathways were upregulated in patients with FH-E (Appendix 6).

Genes differentially expressed in FH-B but not in FH-E: Pathway analysis of these 430 entities did not reveal significant change in any pathway.

Validation using qRT-PCR

The fold-change values of mRNA for selected genes (Appendix 1) in livers from FH-E than in normal liver tissue determined using qRT-PCR showed excellent concordance with those at microarray (Figure 3), using either of the two housekeeping genes.

Immunohistochemistry

IHC showed a few $CD4^+$ positive T-cells and occasional NK cells in all the biopsies. In FH-B as well as FH-E, $CD8^+$ T-cells were found in both the liver parenchyma and the portal areas (Figure 4). The median number of $CD8^+$ T cells was greater in liver tissues from both FH-E [median 53.4 (range 31.2-99.9)]; $P = 0.005$, 2-sided Mann-Whitney *U* test) and FH-B [49.3 (19.3-51.0); $P = 0.005$] than in controls 6.9 (3.1-14.9); however, there was no significant difference between FH-E and FH-B.

DISCUSSION

Host tissue injury during a viral infection may be caused either by virus-induced cell death, or by immune-mediated killing of infected cells. Understanding the underlying mechanism may not only help understand the pathogenesis of a particular viral disease but also provide a lead to development of strategies for countering the host injury. In infection with hepatitis A, B or C virus, liver injury is mediated primarily by the host immune response[7-11]. However, the mechanisms of liver injury in HEV infection remain unclear.

Initial attempts at delineation of mechanism of liver injury caused by HEV were based on observations during experimental HEV infection in non-human primates. In these studies, liver injury appeared after viremia and fecal viral excretion had started declining

Table 2 Gene Ontology analysis (biological processes) for gene entities differentially expressed in liver tissue from fulminant hepatitis E as compared to normal liver tissue, as well as in fulminant hepatitis B as compared to normal liver tissue

Gene ontology accession	Gene Ontology term	*P* value
GO:0055114	Oxidation-reduction process	2.68E-40
GO:0006082	Organic acid metabolic process	4.75E-39
GO:0043436	Oxoacid metabolic process	2.17E-38
GO:0019752	Carboxylic acid metabolic process	4.09E-38
GO:0006629	Lipid metabolic process	3.94E-28
GO:0044281	Small molecule metabolic process	9.06E-27
GO:0032787	Monocarboxylic acid metabolic process	3.56E-22
GO:0016054	Organic acid catabolic process	4.22E-19
GO:0046395	Carboxylic acid catabolic process	4.22E-19
GO:0044255	Cellular lipid metabolic process	1.35E-17
GO:0044282	Small molecule catabolic process	5.16E-17
GO:0044712	Single-organism catabolic process	5.16E-17
GO:0006631	Fatty acid metabolic process	2.71E-16
GO:0006520	Cellular amino acid metabolic process	4.43E-16
GO:0044711	Single-organism biosynthetic process	8.25E-14
GO:1901605	Alpha-amino acid metabolic process	1.07E-13
GO:0044283	Small molecule biosynthetic process	1.60E-13
GO:1901564	Organonitrogen compound metabolic process	2.46E-11
GO:0009063	Cellular amino acid catabolic process	4.46E-11
GO:0008610	Lipid biosynthetic process	8.46E-11
GO:0072376	Protein activation cascade	3.87E-10
GO:0008202	Steroid metabolic process	4.10E-10
GO:0016053	Organic acid biosynthetic process	6.26E-10
GO:0046394	Carboxylic acid biosynthetic process	6.26E-10
GO:1901566	Organonitrogen compound biosynthetic process	1.94E-08
GO:0008152	Metabolic process	4.34E-08
GO:1901606	Alpha-amino acid catabolic process	4.58E-08
GO:0072329	Monocarboxylic acid catabolic process	1.40E-07
GO:0007596	Blood coagulation	1.98E-07
GO:0050817	Coagulation	1.98E-07
GO:0007599	Hemostasis	2.83E-07
GO:1901615	Organic hydroxy compound metabolic process	4.14E-07
GO:0042060	Wound healing	4.86E-07
GO:0006956	Complement activation	6.77E-07
GO:0009062	Fatty acid catabolic process	1.71E-06
GO:0006066	Alcohol metabolic process	1.82E-06
GO:0008652	Cellular amino acid biosynthetic process	2.42E-06
GO:0050878	Regulation of body fluid levels	5.46E-06
GO:0051186	Cofactor metabolic process	5.50E-06
GO:0006694	Steroid biosynthetic process	8.32E-06
GO:0044242	Cellular lipid catabolic process	1.09E-05
GO:0005996	Monosaccharide metabolic process	0.000012
GO:0051346	Negative regulation of hydrolase activity	1.28E-05
GO:0005975	Carbohydrate metabolic process	1.35E-05
GO:0010876	Lipid localization	5.30E-05
GO:0044710	Single-organism metabolic process	5.82E-05
GO:0006732	Coenzyme metabolic process	6.57E-05
GO:0010951	Negative regulation of endopeptidase activity	7.22E-05
GO:0019318	Hexose metabolic process	7.95E-05
GO:0006869	Lipid transport	8.02E-05
GO:0065008	Regulation of biological quality	8.96E-05
GO:0010466	Negative regulation of peptidase activity	0.0001
GO:0006006	Glucose metabolic process	0.0001
GO:0042558	Pteridine-containing compound metabolic process	0.0002
GO:0043648	Dicarboxylic acid metabolic process	0.0002
GO:0006958	Complement activation, classical pathway	0.0004
GO:0006091	Generation of precursor metabolites and energy	0.0004
GO:0044262	Cellular carbohydrate metabolic process	0.0004
GO:0006575	Cellular modified amino acid metabolic process	0.0005
GO:0016051	Carbohydrate biosynthetic process	0.0005
GO:0072330	Monocarboxylic acid biosynthetic process	0.0006
GO:0002455[1]	Humoral immune response mediated by circulating immunoglobulin	0.0010
GO:0044723	Single-organism carbohydrate metabolic process	0.0010
GO:0019320	Hexose catabolic process	0.0011
GO:0016052	Carbohydrate catabolic process	0.0014
GO:0044724	Single-organism carbohydrate catabolic process	0.0014
GO:0006820	Anion transport	0.0016
GO:0006007	Glucose catabolic process	0.0017
GO:0046365	Monosaccharide catabolic process	0.0018
GO:0046835	Carbohydrate phosphorylation	0.0020
GO:1901617	Organic hydroxy compound biosynthetic process	0.0020
GO:0002253[1]	Activation of immune response	0.0021
GO:0016125	Sterol metabolic process	0.0022
GO:0006096	Glycolysis	0.0023
GO:0019395	Fatty acid oxidation	0.0037
GO:0034440	Lipid oxidation	0.0037
GO:0046165	Alcohol biosynthetic process	0.0038
GO:0006959[1]	Humoral immune response	0.0042
GO:0009611	Response to wounding	0.0044
GO:0006957	Complement activation, alternative pathway	0.0044
GO:0006760	Folic acid-containing compound metabolic process	0.0044
GO:0045087[1]	Innate immune response	0.0053
GO:0052548	Regulation of endopeptidase activity	0.0077
GO:0046364	Monosaccharide biosynthetic process	0.0077
GO:0006790	Sulfur compound metabolic process	0.0078
GO:0051179	Localization	0.0079
GO:0052547	Regulation of peptidase activity	0.0083
GO:0046942	Carboxylic acid transport	0.0083
GO:0015849	Organic acid transport	0.0095
GO:0016042	Lipid catabolic process	0.0113
GO:0015711	Organic anion transport	0.0113
GO:0051234	Establishment of localization	0.0113
GO:0006810	Transport	0.0119
GO:0044092	Negative regulation of molecular function	0.0126
GO:0019319	Hexose biosynthetic process	0.0131
GO:0072378	Blood coagulation, fibrin clot formation	0.0132
GO:0006558	L-phenylalanine metabolic process	0.0146
GO:0006559	L-phenylalanine catabolic process	0.0146
GO:0000038	Very long-chain fatty acid metabolic process	0.0146
GO:0051289	Protein homotetramerization	0.0146
GO:0002252[1]	Immune effector process	0.0153
GO:0008203	Cholesterol metabolic process	0.0153
GO:0009074	Aromatic amino acid family catabolic process	0.0212
GO:0048806	Genitalia development	0.0212
GO:0016064[1]	Immunoglobulin mediated immune response	0.0214
GO:0050819	Negative regulation of coagulation	0.0214

GO:0043086	Negative regulation of catalytic activity	0.0224
GO:0034754	Cellular hormone metabolic process	0.0243
GO:0019724[1]	B cell mediated immunity	0.0263
GO:0051262	Protein tetramerization	0.0279
GO:0050778[1]	Positive regulation of immune response	0.0292
GO:0050776[1]	Regulation of immune response	0.0341
GO:0006633	Fatty acid biosynthetic process	0.0372
GO:0003333	Amino acid transmembrane transport	0.0389
GO:0016126	Sterol biosynthetic process	0.0416
GO:0006811	Ion transport	0.0420
GO:0006094	Gluconeogenesis	0.0474
GO:0001676	Long-chain fatty acid metabolic process	0.0474

[1]These pathways are related to immune responses.

and coincided with the appearance of anti-HEV antibodies[17-19]. These findings suggested that host immune response was responsible for the hepatocyte killing. This was followed by attempts to determine whether HEV was capable of producing a cytopathic effect in *in vitro* models. However, these efforts were largely unsuccessful due to failure of this virus to grow well in cell culture. Recent successful culture[20], though in low titers, of some genotypes 3 and 4 HEV isolates has revealed absence of cytotoxicity[21,22], providing some support to the hypothesis that liver injury in HEV infection is unlikely to be virus-mediated.

Human data on cellular immune responses during HEV infection are limited to studies on peripheral blood mononuclear cells (PBMCs). In these studies, we and others have shown proliferation of HEV-specific $CD4^+$ and $CD8^+$ cells, and increased production of various cytokines by PBMCs during acute and convalescent phases of hepatitis E[14,16]. In a study that looked at patients with different disease severities, HEV-specific T-cell responses in PBMCs were weaker in patients with FH-E than in those with uncomplicated acute hepatitis E[12]. However, observations made on circulating immune cells may not faithfully represent those in the immune cells infiltrating the liver, because of preferential localization of certain cells, including virus-specific T cell subsets, in the inflamed tissue[23,24]. Thus, there is a need to study changes in the liver tissue from patients with hepatitis E.

In the current study, we found changes in expression of genes involved in a variety of immune responses, including the innate, humoral and cellular immune pathways in liver tissue from patients with FH-E. Of these, changes in innate and humoral immune responses were also observed in FH-B, indicating that HEV infection may share some of the pathways causing liver cell injury with HBV infection. In contrast, activation of cellular immune pathways was found only in FH-E, and not in FH-B, indicating a specific role for cellular immune response in inducing liver injury during HEV infection.

In particular, we found several T-cell surface molecules such as CD2, CD3 (γ, δ and ε chains) and CD8-alpha to be up-regulated in liver tissue from patients with FH-E. Furthermore, several T-cell signalling molecules, such as Fyn, phospholipase C gamma-1, NFκB1, protein kinase C and ras-related C3 botulinum

Table 3 Results of Gene Ontology analysis (biological processes) for gene entities differentially expressed in liver tissue from fulminant hepatitis E as compared to normal liver, but not in that from fulminant hepatitis B as compared to normal liver

Gene Ontology term	*P* value
Immune system process[1]	1.67E-11
Immune response[1]	4.81E-09
Regulation of immune system process[1]	5.06E-08
Positive regulation of immune system process[1]	2.72E-06
Defense response	1.04E-05
Response to wounding	1.90E-05
Signaling	7.78E-05
Adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains[1]	2.40E-04
Adaptive immune response[1]	2.40E-04
Response to stimulus	3.53E-04
Regulation of cell activation	4.65E-04
Inflammatory response[1]	6.89E-04
Cell adhesion	0.0010
Biological adhesion	0.0010
Positive regulation of immune response[1]	0.0015
Cell activation	0.0018
Signal transduction	0.0019
Regulation of leukocyte activation[1]	0.0020
Activation of immune response[1]	0.0021
Oxidation reduction	0.0021
B cell mediated immunity[1]	0.0023
Leukocyte mediated immunity[1]	0.0026
Positive regulation of response to stimulus	0.0027
Regulation of locomotion	0.0028
Regulation of immune response[1]	0.0030
Phospholipid efflux	0.0032
Lymphocyte mediated immunity[1]	0.0032
Regulation of cell migration[1]	0.0048
Regulation of cell motility[1]	0.0048
Response to stress	0.0050
Regulation of lymphocyte activation[1]	0.0056
Regulation of cellular component movement	0.0064
Signal transmission	0.0067
Immunoglobulin mediated immune response[1]	0.0067
Signaling process	0.0067
Small molecule metabolic process	0.0083
Cholesterol efflux	0.0124
Immune effector process[1]	0.0154
Negative regulation of apoptosis	0.0161
Positive regulation of cell activation	0.0176
Positive regulation of leukocyte activation[1]	0.0176
Regulation of response to stimulus	0.0177
Negative regulation of cell death	0.0179
Negative regulation of programmed cell death	0.0179
Regulation of lipid metabolic process	0.0180
Signaling pathway	0.0206
Acute inflammatory response[1]	0.0241
Phospholipid transport	0.0257
Regulation of fatty acid metabolic process	0.0273
Alcohol metabolic process	0.0282
Cholesterol transport	0.0310
Sterol transport	0.0472

[1]These pathways are related to immune responses.

Table 4 Pathways whose genes were found to be over-represented among entities differentially expressed in both fulminant hepatitis E and fulminant hepatitis B, and in fulminant hepatitis E but not in fulminant hepatitis B (derived using BioCarta through DAVID resource)

BioCarta pathway	Gene count	*P* value
In both FH-B *vs* normal, and FH-E *vs* normal		
Complement pathway	10	0.0002
Intrinsic prothrombin activation pathway	10	0.0002
Nuclear receptors in lipid metabolism and toxicity	13	0.0022
Alternative complement pathway	6	0.0069
Extrinsic prothrombin activation pathway	6	0.0113
Lectin induced complement pathway	6	0.0172
Classical complement pathway	6	0.0172
Acute myocardial infarction	6	0.0248
Vitamin C in the brain	4	0.0385
CBL mediated ligand-induced downregulation of epidermal growth factor receptors	5	0.0543
Catabolic pathways for methionine, isoleucine, threonine and valine	3	0.0955
In FH-E *vs* normal but not in FH-B *vs* normal		
T cytotoxic cell surface molecules	7	0.0005
T helper cell surface molecules	6	0.0043
Cytotoxic T lymphocyte mediated immune response against target cells	7	0.0022
Co-stimulatory signal during T-cell activation	7	0.0085
Role of epidermal growth factor receptor transactivation by G protein-coupled receptors in cardiac hypertrophy	6	0.0092
T cell receptor signaling pathway	10	0.0049
Lck and Fyn tyrosine kinases in initiation of T cell receptor activation	5	0.0187
Interleukin-7 signal transduction	5	0.0446
Mitochondrial carnitinepalmitoyltransferase system	3	0.0529
T cell receptor and CD3 complex	3	0.0529
Interleukin-17 signaling pathway	5	0.0562

toxin substrate (rac) were also overexpressed in these livers. These findings may indicate a role for activated $CD8^+$ T cells in the causation of liver injury in HEV infection. An over-expression of CTLA4, which is expressed only on activated T cells, also supports this hypothesis. An alternative explanation for the overexpression of CD2 in liver tissue could be the presence of this marker on the NK cells. Importantly, the increased expression of CD2 in the liver tissue was also associated with over-expression of perforin, the main cytolytic protein contained in the $CD8^+$ T cells and NK cells. The above findings, taken together, indicate that immune-mediated cytotoxicity of $CD8^+$ or NK cells against virus-infected cells may play a role in producing liver damage in HEV infection.

Some other findings in our study may also point to alterations in $CD8^+$ cells. In viral infections, the number of effector CD8 T cells contracts over time with the formation of a population of protective memory cells, which is maintained by IL-7 and IL-15. Our finding of activation of IL-7-mediated signalling in patients with hepatitis E possibly reflects this phenomenon. In addition, the genes for signal transducer and activator of transcription 5 (STAT5), a key molecule involved in the survival of effector and memory CD8 cells[25], and for BCL2, a key survival molecule that is upregulated by STAT5, were overexpressed in FH-E.

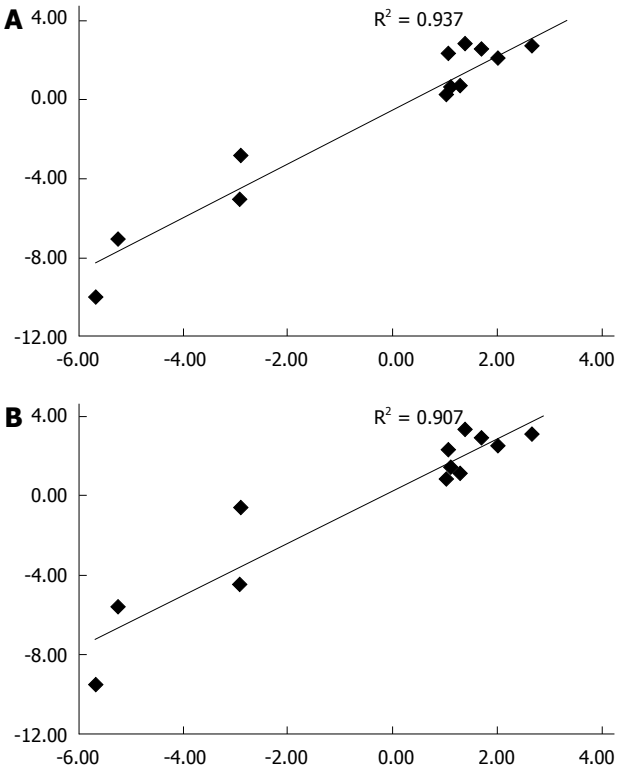

Figure 3 Relationship of fold-change in RNA expression of selected genes in patients with fulminant hepatitis E and fulminant hepatitis B on real-time polymerase chain reaction with fold-change in RNA expression of the same genes on microarray analysis. A and B show the data when reverse transcription polymerase chain reaction results were normalized using the GAPDH gene and 18S rRNA gene as housekeeping gene, respectively.

The differential expression in livers from patients with FH-E of several genes which were unaffected in FH-B could have been because of changes in any of the several cell types present in the liver, including hepatocytes, Kupffer cells, cholangiocytes, endothelial cells, stellate cells and a variety of immune cells. However, the demonstration of predominant infiltration with $CD8^+$ T cells in the FH-E livers in the current study as well as in two previous reports[26,27], and the fact that the genes that showed differential expression are not expressed much in other cell types, indicate the $CD8^+$ T cells were the most likely source. It may be pertinent to note that since the immune cells constitute only a minority of all the cells in the liver, the absolute gene expression changes within the intrahepatic immune cells must be more marked than is indicated by the overall gene expression data. It may thus be interesting in future to undertake studies on such immune cells after recovering these using techniques such as laser dissection microscopy.

It may be pertinent to compare our results with those from a recent study of gene expression in serial

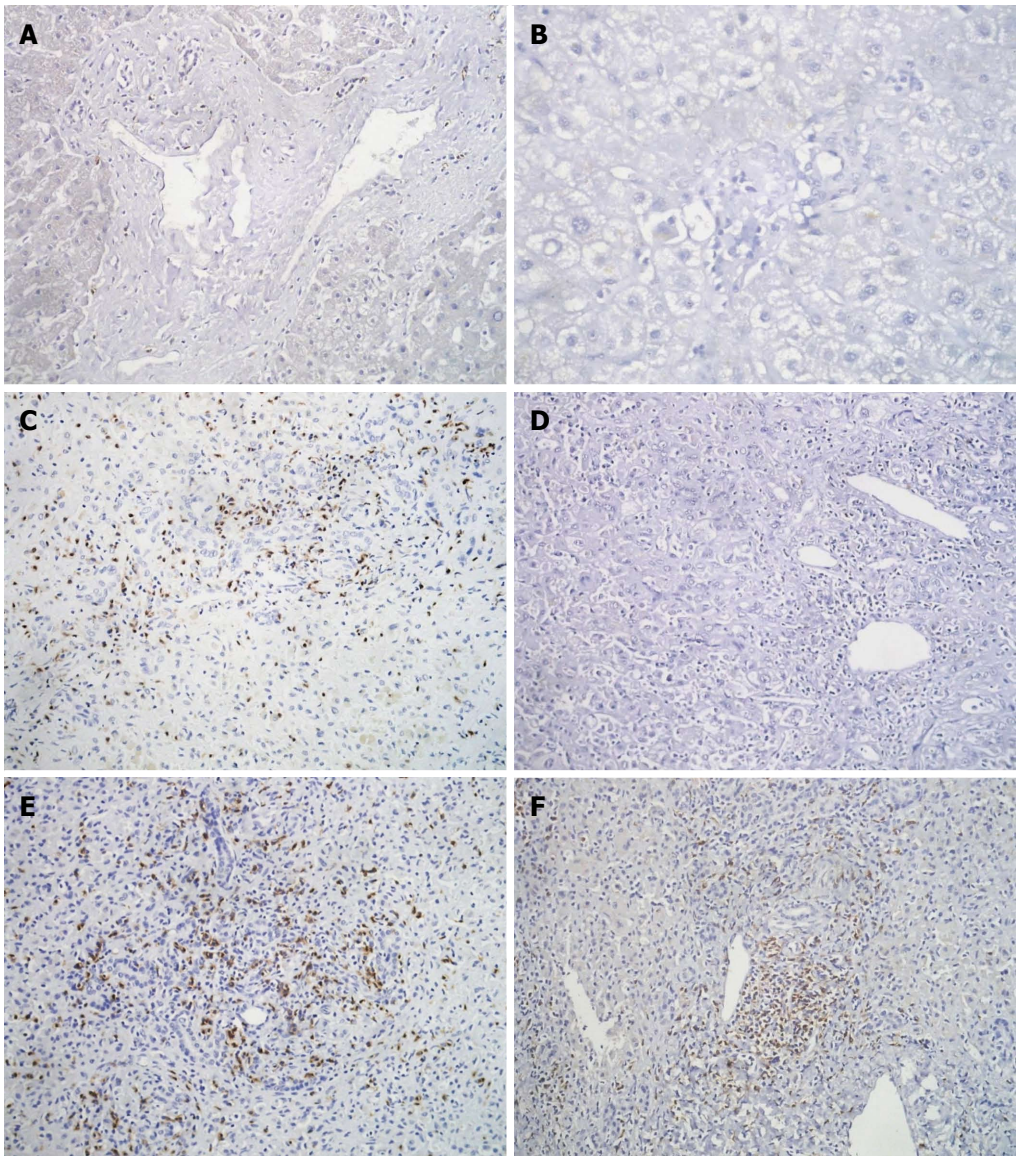

Figure 4 Immunohistochemistry for CD8$^+$ cells in liver biopsies shows occasional cytotoxic T-cells in portal tracts of controls (A), and moderate infiltration in HBV (C) and HEV (E); immunostaining for CD4 shows absence of helper T-cells in portal areas of control (B), an occasional cell in HBV (D) and some small aggregates of helper T-cells in HEV (F). A and B: Diaminobenzidine × 400; C-F: Diaminobenzidine × 200. HBV: Hepatitis B virus; HEV: Hepatitis E virus.

liver tissues from chimpanzees with experimental HEV infection[28]. In this study, differential expression was limited to a few genes that belonged predominantly to the innate immune response pathways, and was weaker than that observed in chimpanzees with HCV infection. Further, the number of upregulated genes peaked sooner after the onset of viremia in HEV infection than with HCV infection. We did find altered expression of some genes involved in innate immune responses; however, these genes were different from those showing significant changes during HEV infection in the chimpanzee study. In this context, it is important to note that comparison of our data with those from the experimentally-infected chimpanzees may not be valid. Our patients had severe liver disease, whereas experimental HEV infection in primates is milder[19,29]. Further, time-kinetics of different types of immune responses vary, with innate immune responses being prominent during the initial phase after viral infection; thus, in human HEV infection, such responses may occur before an infected person becomes symptomatic and hence not picked up. Also, the experimental animals had been inoculated by the intravenous route, whereas humans acquire infection through the oral route.

Though we studied patients with FH-B primarily as disease controls, it may be interesting to compare liver in this condition with healthy livers. Livers from patients with FH-B showed infiltration with CD8$^+$ cells, but no differential expression of genes belonging to cellular immune response pathways. Previous gene expression data in this disease are available from only two Italian patients[30,31]. These patients too showed prominent CD8$^+$ T cell infiltration with little change in expression of genes associated with T cell activity and a prominent upregulation of B cell response, similar to

our observations.

Interestingly, though liver tissues from both FH-E and FH-B in our study showed prominent $CD8^+$ T cell infiltration, only FH-E was associated with a cytotoxic T cell transcriptional signature *e.g.,* increased expression of perforin. This suggests that the infiltrating $CD8^+$ T cells in the two diseases behave quite differently. This may hold the key to pathogenesis of liver injury in HEV infection.

We also found reduced expression of several genes associated with metabolic, hemostatic and complement pathways in liver tissue from FH-E as well as FH-B. Liver is a metabolically active organ which produces several body proteins. Since FH, irrespective of its cause, is characterized by marked destruction of hepatocytes, reduction in the expression of these proteins was thus expected in both FH-E and FH-B, as a consequence of the massive liver injury.

Our data are limited by a small sample size, the use of post-mortem tissue, and of patients with severe liver injury at only one time-point. These limitations are related to the fact that liver biopsy, being invasive, is ethically unacceptable in patients with acute liver disease, forcing us to use tissue collected immediately post-mortem. Though our findings may not be entirely applicable to acute uncomplicated hepatitis E, these should be seen in light of the current absence of any human data on immune events in the liver in acute hepatitis E. Further, we compared our data to those from a control group of post-mortem biopsies from FH-B, which would have been susceptible to similar artefacts as those in FH-E.

In conclusion, liver tissue from our patients with FH-E showed infiltration with $CD8^+$ T cells and overexpression of genes involved in T cell immune responses, especially those related to T cell activation, cytotoxicity and IL-7 signalling. The latter changes were not observed in FH-B, and hence were specific to hepatitis E. These data suggest that the severe liver damage in FH-E is mediated by the host T-cell immune response. Further work on this aspect should help us better understand the pathogenesis of liver injury in hepatitis E.

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COMMENTS

Background

Infection with hepatitis E virus is the most common cause of acute hepatitis in the world. The mechanism of liver injury in this disease and its pathogenesis are yet fully understood. A better understanding of these may allow attempts at therapeutic intervention in patients with this disease.

Research frontiers

Microarray techniques allow study of expression of several genes in tissues from patients with a particular disease and its comparison with those from healthy controls and persons with similar diseases. These techniques have been applied for the study of pathogenesis of several diseases.

Innovations and breakthroughs

The authors used microarray techniques to determine the expression of various genes in the liver tissues from patients dying of hepatitis E and compared this to that in healthy liver tissue (control tissue) and liver tissues from patients dying of a disease with similar morphologic changes but caused by infection with another virus (hepatitis B; disease controls). The data showed several differences in gene expression between these groups. In particular, livers of patients with fulminant hepatitis E, but not those of hepatitis B, showed activation of several immune response pathways, particularly those involving cytotoxic T cells. This difference was observed even though tissues from both hepatitis B and E showed infiltration with cytotoxic T cells.

Applications

All data suggest that immune cells may play a role in the pathogenesis of hepatitis E, though further work is required in this regard.

Peer-review

Minor improvements, such as addition of statistical methods to the manuscript.

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ORIGINAL ARTICLE

Basic Study

Contrast-enhanced micro-computed tomography using ExiTron nano6000 for assessment of liver injury

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Abstract

AIM: To explore the potential of contrast-enhanced computed tomography (CECT) using ExiTron nano6000 for assessment of liver lesions in mouse models.

METHODS: Three mouse models of liver lesions were used: bile duct ligation (BDL), lipopolysaccharide (LPS)/D-galactosamine (D-GalN), and alcohol. After injection with the contrast agent ExiTron nano6000, the mice were scanned with micro-CT. Liver lesions were evaluated using CECT images, hematoxylin and eosin staining, and serum aminotransferase levels. Macrophage distribution in the injury models was shown by immunohistochemical staining of CD68. The *in vitro* studies measured the densities of RAW264.7 under different conditions by CECT.

RESULTS: In the *in vitro* studies, CECT provided specific and strong contrast enhancement of liver in mice. CECT could present heterogeneous images and

densities of injured livers induced by BDL, LPS/D-GalN, and alcohol. The liver histology and immunochemistry of CD68 demonstrated that both dilated biliary tracts and necrosis in the injured livers could lead to the heterogeneous distribution of macrophages. The *in vitro* study showed that the RAW264.7 cell masses had higher densities after LPS activation.

CONCLUSION: Micro-CT with the contrast agent ExiTron nano6000 is feasible for detecting various liver lesions by emphasizing the heterogeneous textures and densities of CECT images.

Key words: Micro-computed tomography; ExiTron Nano6000; Liver injury

Core tip: Noninvasive methods have been extensively studied for examining injuries in small animals in preclinical research. Contrast-enhanced computed tomography (CECT) with ExiTron nano6000 could detect various liver lesions by emphasizing the heterogeneous textures and densities of CECT images. The phenomenon is probably due to the changes in macrophage distribution, number, and function.

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INTRODUCTION

Small animal models have significantly contributed to the study of liver lesions. Mouse models of liver lesions are frequently limited by difficulties in monitoring disease progression in a longitudinal and noninvasive manner. The assessment of liver lesions during autopsy is time consuming and unfavorable for the principles of animal welfare. Noninvasive methods, including micro-computed tomography (micro-CT), magnetic resonance imaging, positron emission tomography, and ultrasound, have been extensively studied for examining injuries in small animals and are widely used for the diagnosis of organ or tissue damage in clinics[1-4]. Micro-CT is the best noninvasive method used in preclinical research of animal models because of its excellent spatial resolution[5]. Initially, micro-CT was implemented in the evaluation of bones, implants and other high-contrast structures because of its poor soft tissue contrast[6,7]. With the development of contrast agent and X-ray detector sensitivity, micro-CT has been facilitated to enable the imaging of soft tissues and vessels[8-11]. Many studies have explored the micro-CT system in the evaluation of liver lesions. Micro-CT was primarily used to detect tumor lesions[12-14], but recent studies have applied micro-CT to distinguish other types of liver lesions. The degree of liver fibrosis in small animals has been successfully estimated by micro-CT[15]. Choukèr *et al*[16] reported that Contrast-enhanced computed tomography (CECT) with the contrast agent Fenestra VC was available to monitor and localize liver ischemic reperfusion (IR) injury in a murine model. ExiTron nano6000 is a novel liver- and spleen-specific contrast agent that can be administered at a low dosage (100 μL per mouse) and has been shown to be an effective and long-term contrast for detecting liver metastatic tumors[17,18]. ExiTron nano6000 is primarily taken up by cells in the reticuloendothelial system (RES), including macrophages, which are distributed extensively in the liver as Kupffer cells. Liver lesions could influence the distribution and function of macrophages within the liver. Most studies involving micro-CT have focused on either the imaging of injuries or the characteristics of contrast agents, such as the time course. However, the detailed imaging mechanism has not been discussed. Here, micro-CT with the contrast agent ExiTron nano6000 was applied to assess three types of liver lesions (other than tumor burden): bile duct ligation (BDL), LPS/D-galactosamine (D-GalN), and alcohol. We discuss the related mechanisms of these treatments through the distribution, number, and functional changes of macrophages, which are the major cells that take up ExiTron nano6000.

MATERIALS AND METHODS

Animal care and use

The animal protocol was designed to minimize pain or discomfort to the animals. The animals were acclimatized to laboratory conditions (23 ℃, 12 h/12 h light/dark, 50% humidity, *ad libitum* access to food and water) for 2 wk prior to experimentation. Intragastric gavage administration was carried out with conscious animals, using straight gavage needles appropriate for the animal size (15-17 g body weight: 22 gauge, 1 inch length, 1.25 mm ball diameter). All animals were euthanized by pentobarbital (50 mg/kg) for tissue collection.

Animal models

Male C57BL/6 mice (8-10 wk old, weight range 20-25 g) were obtained from the Department of Laboratory Animal Science of Shanghai Jiao Tong University School of Medicine. Induction of cholestatic liver lesions was performed in age-matched male mice (n = 5 per group) by ligating the common bile duct (BDL). The mice were anesthetized *via* intraperitoneal injection of pentobarbital (50 mg/kg). After making the abdominal midline incision, the common bile duct

was ligated with 8-0 nylon sutures and transected between the ligatures. The control animals underwent sham operations, whereby the common bile duct was exposed without ligation. Several drops of bupivacaine were applied on the suture line after the muscle layer was closed before closing of the skin wound. These efforts were designed to minimize the suffering of the mice. For fulminant liver lesions, the mice were administered an intraperitoneal injection of D-GalN (700 mg/kg) and *Escherichia coli* lipopolysaccharide (LPS, 10 μg/kg), and the control group received an identical volume of phosphate-buffered saline (PBS) (n = 5 per group). For alcohol-induced liver lesions, the mice received one dose of alcohol (5 g/kg body weight, diluted 25:75 vol:vol in water) by gavage (n = 5) once daily for four consecutive days. The mice were permitted water and standard pelleted feed during alcohol administration.

Contrast agent and micro-CT images of mice model

ExiTron nano6000 (130-095-146; Miltenyi Biotec) is an alkaline earth metal-based nanoparticulate contrast agent specifically formulated for preclinical CT. It shows strong X-ray absorption due to the high metal load of the particles. Approximately 100 μL of this contrast agent was injected into the tail vein of the mice 4 h before the micro-CT procedure, as previously described, because the density in the liver would reach the highest contrast levels at 4 h after ExiTron nano6000 injection; this effect can last for many days. Upon intravenous injection, ExiTron nano6000 circulates in the bloodstream and is taken up by Kupffer cells (liver macrophages). After ExiTron nano6000 injection, serial micro-CT images of the mice were obtained to observe the macrophage-rich liver. The parameters of the micro-CT scans were as follows: tube voltage: 80 kV; tube current: 0.45 mA; number of views: 400; exposure time: 400 ms; detector bin mode: 2 × 2; and effective pixel size: 0.045 mm. The total scan time was about 20 min for the liver. Analysis of the reconstructed images was performed using Launch GEHC Micro View.

Liver enzyme chemistry and histological analysis

Blood was collected from the retro-orbital sinus to determine the serum alanine aminotransferase (ALT) activity using the Infinity ALT Liquid Stable Reagent (Thermo Fisher Scientific) on a spectrophotometer. The liver tissues were removed from a portion of the left lobe and fixed immediately in 10% neutral-buffered formalin, subsequently dehydrated, and embedded in paraffin. The formalin-fixed and paraffin-embedded tissues were cut serially into 5-μm sections and stained with hematoxylin and eosin (HE). Distribution of the macrophages was detected by immunohistochemistry against CD68 (Gene Tech, Shanghai, China)[19]. After deparaffinization and rehydration, the sections were soaked in 10 mmol/L citrate buffer (pH 6.0) for antigen retrieval. To block endogenous peroxidase, the sections were placed in 3% H_2O_2 for 5 min and then washed with PBS. The slides were blocked with 10% normal goat serum for 10 min at 37 ℃ and incubated overnight at 4 ℃ with primary antibody. After rinsing with PBS, the sections were incubated with a horseradish-peroxidase-conjugated secondary antibody (Changdao, Shanghai, China) for 30 min at room temperature and then stained with 3,3'-diaminobenzidine (DAB; Maixin-Bio, Guangzhou, China). Hematoxylin was applied for the nuclear staining. Five fields in each liver sample were randomly selected for observation.

Cell culture and micro-CT images of the RAW264.7 cell mass

The murine macrophage cell line RAW264.7, a murine macrophage cell line[20], was kindly provided by Dr. X. Ma (Key Laboratory of Gastroenterology and Hepatology, Ministry of Health, Shanghai Jiao-Tong University, Shanghai, China). The cells were cultured in DMEM supplemented with 10% FBS, 10 mmol/L L-glutamine, 100 U/mL of penicillin, and 0.1 mg/mL of streptomycin (all purchased from Invitrogen Life Technologies, Carlsbad, CA, United States), at 5% CO_2 and 37 ℃. The cells were plated in 60-mm dishes at a density of 0.3 × 10^6/mL 1 d before stimulation. The cells were stimulated with 1 μg/mL LPS (Sigma, China) for 12 h followed by co-incubation with ExiTron nano6000 (1:500, vol:vol in DMEM) for 4 h. For the micro-CT images, the cells were washed three times with PBS buffer. Then, the cells were trypsinized and centrifuged at 600 rpm for 5 min to wash out any unendocytosed contrast agent. The cells was resuspended with 1 mL PBS buffer, transferred to 1.5 mL Eppendorf tubes, and centrifuged at 300 g for 5 min. The tubes were held on a foam board and scanned by a micro-CT imaging system with the following parameters: tube voltage, 80 kV; current intensity, 0.45 mA.

Statistical analysis

The probabilities were two-sided and expressed as the mean ± SD. The data were analyzed with Student's t test. We conducted the statistical analysis with SPSS version 19.0 software. We considered values of P < 0.05 as statistically significant.

RESULTS

Evaluation of liver lesions with CECT

ExiTron nano6000 as a non-toxic and targeted agent to the liver: The mice showed no observable adverse events or abnormal behavior after injection with ExiTron nano6000[17]. As shown in Figure 1, ExiTron nano6000 provided specific and strong contrast enhancement of the micro-CT images of the liver and spleen.

CECT images in cholestasis: CECT was first performed on the BDL-treated mice, which are extensively used as

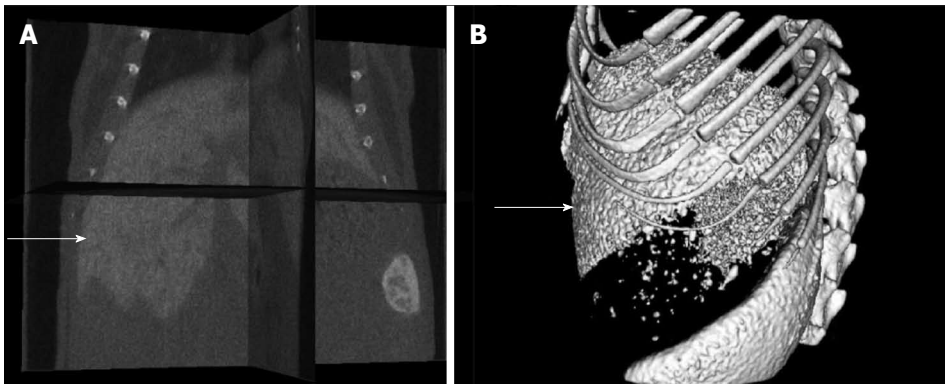

Figure 1 Contrast enhanced computed tomography images of a normal liver. A: 3D image of a liver (arrow); B: Perspective view of a liver (arrow).

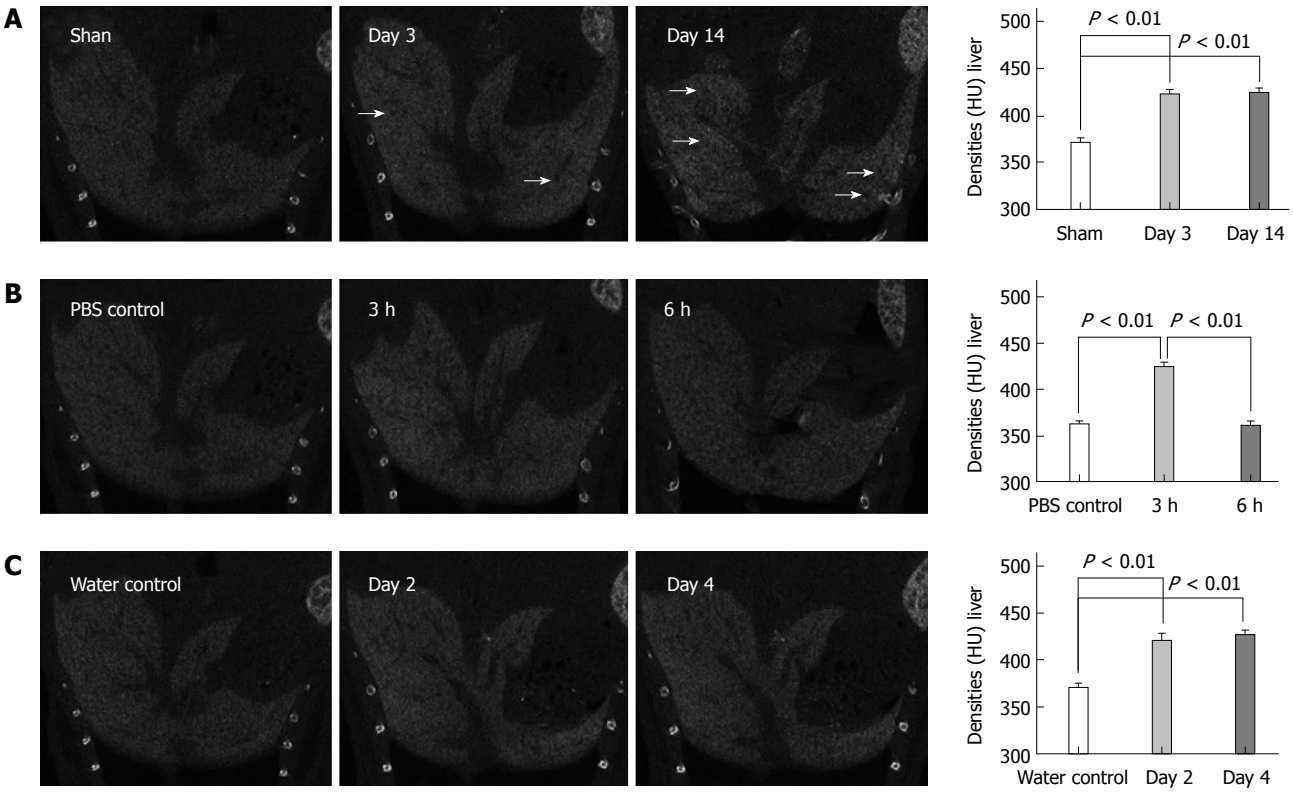

Figure 2 Contrast-enhanced computed tomography images of liver lesions induced by bile duct ligation (A), lipopolysaccharide/D-GalN (B) and alcohol (C); density measured in the livers are reported as HU. The black arrows indicate black regions with low densities. Values are represented as the means of triplicate values and presented as the mean ± SD.

a cholestasis model. On day 3 after BDL treatment, the texture of the liver became heterogeneous and black regions appeared. The liver density significantly increased compared to that of the sham controls (422.7 ± 7.8 HU *vs* 374.7 ± 11.4 HU, respectively; $P < 0.001$). On the day 14 after BDL treatment, the texture became more heterogeneous, whereas the black regions became more extensive and larger in the CECT images. Similarly, the liver density was significantly greater compared to that of the sham controls (423.7 ± 8.3 HU *vs* 367.7 ± 7.8 HU, respectively, $P < 0.001$) (Figure 2A).

CECT applied in LPS/D-GalN and alcohol-induced liver lesions: We performed CECT imaging of LPS/D-GalN-induced liver lesions. The CECT images in Figure 2B show that the texture of the liver became increasingly more heterogeneous, and the black regions became more numerous with the advancement of LPS/D-GalN-induced liver lesions. The liver density showed an up-down trend in that it increased in the early period and decreased in the advanced stages of liver lesions (423.7 ± 8.5 HU for 3 h injury *vs* 365.0 ± 7.6 HU for PBS control, respectively, $P < 0.001$; 360.7 ± 6.7 HU for 6 h injury *vs* 360.0 ± 7.2 HU

for PBS control, respectively, $P = 0.91$). For acute alcohol-induced liver lesions, Figure 2C shows that the predominant changes were the increased density of the injured liver compared to that of the sham controls (420.7 ± 11 HU for 2 d injury *vs* 363.3 ± 8.3 HU for water control, respectively, $P < 0.001$; 426.7 ± 8.0 HU for 4 d injury *vs* 377.0 ± 9.0 HU for water control, respectively, $P < 0.001$).

Morphological changes influencing the texture of the CECT images: Histology was performed to identify the morphological changes related to the texture of the CECT images, and the serum ALT activities were measured to examine physiologically the extent of the liver lesions. For cholestasis, HE staining showed an increasing bile infarct, dilated biliary tract, and portal liver inflammation with the advancement of cholestasis (Figure 3A). Because ExiTron nano6000 is predominantly taken up by cells of the RES (particularly macrophages within the liver), we studied the effects of liver lesions on the distribution of macrophages in the liver by staining for CD68[21,22]. This staining showed that macrophages were not present in the area of either the bile infarct or the dilated biliary tract (Figure 3B).

For animals subjected to LPS/D-GalN-induced liver lesions, HE staining primarily showed slight hepatic necrosis accompanying inflammatory cell infiltration at 3 h, and displayed massive necrosis and destruction of the hepatic architecture at 6 h (Figure 3A), which correlated with the serum ALT activities (Figure 4). No macrophages were present in the area of necrosis in CD68-immunostained samples (Figure 3B). For animals subjected to alcohol-induced liver lesions, periportal microvesicular steatosis was observed in the histological examination, and there was no significant change in the distribution of macrophages (Figure 3B).

These results suggest that CECT using ExiTron nano6000 could identify liver lesions such as necrosis and dilated biliary tract by monitoring the distribution of macrophages, which cause various CECT liver textures.

Number and function of macrophages influencing tissue density

Based on the *in vivo* observations described above, we attempted to determine a relationship between the CECT densities of various injured livers and the recruitment of macrophages to the injured livers. Figure 5A shows that the livers from mice subjected to LPS/D-GalN-induced injury for 6 h had significantly fewer macrophages compared to those of the PBS control. Other injuries did not induce any significant changes in the number of macrophages in the livers (Figure 5A), which is consistent with previous studies[23,24]. We speculated whether liver lesions could improve the endocytotic function of macrophages regarding ExiTron nano6000 uptake and affect the observed increased densities of the injured liver.

We performed CECT of RAW264.7 cells in different states (*i.e.*, quiet or LPS-activated) to confirm the endocytotic ability of macrophages to take up ExiTron nano6000. The results showed that the density of the RAW264.7 cell masses increased significantly after co-incubation with ExiTron nano6000 (135.0 ± 12.8 HU *vs* -37.00 ± 11.4 HU, respectively, $P < 0.001$). The LPS-activated RAW264.7 cells had a higher density compared to the quiet RAW264.7 cells (184.7 ± 11.0 HU *vs* 135.0 ± 12.8 HU, respectively, $P < 0.01$) indicating a significant accumulation of ExiTron nano6000 in the LPS-treated RAW264.7 cells (Figure 5B). Besides, hepatocytes were not found to have endocytotic ability when CECT images were performed on the HepG2 cells (data not shown). We concluded that the CECT liver densities were positively correlated with the number and function of macrophages in the liver.

DISCUSSION

Noninvasive detection has become an attractive field in preclinical translational studies[25-28]. For small animals, a contrast agent is necessary for micro-CT to improve the imaging of soft tissue[29]. By contrasting macrophages, studies have noninvasively investigated macrophage-rich injuries of soft tissues such as atherosclerotic plaques[30,31]. Our study has shown that micro-CT using the ExiTron nano6000 contrast agent could detect liver lesions induced by BDL, LPS/D-GalN and alcohol.

Based on the CECT images obtained, ExiTron nano6000 successfully targets and highlights the morphology of the macrophage-rich liver. Extrahepatic cholestasis is a common liver disease and can be caused by diseases including choledocholithiasis and pancreatic disease. BDL-induced liver lesions is a classic model for studying extrahepatic cholestasis and related liver lesions[32]. In this experiment, CECT showed dark regions that were increased in number and size with the advancement of cholestasis after BDL. Because CECT predominantly shows the areas rich in macrophages (due to ExiTron nano6000 uptake), the dark regions are suggestive of areas that are deficient in macrophages. The increased density of liver after BDL is also an important of indication of cholestatic liver lesions.

LPS/D-GalN-induced liver lesions are a useful model for studying fulminant liver failure[33]. CECT images show a more heterogeneous texture and expanded sporadic black regions with the progression of liver lesions. The liver density in the CECT images significantly increased at 3 h after LPS/D-GalN treatment and then returned to baseline at 6 h. We hypothesize that the enlarged necrotic area accounts for the low density of the liver at 6 h after LPS/D-GalN injection. For the alcohol-induced liver lesions model, the primary change in the CECT images was increased

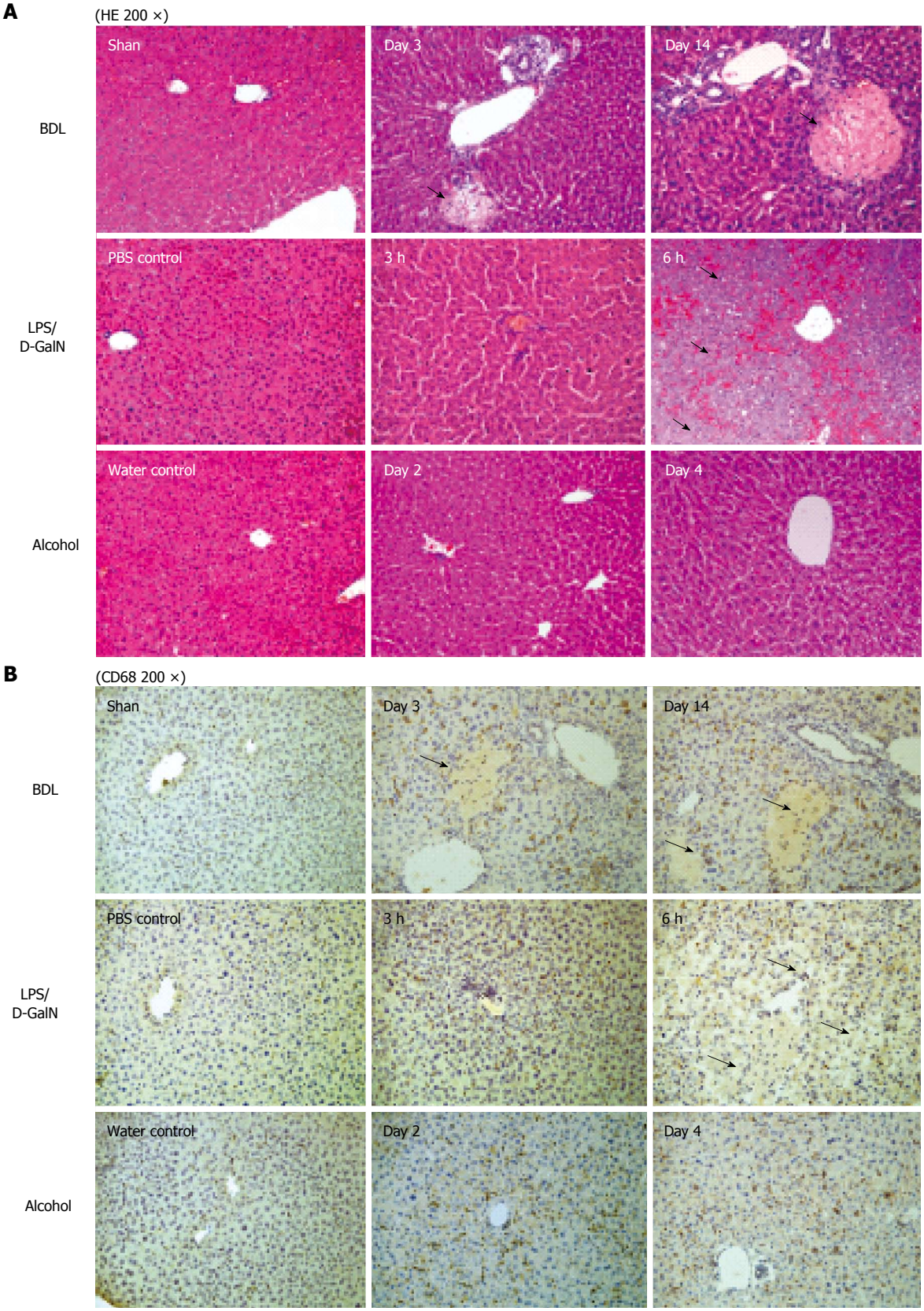

Figure 3 HE stains for liver lesions induced by bile duct ligation, lipopolysaccharide/D-GalN and alcohol (A); Immunostaining of CD68 after liver lesions induced by bile duct ligation, lipopolysaccharide/D-GalN or alcohol (B). The black arrows indicate necrosis, and the white arrows indicate dilated biliary tracts.

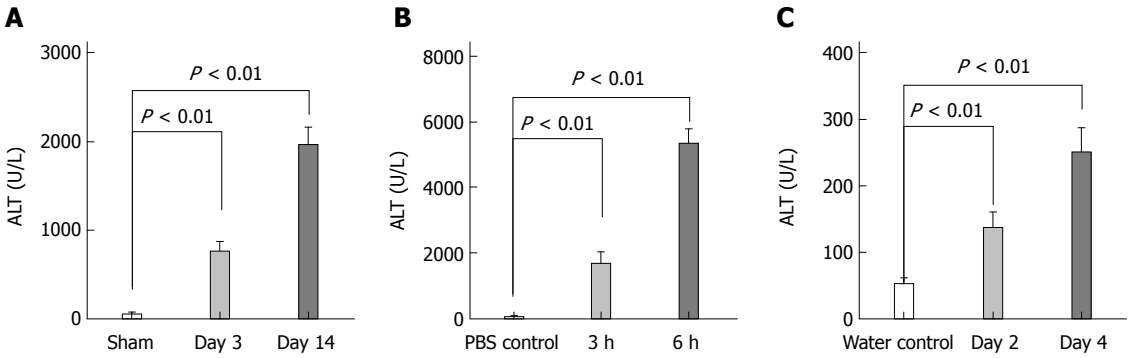

Figure 4 ALT levels in mouse models induced by bile duct ligation (A), lipopolysaccharide/D-GalN (B) and alcohol (C), $^{b}P < 0.01$.

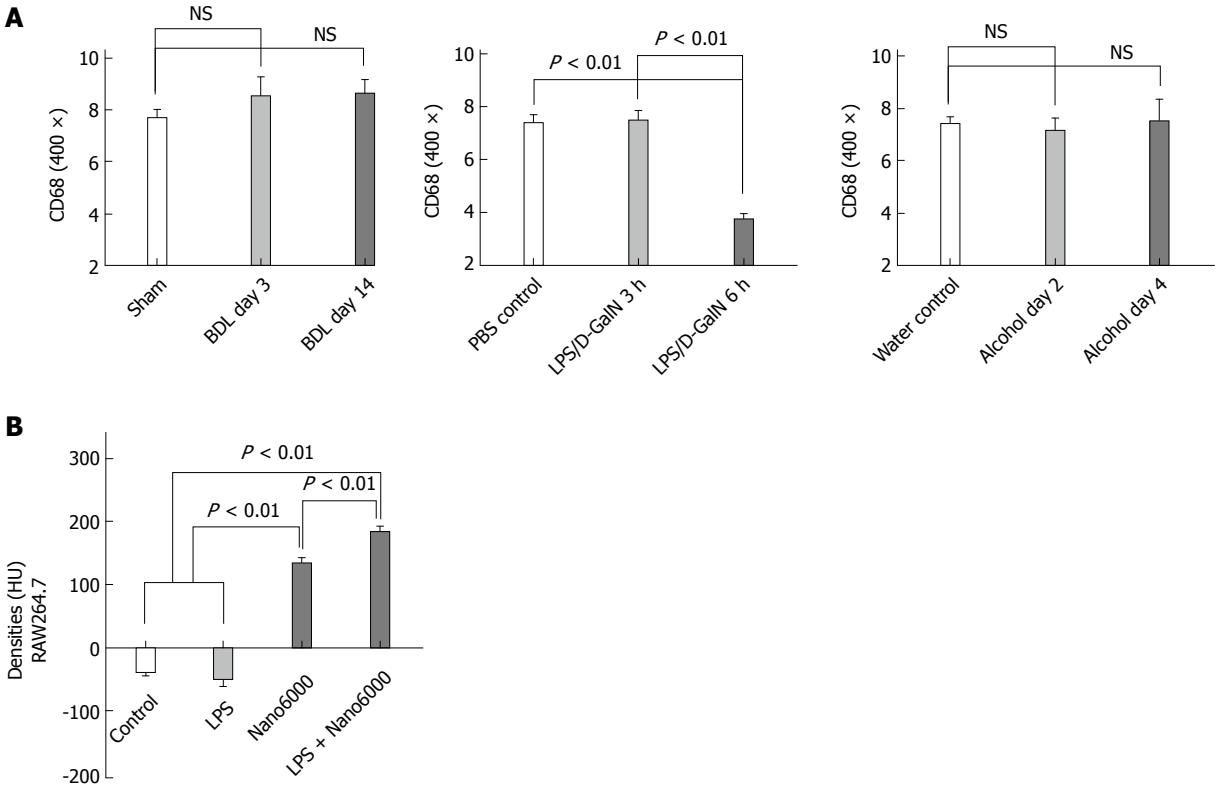

Figure 5 Comparison of the number of CD68+ cells in the injured livers of the three models (A); comparison of the densities of RAW264.7 cell mass co-cultured with nano6000, lipopolysaccharide or both (B). Values are represented as the means of triplicate values and presented as the mean ± SD.

liver density.

Next, we compared the CECT images and the pathological results, which is the gold standard for the assessment of liver lesions. Comparison demonstrated that the increased density of the CECT images was nearly completely consistent with the pathological results; however, the increased density decreased significantly upon with serious liver lesions such as at 6 h after LPS/D-GalN injection.

Because the macrophages within the liver represent a major cell type that takes up ExiTron nano6000, we conducted an additional study of the pathology to explore the effect of liver lesions on the distribution and number of macrophages within the liver. By comparing HE and CD68 staining, we determined that various types of liver lesions could cause the heterogeneous distribution of macrophages instead of changing their numbers. Macrophages were absent in the necrotic area or dilated biliary tract, whereas no significant changes were observed in the healthy regions. By comparing the pathological findings with the CECT images, we concluded that the heterogeneous distribution of macrophages contributed to the heterogeneous texture of the injured livers in the CECT images, and the increased sporadic black regions in the CECT images indicated the areas of either necrosis or dilated biliary tracts.

The unchanged number of macrophages within the injured livers prompted us further to examine changes in macrophage function. The endocytotic ability of

macrophages has been shown to increase significantly after activation[34]. To understand the relationship between the endocytotic function of macrophages for ExiTron nano6000 and liver lesions, we performed an *in vitro* study using LPS to stimulate RAW246.7 cells[35,36]. The RAW246.7 cell mass presented a significantly increased density on the CECT images after co-incubation with ExiTron nano6000, which confirmed the ability of macrophages to take up this contrast agent. LPS could activate the Toll-like receptor 4 pathway, which is a classic pathway of macrophage activation in various liver lesions. Consistent with our observations, the LPS-activated RAW246.7 cells had increased densities on the CECT images compared to the nonactivated cells. The results of this *in vitro* study demonstrated that macrophages could be activated by liver lesions and were responsible for the increased density of the injured livers on CECT imaging. These *in vitro* studies suggest that the endocytotic ability of macrophages was activated by liver lesions and resulted in increased density of the injured livers, as observed on CECT imaging.

In conclusion, we demonstrated that micro-CT in conjunction with the ExiTron nano6000 contrast agent could provide specific liver imaging without adverse reactions. CECT was able to detect liver lesions objectively based on the texture and density alterations of the CECT image; these alterations were caused by variations in macrophage distribution, number and function. Besides, ExiTron nano6000 and other contrast agents could also provide pronounced contrast for imaging of adrenal glands, vascular structures or other tissues of interest. All of these suggest that the use of micro-CT could be further expanded in future applications.

COMMENTS

Background

Recently, micro-computed tomography (CT) and many other noninvasive methods have been extensively used to detect liver lesions due to the defects of autopsy, such as being time consuming and unfavorable for the principles of animal welfare. ExiTron nano6000 is an alkaline earth metal-based nanoparticulate contrast agent specifically formulated for preclinical CT. Studies have reported that ExiTron nano6000 could be used to monitor the progress of liver tumor, but liver lesions (other than tumor burden) have not been studied and the detailed imaging mechanism remains to be discussed.

Research frontiers

Micro-CT has been widely used in preclinical small animal studies. Various contrast agents were created to detect the lesions of soft tissues such as blood vessels, brain, liver, and kidney. However, most of them focused on tumor studies. To expand the application of micro-CT are of major interest.

Innovations and breakthroughs

At first, micro-CT using ExiTron nano6000 was successfully implied to detect liver lesions induced by BDL, LPS/D-GalN and alcohol through emphasizing the heterogeneous textures and densities of CECT images. Then, the changes in macrophage distribution, number, and function of liver lesions were found to be the related mechanisms. More importantly, of the mechanisms, we are the first to discuss the role of cellular function in detecting liver lesions by CECT images.

Applications

The study results suggest that micro-CT with the contrast agent ExiTron nano6000 is a feasible method for detecting various liver lesions.

Terminology

ExiTron nano6000 is an alkaline earth metal-based nanoparticulate contrast agent specifically formulated for preclinical CT. It shows strong X-ray absorption due to the high metal load of the particles. Upon intravenous injection, ExiTron nano6000 circulates in the bloodstream and is primarily taken up by cells in the reticuloendothelial system, including macrophages, which are distributed extensively in the liver as Kupffer cells.

Peer-review

This is a well-done, thoughtful manuscript that is well written. The authors showed that micro-CT with the contrast agent ExiTron nano6000 is potentially useful for detecting various liver lesions such as alcoholic liver changes by the heterogeneous textures and densities images, depending on the distribution, number, and function of macrophages. As a reviewer, I also believe that it has the potential to provide important information about the importance of new technique for detecting different liver lesions.

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ORIGINAL ARTICLE

Basic Study

Conjugation of toll-like receptor-7 agonist to gastric cancer antigen MG7-Ag exerts antitumor effects

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Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at gyjin@szu.edu.cn. No additional data are available.

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Abstract

AIM: To investigate the effects of our tumor vaccines on reversing immune tolerance and generating therapeutic response.

METHODS: Vaccines were synthesized by solid phase using an Fmoc strategy, where a small molecule toll-like receptor-7 agonist (T7) was conjugated to a monoclonal gastric cancer 7 antigen mono-epitope (T7-MG1) or tri-epitope (T7-MG3). Cytokines were measured in both mouse bone marrow dendritic cells and mouse spleen lymphocytes after exposed to the vaccines. BALB/c mice were intraperitoneally immunized with the vaccines every 2 wk for a total of three times, and

then subcutaneously challenged with Ehrlich ascites carcinoma (EAC) cells. Three weeks later, the mice were killed, and the tumors were surgically removed and weighed. Serum samples were collected from the mice, and antibody titers were determined by ELISA using an alkaline phosphate-conjugated detection antibody for total IgG. Antibody-dependent cell-mediated cytotoxicity was detected by the lactate dehydrogenase method using natural killer cells as effectors and antibody-labeled EAC cells as targets. Cytotoxic T lymphocyte activities were also detected by the lactate dehydrogenase method using lymphocytes as effectors and EAC cells as targets.

RESULTS: Vaccines were successfully synthesized and validated by analytical high performance liquid chromatography and electrospray mass spectrometry, including T7, T7-MG1, and T7-MG3. Rapid inductions of tumor necrosis factor-α and interleukin-12 in bone marrow dendritic cells and interferon γ and interleukin-12 in lymphocytes occurred *in vitro* after T7, T7-MG1, and T7-MG3 treatment. Immunization with T7-MG3 reduced the EAC tumor burden in BALB/c mice to 62.64% ± 5.55% compared with PBS control ($P < 0.01$). Six or nine weeks after the first immunization, the monoclonal gastric cancer 7 antigen antibody increased significantly in the T7-MG3 group compared with the PBS control ($P < 0.01$). As for antibody-dependent cell-mediated cytotoxicity, antisera obtained by immunization with T7-MG3 were able to markedly enhance cell lysis compared to PBS control (31.58% ± 2.94% *vs* 18.02% ± 2.26%; $P < 0.01$). As for cytotoxic T lymphocytes, T7-MG3 exhibited obviously greater cytotoxicity compared with PBS control (40.92% ± 4.38% *vs* 16.29% ± 1.90%; $P < 0.01$).

CONCLUSION: A successful method is confirmed for the design of gastric cancer vaccines by chemical conjugation of T7 and multi-repeat-epitope of monoclonal gastric cancer 7 antigen.

Key words: Gastric cancer; Immunotherapy; Monoclonal gastric cancer 7 antigen; Toll-like receptor-7; Vaccine

Core tip: Immunization with toll-like receptor-7 agonist conjugated with a monoclonal gastric cancer 7 antigen tri-epitope was efficacious in reversing tolerance and generating a therapeutic response in Ehrlich ascites carcinoma tumor-bearing mice. This occurred by enhancing the specific humoral and cellular immunity, which were displayed as higher antibody titers, antibody-dependent cell-mediated cytotoxicity, and cytotoxic T lymphocyte activity.

Wang XD, Gao NN, Diao YW, Liu Y, Gao D, Li W, Wan YY, Zhong JJ, Jin GY. Conjugation of toll-like receptor-7 agonist to gastric cancer antigen MG7-Ag exerts antitumor effects. *World J Gastroenterol* 2015; 21(26): 8052-8060 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i26/8052.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i26.8052

INTRODUCTION

Gastric cancer is one of the most common malignancies and the second leading cause of cancer-related death worldwide. Surgery, chemotherapy, and radiotherapy are frequently used in the treatment of gastric cancer. Immunotherapy, especially tumor vaccine, has now drawn more and more attention for its advantages, such as low toxicity and long-term effect. However, due to the lack of tumor-specific antigens, few effective gastric cancer vaccines have been investigated.

Monoclonal gastric cancer 7 antigen (MG7-Ag), discovered by Dr. Fan Dai-Ming (the Fourth Military Medical University, Xi'an, China), is a tumor-associated antigen with high specificity and selectivity. MG7-Ag expression in gastric mucosa is closely associated with high risk of gastric atypical hyperplasia and malignant change, which suggests that MG7-Ag plays an important role in gastric cancer progression[1,2]. Many studies have been carried out concerning tumor prevention and treatment based on MG7-Ag, including DNA vaccines and recombinant protein vaccines[3,4]. However, weak immunogenicity of tumor-associated antigens is a drawback of tumor vaccines, which requires the addition of adjuvants for optimal performance.

Toll-like receptors (TLRs) are fundamental elements of immune system, which facilitate our understanding of the innate and adaptive immunity. The application of TLR ligands as adjuvants in vaccine development is currently under intensive investigation, as they are not only capable of protecting against infectious diseases[5,6], but also serving as a promising method for cancer treatment[7,8]. Evidence has revealed that vaccine-mediated tumor inhibition was caused by specific immunity against MUC1 and nonspecific adjuvant effects of the TLR2 agonist[9]. Among all kinds of TLR ligands, the TLR7 agonist is the only choice of small molecule synthetic compound, which is more convenient to obtain than other biomacromolecules, such as TLR4 and TLR9 ligands. The TLR7 agonist has also been reported with immunostimulatory capacity, and applied in the research of vaccines against several types of tumors, such as leukemia and breast cancer[10,11]. Imiquimod, the classic TLR7 agonist, lacks the chemical group to couple with proteins or peptides. Therefore, we adopted another well-studied TLR7 agonist (T7; chemical structure is shown in Figure 1) where its free carboxyl group can link to the amino group of peptides[12].

Here, we constructed a novel gastric cancer vaccine by covalently coupling the small molecule T7 with MG7-Ag mono-epitope (MG1; sequence used widely in

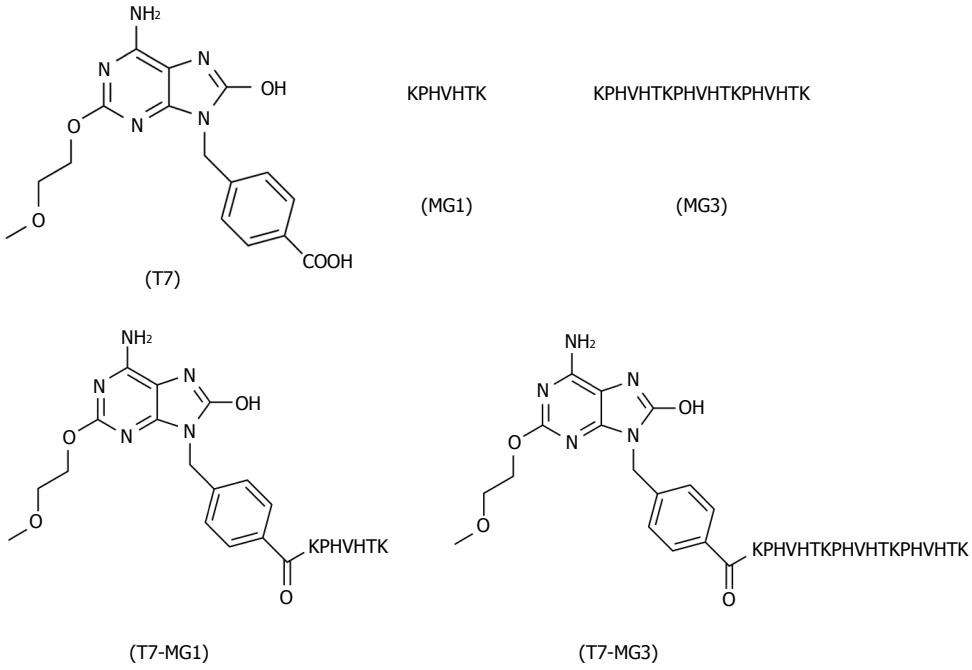

Figure 1 Chemical structures of the synthetic vaccines. MG1: Monoclonal gastric cancer 7 antigen mono-epitope; MG3: Monoclonal gastric cancer 7 antigen tri-epitope; T7: Toll-like receptor-7 agonist.

tumor vaccines), and examined its efficiency in eliciting humoral and cellular immune response, reversing tumor tolerance, and generating a protective effect. Furthermore, we designed an MG7-Ag tri-epitope (MG3; epitope sequence repeated three times) as a superantigen, and conjugated it with T7 to constitute a more potent vaccine. Our research revealed that conjugation of T7 and MG3 was critical for inducing optimal immune responses, displayed by cytokine detection, antibody titer determination, antibody-dependent cell-mediated cytotoxicity (ADCC), cytotoxic T lymphocyte (CTL) activity, and a tumor challenge assay.

MATERIALS AND METHODS

Chemical synthesis

T7 was synthesized as described before[12]. The peptides were synthesized by solid phase using an Fmoc strategy[13]. 2-Chlorotrityl chloride resin (GL BIO Company, Shanghai, China) was the solid support loaded at 1.0 mmol/g. The following N-protected Fmoc amino acids were applied as the functionalized amino acids: Fmoc-Lys(Boc)-OH, Fmoc-Thr(tBu)-OH, Fmoc-His(Trt)-OH, Fmoc-Val-OH, Fmoc-Pro-OH (GL BIO Company, Shanghai, China) and T7. For the synthesis, 2-(1 H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate was used as the coupling reagent, while each amino acid was used at eight equal molar quantities. Fmoc deprotection was carried out in a mixture of piperidine and dimethylformamide at a ratio of 2:8 (v/v). Disaggregation of the peptide from the resin was performed for 3.5 h in a solution containing trifluoroacetic acid, phenol, water, and triisprpylsilane at a ratio of 88:5:5:2 (v/v). Following addition of cold diethyl ether for precipitation, peptides were dissolved with 0.1% trifluoroacetic acid in water/acetonitrile and lyophilized. Finally, a purity of at least 95% (UV detection at 214 nm and 254 nm) in all peptides was validated by analytical high performance liquid chromatography using a C18 column (5 μm, 300 Å, 10 mm × 200 mm). Electrospray mass spectrometry was also performed to further identify the peptides.

Cytokine assays

Cytokines were measured in both mouse bone marrow dendritic cells (BMDCs) and mouse spleen lymphocytes. BMDCs were generated as described previously[14]. Briefly, bone marrow cells from femurs and tibiae of BALB/c mice were cultured at 37 ℃ for 6 d in X-vivo 15 medium (Lonza Group, Basel, Switzerland) containing granulocyte-macrophage colony-stimulating factor (GM-CSF) (10 ng/mL) and interleukin (IL)-4 (10 ng/mL). Spleen lymphocytes were isolated from BALB/c mice using Mouse Lymphocyte Separation Medium (Dakewe, Beijing, China), according to the supplier's manual. BMDCs and lymphocytes were seeded in 96-well plates at a density of 5 × 10^4 cells per well. Vaccines were added at a final concentration ranging from 0.01 μmol/L to 10 μmol/L and incubated for 24 h. Culture supernatants were collected and cytokine quantification of tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and IL-12 was performed using Mouse TNF-α, IFN-γ, and IL-12 p70 ELISA Ready-SET-Go reagent sets (eBioscience, San Diego, CA, United States), according to the supplier's

manual. Briefly, an ELISA plate was first coated with capture antibody overnight at 4 ℃, and then filled successively with block solution, culture supernatant, and detection antibody for 1 h at room temperature. Finally, substrate and stop solution were added to each well, and the optical density was measured at 450 nm with a spectrophotometer (BioTek, Winooski, VT, United States).

Immunization of mice

This study was approved by the Laboratory Animal Ethics Committee of Shenzhen University. The animal protocol was designed to minimize pain or discomfort to the animals. Female 4-wk-old BALB/c mice weighing 15-20 g were purchased from the Medical Laboratory Animal Center (Guangzhou, Guangdong, China). All mice were housed in constant laboratory conditions of a 12 h light/dark cycle and specific pathogen-free conditions, and fed with water and food *ad libitum*. After being acclimated for 1 wk, BALB/c mice were used to evaluate the immunogenicity of the vaccines, by immunizations with T7, MG1, T7+MG1 (unconjugated), T7-MG1 (conjugated), MG3, T7+MG3 (unconjugated), T7-MG3 (conjugated), and PBS as control. For these experiments, 25 μg of T7-MG3 or equal molar quantities of other vaccines were administered i.p. to each mouse every 2 wk for a total of three times.

Tumor challenge assays

After 6 wk immunizations, each BALB/c mouse was subcutaneously challenged with 1×10^7 Ehrlich ascites carcinoma (EAC) cells, and the expression of MG7-Ag was assessed by Western blot using MG7-Ag antibody (a gift from Dr. Fan Dai-Ming and Dr. Nie Yong-Zhan). Three weeks after challenge, the mice were killed, and the tumors were surgically removed and weighed.

Determination of antibody titers

After each immunization, blood samples were collected from the mice and centrifuged at 3000 *g* for 15 min to obtain serum samples. Antibody titers in serum were determined by ELISA using an alkaline phosphate-conjugated detection antibody for total IgG (Millipore Corp., Billerica, MA, United States). Briefly, an ELISA plate was coated with BSA-MG1 (peptide sequence is BSA-KPHVHTK) overnight at 4 ℃, then incubated successively with block solution for 2 h, serum samples (1:50 diluted), and detection antibody for 1 h at room temperature. Finally, p-NPP substrate (Millipore Corp.) and stop solution were added to each well, and the optical density was measured at 405 nm with a spectrophotometer (BioTek).

Determination of ADCC

At the time of sacrifice, serum samples from the mice were diluted 1:25 and incubated with EAC tumor cells for 30 min at 37 ℃. Natural killer (NK) cells, isolated from normal BALB/c mice using a Mouse NK Cell Separation Kit (Hao Yang, Tianjin, China), were used as effectors and seeded with the antibody-labeled EAC cells for 4 h at an effector-to-target cell ratio of 50:1. Cytotoxicity was measured by the lactate dehydrogenase (LDH) method using Non-Radioactive Cytotoxicity Assay (Promega Corp., Madison, WI, United States), according to the supplier's manual. Briefly, after incubation, culture supernatant was transferred to an ELISA plate, followed by the addition of substrate solution for 30 min at room temperature. Finally, stop solution was filled, and the optical density was measured at 490 nm with a spectrophotometer (BioTek).

Determination of CTL

At the time of sacrifice, lymphocytes, separated from the spleen of each mouse by Mouse Lymphocyte Separation Medium (Dakewe, Beijing, China), were used as effectors. EAC tumor cells were used as target cells and incubated with lymphocytes for 4 h at an effector-to-target cell ratio of 100:1. Cytotoxicity was also measured by the LDH method as described above.

Statistical analysis

Data are expressed as mean ± SE for the indicated number of independently performed experiments. Student's *t* test was used for the determination of statistical significance. The difference was considered to be statistically significant at $P < 0.05$. The statistical methods of this study were reviewed by Dr. Gao Kai-Ping from the School of Medicine, Shenzhen University.

RESULTS

Chemical synthesis of vaccines

T7 was synthesized as described above and used in the preparation of other vaccines (Figure 1). The following four peptides were synthesized by solid phase method: MG1 (peptide sequence is KPHVHTK), MG3 (peptide sequence is KPHVHTKPHVHTKPHVHTK), T7-MG1, and T7-MG3. T7 and MG7-Ag were also used in combination without chemical conjugation by mixing equal molar quantities of T7 and MG1 (T7+MG1) or MG3 (T7+MG3). All of above-mentioned compounds were confirmed by mass spectrometry and high performance liquid chromatography.

In vitro cytokine release by vaccines

To examine the activity on initiating the production of necessary cytokines, mouse BMDCs and spleen lymphocytes were exposed to vaccines at indicated concentrations. As shown in Figure 2A and B, the levels of two cytokines (TNF-α and IL-12) remained unchanged when the BMDCs were incubated with MG1 or MG3 alone. However, T7 displayed dose-dependent increases of TNF-α release in the range of 0.1 μmol/L to 10 μmol/L, and IL-12 release in the range of 1 μmol/L to

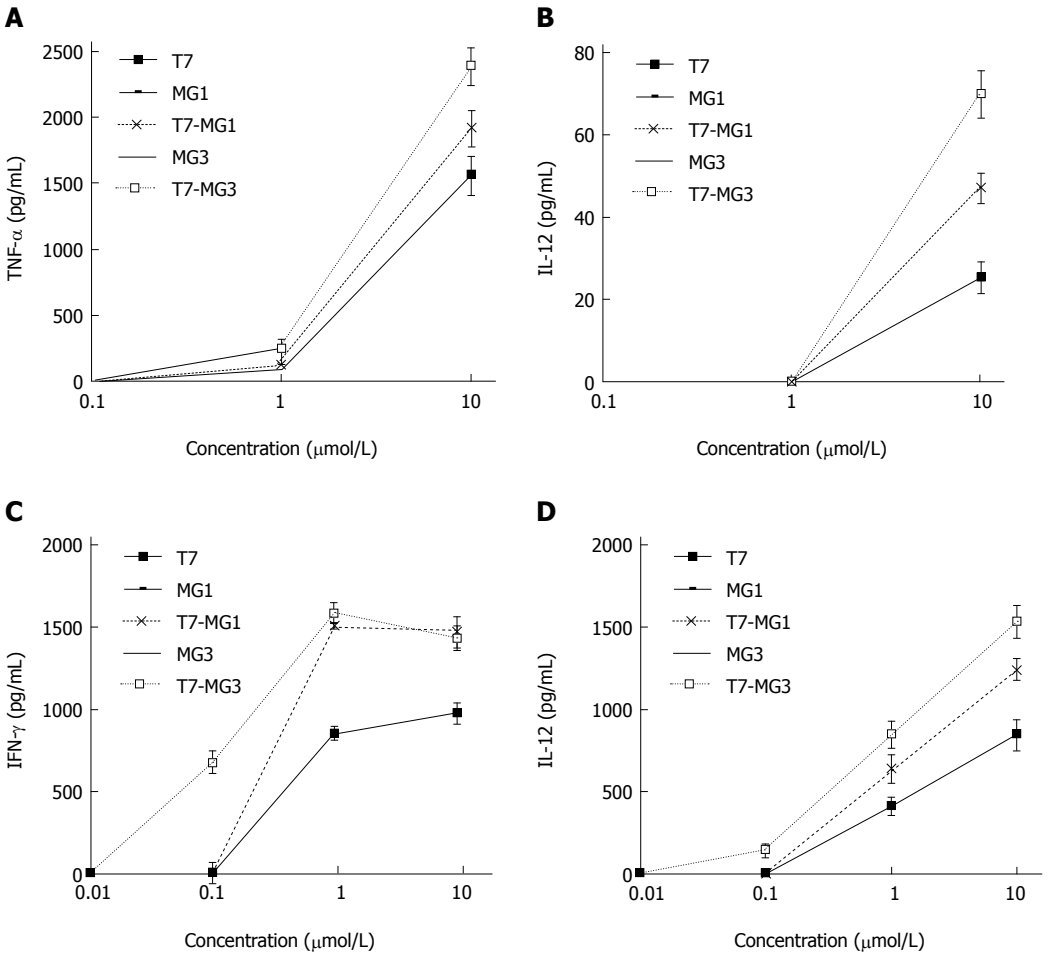

Figure 2 Vaccines induced cytokine release of mouse bone marrow dendritic cells and mouse spleen lymphocytes *in vitro*. Cells were incubated with vaccines for 24 h. Quantification of A: Tumor necrosis factor-α (TNF-α); and B: Interleukin-12 (IL-12) in bone marrow dendritic cells; and C: Interferon-γ (IFN-γ); and D: IL-12 in lymphocytes. Data are presented as mean ± SE (n = 3). MG1: Monoclonal gastric cancer 7 antigen mono-epitope; MG3: Monoclonal gastric cancer 7 antigen tri-epitope; T7: Toll-like receptor-7 agonist.

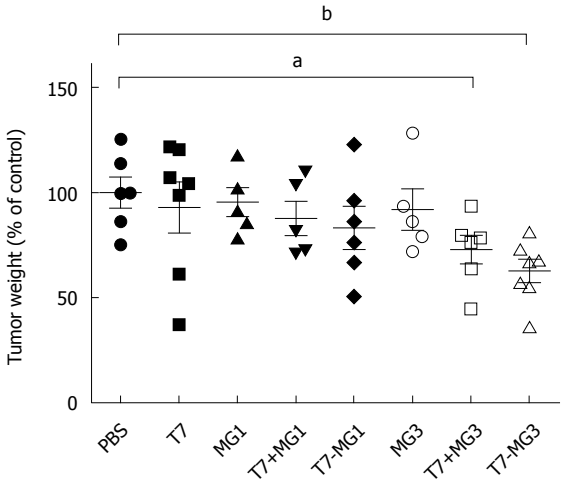

Figure 3 Vaccines reduced Ehrlich ascites carcinoma tumor burden in BALB/c mice. Mice were immunized with vaccines or PBS as control every 2 wk for a total of three times. Data are presented as mean ± SE ($n \geqslant 5$); $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control group. MG1: Monoclonal gastric cancer 7 antigen mono-epitope; MG3: Monoclonal gastric cancer 7 antigen tri-epitope; T7: Toll-like receptor-7 agonist.

10 μmol/L. Significantly higher levels of cytokines were detected when the MG7-Ag epitope was conjugated to T7 (T7-MG1 or T7-MG3) compared to T7 alone ($P < 0.05$). Moreover, 10 μmol/L T7-MG3 was more potent in raising cytokine release levels of TNF-α and IL-12 than T7-MG1. Similar results were demonstrated for cytokines released in lymphocytes (Figure 2C and D). Furthermore, 0.1 μmol/L T7-MG3, rather than 0.1 μmol/L T7-MG1, increased the levels of IFN-γ and IL-12, indicating that T7-MG3 had a stronger stimulating effect on mouse lymphocytes than T7-MG1.

Protection of vaccines against tumor challenge

EAC cells, which have been verified for the presence of MG7-Ag[3], were used to challenge the BALB/c mice to investigate the protective ability of tumor vaccines. We also confirmed the expression of MG7-Ag in EAC cells by Western blot (data not shown). As shown in Figure 3, immunization with T7, MG1, or MG3 alone did not exhibit much improvement of antitumor properties as compared with PBS control. Although T7+MG1 and T7-

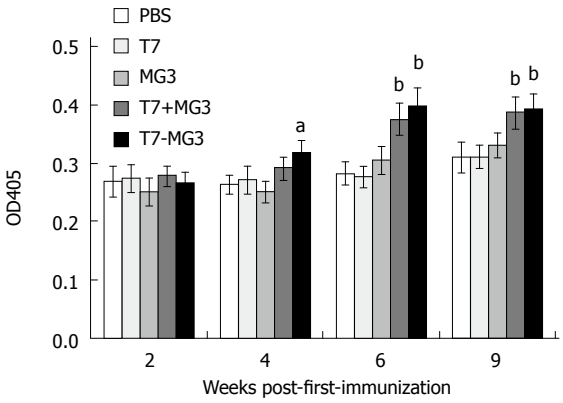

Figure 4 Vaccines induced serum antibody against monoclonal gastric cancer 7 antigen in BALB/c mice. Every two weeks after immunization (week 2, 4, 6) and after sacrifice (week 9), serum samples were collected and antibody titers were determined by ELISA. Data points represent mean ± SE (n = 5); $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control group. MG3: Monoclonal gastric cancer 7 antigen tri-epitope; T7: Toll-like receptor-7 agonist.

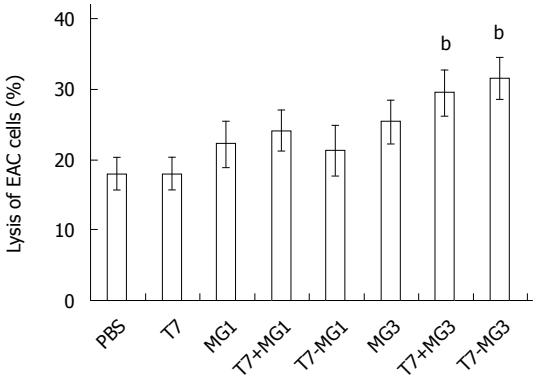

Figure 5 Vaccines induced antibody-dependent cell-mediated cytotoxicity. Serum samples were collected from BALB/c mice immunized with vaccines or PBS controls. Antibody-dependent cell-mediated cytotoxicity activities were indicated by lysis of Ehrlich ascites carcinoma (EAC) cells using the lactate dehydrogenase method. Data are presented as mean ± SE (n = 5); $^{b}P < 0.01$ *vs* control group. MG1: Monoclonal gastric cancer 7 antigen mono-epitope; MG3: Monoclonal gastric cancer 7 antigen tri-epitope; T7: Toll-like receptor-7 agonist.

MG1 led to somewhat smaller tumors, their differences were not significant compared with PBS control. Obvious reduction of tumor weight was observed when T7 was used together with the MG3, whether as a commixture (T7+MG3) or chemical conjugation (T7-MG3); the difference between T7+MG3 and T7-MG3 was not significant. Meanwhile, mouse breast cancer 4T1 cells (absence of MG7-Ag expression ascertained by Western blot) were included as a negative control. Neither T7+MG3 nor T7-MG3 could reduce the 4T1 tumor burden (data not shown), indicating the MG7-Ag specificity of the observed effects in EAC cells.

Serum antibody induced by vaccines

Serum antibody titers against MG7-Ag were determined by ELISA assay. Six or nine weeks after first immunization, MG7-Ag antibody increased significantly in T7+MG3 and T7-MG3 groups compared to PBS control, while the difference of immune effect was negligible between chemical conjugation (T7-MG3) and simple commixture (T7+MG3). On the other hand, T7, MG1, or MG3 alone exerted little impact on MG7-Ag antibody (Figure 4). Vaccines constructed with MG1 (T7+MG1 and T7-MG1) also could not elicit antibody response, independent of the times of immunization (data not shown).

ADCC induced by vaccines

ADCC was determined by addition of serum samples and NK cells (cytotoxic effector cells) to EAC tumor cells (target cells), and measurement of released LDH activity (Figure 5). Antisera collected from the mice immunized with T7 and MG1, whether used alone or in combination (T7+MG1 or T7-MG1), did not induce significant cancer cell lysis compared with PBS control. Antibodies derived from MG3 displayed stronger cytotoxicity to a certain degree, yet still not significantly different from the PBS control. However, antisera obtained by immunization with T7+MG3 and T7-MG3 were able to markedly increase cell lysis compared to PBS or MG3 alone. No remarkable ADCC effect of vaccines was found when mouse breast cancer 4T1 cells were used as target cells for negative control (data not shown).

CTL induced by vaccines

To assess the ability of vaccines to activate cellular immunity, CTL activity was determined by LDH assay, which is identically sensitive to the ^{51}Cr release assay when measuring cytotoxicity. As shown in Figure 6, immunization with T7, MG1, or MG3 alone did not induce more CTL in BALB/c mice than in PBS control. As expected, CTL activated by T7+MG3 and T7-MG3 exhibited obviously greater cytotoxicity compared with control, being significantly higher with T7-MG3 compared to T7+MG3 ($P < 0.05$). However, vaccines constructed with MG1, both T7+MG1 and T7-MG1, did not display similar high lytic activity as with T7+MG3 and T7-MG3. Similar with ADCC, no CTL effect of vaccines was detected with 4T1 cells as negative control (data not shown).

DISCUSSION

Gastric cancer is the second leading cause of cancer-related death worldwide, and therapeutic effect was poor by traditional treatment (surgery, chemotherapy, and radiation) with a low five-year survival rate. Therefore, immunotherapy offers another direction in prevention and treatment of gastric cancer. Nowadays, adoptive cell therapy, gene therapy, monoclonal antibody therapy, and cancer vaccines are frequently used with some success[15]. Clinical studies of adoptive immunotherapies have shown that longer survival was achieved in gastric cancer patients treated with

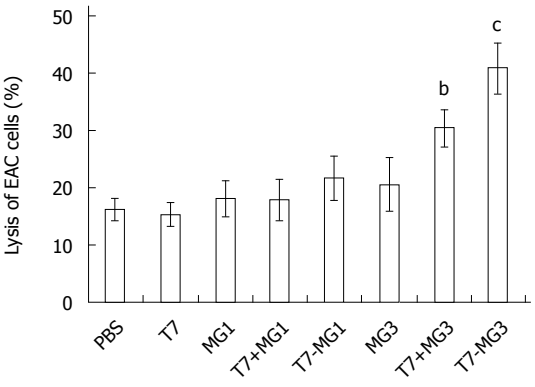

Figure 6 Vaccines induced cytotoxic T cell responses. Lymphocytes were separated from the spleens of mice immunized with vaccines or PBS as control. Cytotoxic T lymphocyte activities were indicated by lysis of Ehrlich ascites carcinoma (EAC) cells using the lactate dehydrogenase method. Data are presented as mean ± SE (n = 5); $^{b}P < 0.01$ *vs* control group; $^{c}P < 0.05$ *vs* T7+MG3 group. MG1: Monoclonal gastric cancer 7 antigen mono-epitope; MG3: Monoclonal gastric cancer 7 antigen tri-epitope; T7: Toll-like receptor-7 agonist.

chemotherapy in combination with tumor-associated lymphocytes or cytokine-induced killer cells than with chemotherapy alone[16,17]. As for cancer vaccines, nanoparticles with the melanoma-associated antigen 3 peptide show the ability to stimulate immune responses *in vivo* and kill mouse fore-stomach carcinoma cells[18]. Vaccination with human vascular endothelial growth factor receptors 1 and 2 combined with chemotherapy is well tolerated and highly effective in advanced or recurrent gastric cancer[19]. Occurrence of novel tumor antigen is one of the most important immunologic characteristics of tumor cells. So far, many tumor-associated antigens have been explored and applied in the research of tumor vaccines in order to elicit specific immune responses in the host, either by themselves or after loading onto antigen presenting cells. Sipuleucel-T, containing prostate acid phosphatase antigen, is the first cell-based immunotherapeutic vaccine approved by the Food and Drug Administration for patients with metastatic castration-resistant prostate cancer[20]. Clinical data have also shown that therapeutic cancer vaccine targeting the MUC1 antigen is effective in non-small-cell lung cancer patients[21].

MG7-Ag, first discovered in the KATO Ⅲ cell line, is one kind of gastric cancer-associated antigen and is expressed at a high level in the serum and tissue of gastric cancer patients[22]. Recently, it has been investigated as a biomarker with high sensitivity and specificity for gastric cancer diagnosis[23], and many methods for MG7-Ag detection are being developed, such as immunohistochemical stain and ELISA[24,25]. It is worth noting that MG7-Ag has also been applied in the development of tumor vaccines. Heterologous prime-boost immunization with oral MG7-Ag DNA vaccine induces significant immune response to gastric cancer[3]. A fusion protein containing MG7-Ag antibody and superantigen staphylococcal enterotoxin B has been constructed with the ability to kill SGC7901 human gastric cancer cells[4]. Despite the potency of the MG7-Ag against cancer shown by previous studies, MG7-Ag epitope peptides are much more widely used in current gastric cancer vaccines, circumventing the difficulty of isolating and purifying MG7-Ag directly from tumor tissue. Moreover, a multi-repeat-epitope is a well-defined strategy of vaccine construction for enhancing the effect of immunization, which has also been adopted in MG7-Ag epitope-based research[26]. Therefore, in this study, MG7-Ag mono-epitope and tri-epitope peptides were used as antigens and expected to achieve specific antitumor effect. However, neither MG1 nor MG3 alone had much impact on immunity enhancement of BALB/c mice, displayed as a minimal change of antibody titers, ADCC, CTL (*in vivo*), and cytokine induction (*in vitro*) after antigen immunization. MG1 and MG3 alone also could not efficiently inhibit the growth of EAC tumor cells *in vivo*. These results confirm the fact that tumor-related antigens, especially epitopes, are often poorly immunogenic and do not sufficiently activate immune cells for recognizing and invading the tumor cells.

Several studies have demonstrated that conjugations of TLR7 ligands and antigens trigger better immunogenicity compared to noncoupled antigens alone[27]. Oh *et al*[28] showed that a TLR7 agonist-Ag conjugate elicited $CD8^+$ T cell responses based on the engagement of DC cross-presentation pathways. Similarly, we used a promising small molecule compound (T7) for the attachment to MG7-Ag in the study of tumor immunotherapy. Chemical conjugation of this agonist and mouse serum albumin resulted in 10-100-fold potent cytokine production *in vitro*, and significant delay in mortality in BALB/c mice inoculated with influenza virus[29]. When the compound was conjugated to phospholipid and administered systemically in mice, prolonged increases occurred in the levels of proinflammatory cytokines. The phospholipid-TLR7 agonist conjugate could be further used as adjuvant during vaccination, which resulted in the boost of both Th1 and Th2 antigen-specific immune responses[12].

The data presented here showed that T7 improved the innate immunity by rapid induction of inflammatory mediators TNF-α and IL-12 in BMDCs and IFN-γ and IL-12 in lymphocytes *in vitro*, while the conjugation (T7-MG1 or T7-MG3) yielded more potent effects. Cytokines produced following the activation of TLR7 can stimulate the expression of costimulatory proteins for optimum interactions between helper T, B, and antigen-presenting cells[9]. Therefore, covalent attachment of T7 and MG7-Ag is critical for the induction of adaptive immunity, including CTLs and antibodies. Indeed, vaccination with T7-MG3 reduced the EAC tumor burden *in vivo*, *via* eliciting cytotoxicity of T lymphocytes and IgG antibodies that could specifically lyse cancer cells. The results of the present study also showed that the differences between T7-MG3 and PBS control in CTL

activities were more noticeable than in antibody titers and ADCC activities, implying that cellular immunity made a greater contribution than humoral immunity to the vaccination of T7-MG3.

Over the past decade, numerous studies have been carried out concerning how to promote antigen presentation for improving immune responses, such as with heat shock proteins or bacterial toxins linked to antigens[30]. These strategies for DC uptake have the disadvantage that immune suppression might occur due to the antigenicity of the targeting device, which could be avoided by the low intrinsic immunity of small molecule T7. Meanwhile, no remarkable effect of T7+MG1 or T7-MG1 was observed on either the promotion of immunity or the reduction of tumor, revealing the importance of multi-repeat-epitope used for vaccine development. As for MG3, chemical conjugation with T7 was more effective than simple commixture when for vaccination of BALB/c mice, especially on the induction of CTLs. Thus, based on the existing reports and the results present here, it is reasonable to suppose that covalent attachment is a better method for the investigation of vaccines than simple mixture, considering that distribution and metabolism of T7 and antigen could be more coincident systemically.

In conclusion, an effective tumor vaccine targeting MG7-Ag has been constructed by chemical conjugation of T7 with MG3. Its capacity was ascertained to generate CTLs and ADCC-mediating antibodies recognizing MG7-Ag and provide a therapeutic benefit in EAC tumor-bearing mice. T7 could elicit the nonspecific antitumor responses and strengthen the specific humoral and cellular immune responses, which is imperative in tumor vaccines due to the fact that tumor antigens rarely possess immunogenicity. This concept of vaccine construction can be further applied in other kinds of tumors, either as single immunotherapy or combined with other therapies.

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COMMENTS

Background

Gastric cancer is one of the most common malignant tumors, in which tumor vaccines play increasingly important roles for their advantages, such as low toxicity and long-term effect. Weak immunogenicity of tumor-associated antigens and lack of immune recognition are main reasons that tumor cell escape surveillance and eradication by the immune system, thus requiring the addition of adjuvant for optimal performance of tumor-associated antigens.

Research frontiers

Recently, monoclonal gastric cancer 7 antigen (MG7-Ag) was investigated as a biomarker with high sensitivity and specificity for gastric cancer diagnosis, and many detecting methods are being developed, such as immunohistochemical stain and ELISA. It is worth noting that MG7-Ag has also been applied in the development of tumor vaccines, such as DNA and recombinant protein vaccines. On the other hand, the applications of toll-like receptor (TLR) ligand as an adjuvant in vaccine development are under intensive investigation, against not only infectious diseases, but also malignant tumors.

Innovations and breakthroughs

In this study, the authors constructed tumor vaccines by covalent attachment of MG7-Ag and a TLR7 agonist (T7). Conjugation of T7 with an MG7-Ag tri-epitope (T7-MG3) significantly increased the release of tumor necrosis factor-α and interleukin (IL)-12 in bone marrow dendritic cells and interferon-γ and IL-12 in mouse lymphocytes *in vitro*. Immunization with T7-MG3 was efficacious in reversing tolerance and generating therapeutic response in Ehrlich ascites carcinoma-bearing mice, through enhancing specific humoral and cellular immunity, which were displayed as higher antibody titers, antibody-dependent cell-mediated cytotoxicity, cytotoxic T lymphocyte activity. Although a simple commixture of T7 and MG3 was able to inhibit tumor growth in tumor-bearing mice because of the immune enhancement, the effects of T7-MG3 were greater, especially on the induction of cytotoxic T lymphocytes. However, vaccines with an MG7-Ag mono-epitope showed no significant effect on either the promotion of immunity or the reduction of tumor size.

Applications

This study demonstrated a successful way for the design of gastric cancer vaccines by combined use of T7 and MG7-Ag, and the importance of multi-repeat-epitopes and chemical conjugation. This concept of vaccine construction can be further applied in other kinds of tumors, either as single immunotherapy or combined with other therapies.

Terminology

MG7-Ag is a tumor-associated antigen with high specificity and selectivity, whose expression in gastric mucosa is closely associated with high risk of gastric atypical hyperplasia and malignant change. Among all kinds of TLR ligands, the TLR7 agonist is the only choice of small molecule synthetic compounds, which is more convenient to obtain than other ligands.

Peer-review

This study aims to develop a vaccine against gastric cancer by conjugating multi-repeat epitopes of gastric cancer antigen MG7-Ag and TLR7 agonist. The authors demonstrate that this conjugate elicits specific humoral, natural killer, and cytotoxic T lymphocyte responses in mice and can reduce the tumor burden. This paper is clearly presented and is of potential interest and clinical relevance.

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ORIGINAL ARTICLE

Basic Study

Ameliorative effects of lutein on non-alcoholic fatty liver disease in rats

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Abstract

AIM: To investigate the therapeutic effects of lutein against non-alcoholic fatty liver disease (NAFLD) and the related underlying mechanism.

METHODS: After 9 d of acclimation to a constant temperature-controlled room (20 ℃-22 ℃) under 12

h light/dark cycles, male Sprague-Darley rats were randomly divided into two groups and fed a standard commercial diet (n = 8) or a high-fat diet (HFD) (n = 32) for 10 d. Animals receiving HFD were then randomly divided into 4 groups and administered with 0, 12.5, 25, or 50 mg/kg (body weight) per day of lutein for the next 45 d. At the end of the experiment, the perinephric and abdominal adipose tissues of the rats were isolated and weighed. Additionally, serum and liver lipid metabolic condition parameters were measured, and liver function and insulin resistance state indexes were assessed. Liver samples were collected and stained with hematoxylin eosin and Oil Red O, and the expression of the key factors related to insulin signaling and lipid metabolism in the liver were detected using Western blot and real-time polymerase chain reaction analyses.

RESULTS: Our data showed that after being fed a high-fat diet for 10 d, the rats showed a significant gain in body weight, energy efficiency, and serum total cholesterol (TC) and triglyceride (TG) levels. Lutein supplementation induced fat loss in rats fed a high-fat diet, without influencing body weight or energy efficiency, and decreased serum TC and hepatic TC and TG levels. Moreover, lutein supplementation decreased hepatic levels of lipid accumulation and glutamic pyruvic transaminase content, and also improved insulin sensitivity. Lutein administration also increased the expression of key factors in hepatic insulin signaling, such as insulin receptor substrate-2, phosphatidylinositol 3-kinase, and glucose transporter-2 at the gene and protein levels. Furthermore, high-dose lutein increased the expression of peroxisome proliferators activated receptor-α and sirtuin 1, which are associated with lipid metabolism and insulin signaling.

CONCLUSION: These results demonstrate that lutein has positive effects on NAFLD *via* the modulation of hepatic lipid accumulation and insulin resistance.

Key words: Lutein; Non-alcoholic fatty liver disease; Insulin resistance; Sirtuin 1; Peroxisome proliferators activated receptor-α

Core tip: Lutein has potential positive effects on chronic diseases. To date, no previous studies have reported the regulatory effects of lutein on non-alcoholic fatty liver disease (NAFLD). We observed that lutein has positive effects on hepatic lipid accumulation, liver function, and insulin resistance induced by a high-fat diet, possibly *via* activation of the expression of sirtuin 1 and, subsequently, peroxisome proliferators activated receptor-α, and other key factors in insulin signaling. These findings provide a new prospect for preventing NAFLD.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a progressive pathological change in chronic liver diseases caused by a disturbance in lipid metabolism[1]. It is the most common type of chronic liver disease in the majority of developed countries[2], possibly due to changes in dietary habits and the increase in sedentary lifestyles[3]. With the spread of the Western lifestyle in developing countries, NAFLD is beginning to affect more people[3]. In Shanghai, Guangdong, and Hong Kong (China), the prevalence of NAFLD has been reported to be 17%, 15%, and 16%, respectively[4]. Therefore, it is important to find effective measures for the control of NAFLD.

The protective effects of phytochemicals on chronic diseases have received much attention from the scientific community in recent decades. Lutein is one of hundreds of known naturally oxygenated carotenoids, and is abundantly present in vegetables, fruits, and egg yolks. Lutein consists of a carbon chain with nine conjugated dienes and a hydroxylated cyclic hexenyl structure at each side; owing to its special chemical structure, it has potential antioxidant properties. Over a long period of time, lutein, as one of the major pigments in the macula lutea on the retina, was found to play a key role in preserving visual performance because of its strong blue light filtering ability[5]. Recently, lutein has drawn increasing attention to its function in chronic diseases other than oculopathy.

The disturbance of lipid metabolism and insulin resistance has an important role in the pathophysiology of NAFLD[1]. While exploring the relationship between serum lutein levels and lipid metabolism, some epidemiological studies found that serum high-density lipoprotein-cholesterol (HDL-C) was positively-associated with serum lutein levels[6]. Changes in oxidized low density lipoprotein (oxLDL) levels were inversely correlated with plasma lutein[7], and an increase in BMI among the population was significantly associated with low levels of serum lutein[8]. Insulin resistance was also found to be inversely related to serum lutein levels[9-11]. To date, there have been very few reports regarding the mechanism and effects of lutein on NAFLD, or on the risk factors of NAFLD. A limited number of researchers have suggested that lutein supplementation may resolve oxidative stress by reducing oxLDL, and that aortic malondialdehyde (MDA) levels were induced by a high-fat diet (HFD)

Table 1 Composition of the experimental diets used in this study (g/kg)

	Experimental diet	
	Normal diet	High-fat diet
Carbohydrate (% of energy)	69.86	48.45
Protein (% of energy)	21.76	17.91
Fat (% of energy)	8.38	33.64
Energy (kcal/100 g)	377.19	450.52
Ingredient (g/kg)		
Cornstarch	397.5	208.7
Casein	200.0	200.0
Dextrose	132.0	69.3
Sucrose	100.0	252.5
Soybean oil	70.0	36.8
Fiber	50.0	26.2
Mineral mix[1]	35.0	18.4
Vitamin mix[1]	10.0	5.3
L-Cystine	3.0	1.6
Choline bitartrate	2.5	1.3
Lard	0.0	150.0
Cholesterol	0.0	11.0
Calcium hydrogen phosphate	0.0	17.0
Sodium cholate	0.0	2.0
Total	1000	1000

[1]Based on the AIN-93G vitamin and mineral mixes.

in guinea pigs[12], as well as decreased TG values in wild-type mice[13]. Therefore, it is critical to explore the mechanism of lutein in NAFLD.

Sirtuin 1 (SIRT1) is reported to have therapeutic potential in NAFLD and play a key role in insulin sensitivity[14]. SIRT1 regulates the expression of peroxisome proliferators activated receptor (PPAR)-α, a key factor in the regulation of lipid metabolism[15,16]. However, to our knowledge, there are few studies regarding the effect of lutein supplements on SIRT1 and PPAR-α.

Based on these findings, we established an NAFLD model in rats fed a HFD[17] and supplied them with different doses of lutein, with the aim to explore the effect of lutein supplementation on NAFLD and to investigate the involved mechanisms.

MATERIALS AND METHODS

Animal treatment protocol

Forty male Sprague-Dawley rats (100 ± 20 g), obtained from Sino-British SIPPK/BK lab, Animal Co., Ltd (Shanghai, China), were maintained in a constant temperature-controlled room (20 ℃-22 ℃) with controlled lighting (12 h light/dark cycles). The animals were cared for according to the guiding principles in the Care and Use of Animals. All experiments were approved by the Tongji Medical College Council's Animal Care Committee. Animals were randomly divided into two groups after acclimation for 9 d. Then, one group was fed a normal diet (ND) (n = 8), while the other group was fed a HFD (n = 32) for 10 d to induce lipid metabolism disturbance. The ND was prepared based on the American Institute of Nutrition-93G (AIN-93G) diet[18], while the HFD consisted of 52.5% standard diet, 20% sucrose, 15% lard, 9.5% casein, 1.7% calcium hydrogen phosphate, 1.1% cholesterol, and 0.2% sodium cholate. The composition of each diet is presented in Table 1. On the 10th day, serum lipid levels were tested after an 8-h fast using blood samples collected *via* the tail tip. The rats fed the HFD were then divided into 4 groups based on total cholesterol and administered 0, 12.5, 25, or 50 mg/kg (body weight) per day lutein [gifted from InnoBio CO (Dalian, China)]. Lutein was suspended in double distilled water. All animals were administered lutein suspension or water daily *via* gavage for the next 45 d. Food intake was recorded every day and body weight was monitored every three days. Energy efficiency was calculated as weight gain (g) divided by energy intake (kcal) during the feeding period[19].

At the end of experiment, rats were sacrificed by decapitation. Perinephric and abdominal adipose tissues were isolated and weighed. Serum and liver samples were collected and stored at -80 ℃ for further use.

Assessment of lipid metabolic condition in liver and serum

Liver samples were homogenized with 9 volumes of isopropanol. After incubation at 4 ℃ for 48 h and centrifugation at 3000 rpm for 15 min at 4 ℃, the supernatant was carefully collected for analysis. TC, TG, HDL-C, and LDL-C were measured using the appropriate kit (Biosino Bio-technology and Science Inc., Beijing, China) in an ELX800 microplate reader (Bio-Tek). All procedures were performed according to the manufacturer's instructions.

Measurement of serum biomarkers for liver function

Serum glutamic pyruvic transaminase (GPT) was measured using a kit (Mindray, Shenzhen, China) and read in a Mindray BS-200 automatic biochemistry analyzer (Shenzhen, China). The results are expressed as units per liter (U/L).

Lipids deposition in liver

Fresh samples from the same location of the liver were divided into two parts. One part was frozen and stained with Oil Red O. The other part was fixed in 4% paraformaldehyde, embedded in paraffin, and stained with hematoxylin and eosin (H&E) and examined by microscopy. Quantification of lipid droplets (area fraction) measured by Oil Red O staining in every group was calculated using Image-Pro Plus 6.0 software.

Determination of fasting glucose and insulin

Fasting glucose was determined directly by glucometer (Abbott Diabetes Care Ltd) when the animals were sacrificed. Fasting insulin was measured using an insulin ELIZA kit (R&D system, United States), following the manufacturer's instructions. HOMA-IR = FIN ×

Table 2 Body weight of rats at different time points

Group	Weight (g)	
	9 d	19 d
ND	201 ± 7.05	254 ± 8.90
HFD	199 ± 7.59	262 ± 15.09

Values represent the mean ± SD. After 9 d of acclimation, the rats were randomly divided into the normal diet (ND) (n = 8) and high-fat diet (HFD) (n = 32) groups. The rats in the HFD group were then fed a high-fat diet for 10 d.

FPG/22.5, HOMA-β = 20 × FIN/(FPG-3.5).

Real-time polymerase chain reaction analysis

Total RNA was extracted from the liver using TRIzol® reagent (Invitrogen, Carlsbad, CA, United States). To quantify the expression of messenger RNA (mRNA), a SYBR green-based qRT-PCR kit (TaKaRa BIO Inc., Dalian) was used according to the manufacturer's instructions in a 7900HT instrument (Applied Biosystems, Forster, CA, United States). The specificity of the product was assessed from melting curve analysis. Gene expression was determined using the $2^{-\Delta\Delta Ct}$ method. Gene expression of insulin receptor substrate-2 (IRS2) (NM_001168633.1), phosphatidylinositol 3-kinase (PI3K, NM_013005.1), and glucose transporter-2 (GLUT2) (NM_012879.2) was presented as fold change relative to the normal control and normalized to β-actin (NM_031144.3). The following forward and reverse primers were used: IRS2, 5′-GGA GCT CTG TTA GCA CCG TT-3′ and 5′-TCC AGT TCC GAG CTT GAG TG-3′, PI3K, 5′-AGG AGC GGT ACA GCA AAG AC-3′ and 5′-CTG CTG TCG ATG ATC TCG CT-3′, GLUT2, 5′-ACC AGC ACA TAC GAC ACC AG-3′ and 5′-ACC ATT CCG CCT ACT GCA AA-3′, and β-actin, 5′-CCC GCG AGT ACA ACC TTC TT-3′ and 5′-CGC AGC GAT ATC GTC ATC CA-3′.

Western blotting

The liver tissue was homogenized and lysed in RIPA Lysis Buffer (1% Triton X-100, 1% deoxycholate, 0.1% SDS). Lysates containing equal protein amounts were separated by 10% SDS-PAGE and transferred onto polyvinylidene difluoride membranes. After blocking, the membranes were incubated with one of the following primary antibodies overnight at 4 ℃: IRS2 (Cell Signaling Technology; Cat. No. 3089), PI3K-P85 (Cell Signaling Technology; Cat. No. 4257), GLUT2 (Santa Cruz Biotechnology, Inc.; Cat. No. sc-9117), PPAR-α (abcam; Cat. No. ab8934), SIRT1 (Santa Cruz Biotechnology, Inc.; Cat. No. sc-15404), or β-actin (Sigma; Cat. No. A1978). The membranes were then incubated with secondary antibodies conjugated to horse-radish peroxidase. Immunoreactive bands were detected by means of an ECL plus Western Blotting Detection System (Amersham Biosciences, Little Chalford, United States), and the band densities were measured using Gel Pro 3.0 software (Biometra, Goettingen, Germany). β-actin served as an internal control protein.

Statistical analysis

All data are expressed as the mean ± SD. Statistical analyses of the data were performed using the SPSS 12.0 software package (SN: 59245 46841 40655 89389 09859 21671 21957 29589 12). The statistical significance of differences among groups was determined by one-way analysis of variance, followed by Student-Newman-Keuls multiple range test to determine the statistical significance between the two groups. The results were considered statistically significant at $P < 0.05$.

RESULTS

HFD increased body weight, energy efficiency, and serum TC and TG levels

After acclimation for 9 d, the weights of the ND and HFD rats increased to 201 g and 199 g (Table 2). The rats in the two groups were then fed a ND or HFD for 10 d, and the weights increased to 254 g and 262 g, respectively (Table 2). The administration of the HFD for 10 d caused significant elevations in weight ($P < 0.01$), energy efficiency ($P < 0.05$), and serum TC ($P < 0.01$) and TG ($P < 0.01$) levels compared to the control group (Figure 1). After dividing into 4 groups, there was no significant difference in TG (Figure 2).

Effect of lutein supplementation on body weight, energy efficiency, and adiposity in rats fed a HFD

After treating with lutein for 45 d, no significant difference was observed in body weight gain or energy efficiency compared with rats feed a HFD (Figure 3A and B), but perinephric fat did decrease notably in the HFD + Lut12.5 group ($P < 0.05$; Figure 3C) and abdominal fat was reduced significantly after treatment with any dose of lutein ($P < 0.01$ for 12.5 and 50 mg/kg, $P < 0.05$ for 25 mg/kg; Figure 3D).

Effect of lutein supplementation on lipid metabolism and liver function

To evaluate the beneficial effect of lutein on lipid metabolism and liver function, the related indices were assessed. The level of TC in the ND group was 1.01 mmol/L and 1.88 mmol/L in the HFD group, which was significantly higher ($P < 0.01$; Table 3). However, in the HFD + Lut25 group, the level of TC only reached 1.53 mmol/L, which was a significant decrease from 1.88 mmol/L ($P < 0.01$; Table 3). Lutein supplementation had a similar effect on hepatic TG (Table 3). HFD feeding induced significant elevations in hepatic TC levels, but down-regulated serum HDL-C levels ($P < 0.01$; Table 3). These alterations were significantly ameliorated by lutein administration (serum HDL-C, $P < 0.01$ for 12.5 and 25 mg/kg;

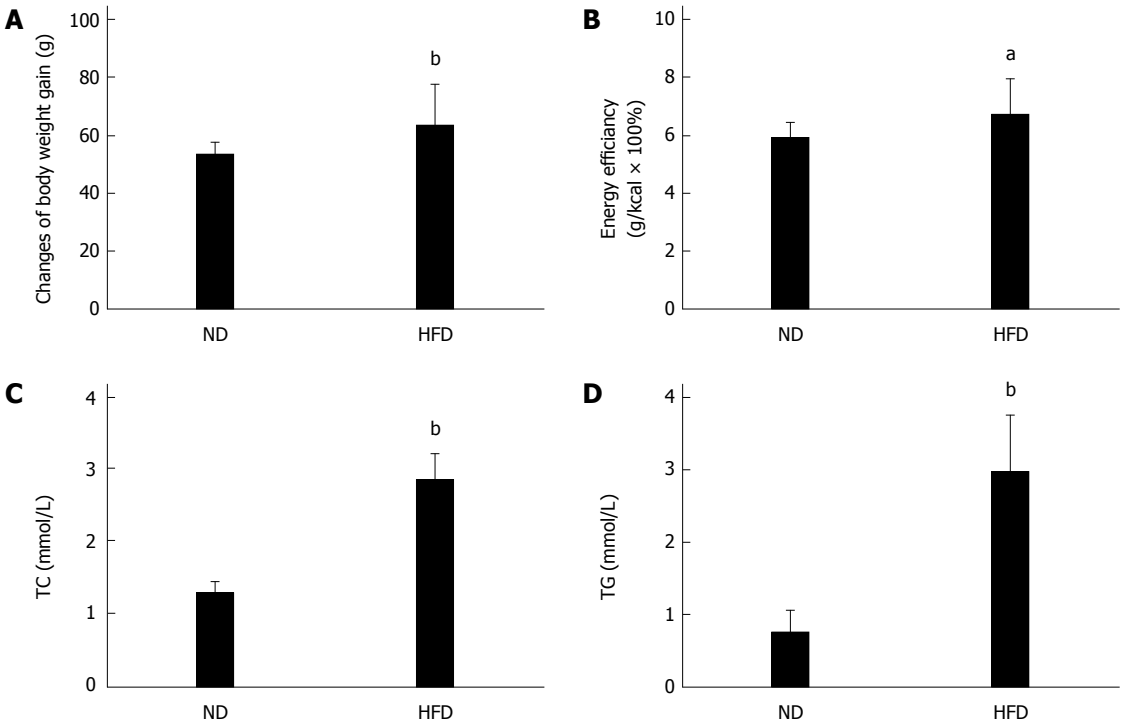


Figure 1 Changes in the basic physiological and biochemical responses of rats fed a high-fat diet. The normal diet (ND) group (n = 8) was fed a standard diet and the high fat diet (HFD) group (n = 32) was fed a HFD for 10 d. The basic indicators included changes in body weight gain (A), energy efficiency (B), serum total cholesterol (TC) (C), and triglyceride (TG) (D). $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* ND group.

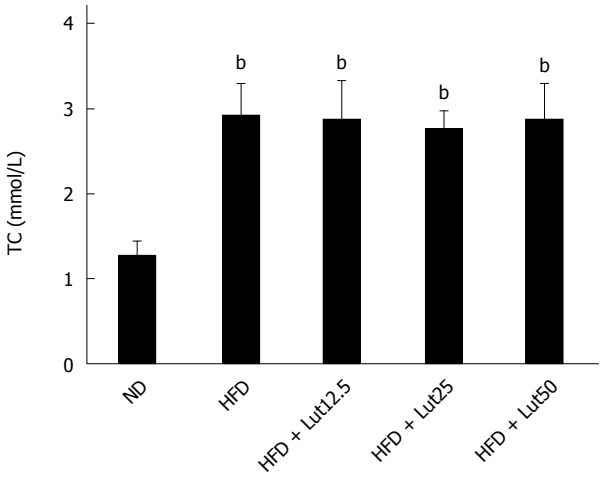


Figure 2 Levels of total cholesterol in rats divided into 4 groups. Rats were fed with normal diet or a high fat diet for 10 d, and then the high fat diet (HFD)-fed rats were divided randomly into four groups based on total cholesterol. Data are expressed as the mean ± SD (n = 8). $^{b}P < 0.01$ *vs* the normal diet (ND) group.

hepatic TC, $P < 0.05$ for 50 mg/kg; Table 3). Similarly, lutein supplementation effectively reversed the increased serum GPT levels caused by the HFD ($P < 0.01$; Figure 4).

Finally, we used H&E and Oil Red O staining of the hepatic tissues to evaluate the extent of fat accumulation. As expected, HFD feeding resulted in severe hepatic lipid accumulation, characterized by an increase in the number and size of accumulated fat droplets in the hepatocytes, while lutein supplementation mitigated hepatic steatosis, especially at medium and high doses ($P < 0.01$; Figure 5).

Lutein improved insulin sensitivity in rats fed a HFD

To elucidate the regulatory effect of lutein on insulin sensitivity, we detected the levels of fasting blood glucose and fasting insulin. We found remarkable increases in fasting blood glucose ($P < 0.01$) and insulin ($P < 0.05$) in the HFD group compared to the ND group. These elevations were significantly ameliorated by lutein treatment (glucose, $P < 0.05$ for 12.5 and 50 mg/kg, $P < 0.01$ for 25 mg/kg; insulin, $P < 0.05$ for 25 and 50 mg/kg, respectively). The HOMA indexes showed that the HFD caused remarkable up-regulation of HOMA-IR ($P < 0.01$) and partly down-regulated HOMA-β compared to the ND group, while lutein supplementation efficiently attenuated these changes ($P < 0.01$ for 12.5, 25, and 50 mg/kg in HOMA-IR; $P < 0.05$ for 25 mg/kg in HOMA-β; Figure 6).

Effects of lutein on mRNA abundance and protein content of hepatic IRS2, PI3K, and GLUT2 in rats fed a HFD

Insulin regulates glucose homeostasis in the liver through binding with its receptor, resulting in tyrosine phosphorylation of IRS2 and activation of PI3K and GLUT2[20]. To explore the effect of lutein on insulin signaling, we detected the expressions of IRS2, PI3K, and GLUT2 in the rat liver. As shown in Figure 7, the mRNA and protein expression of the key factors in insulin signaling were down-regulated markedly by

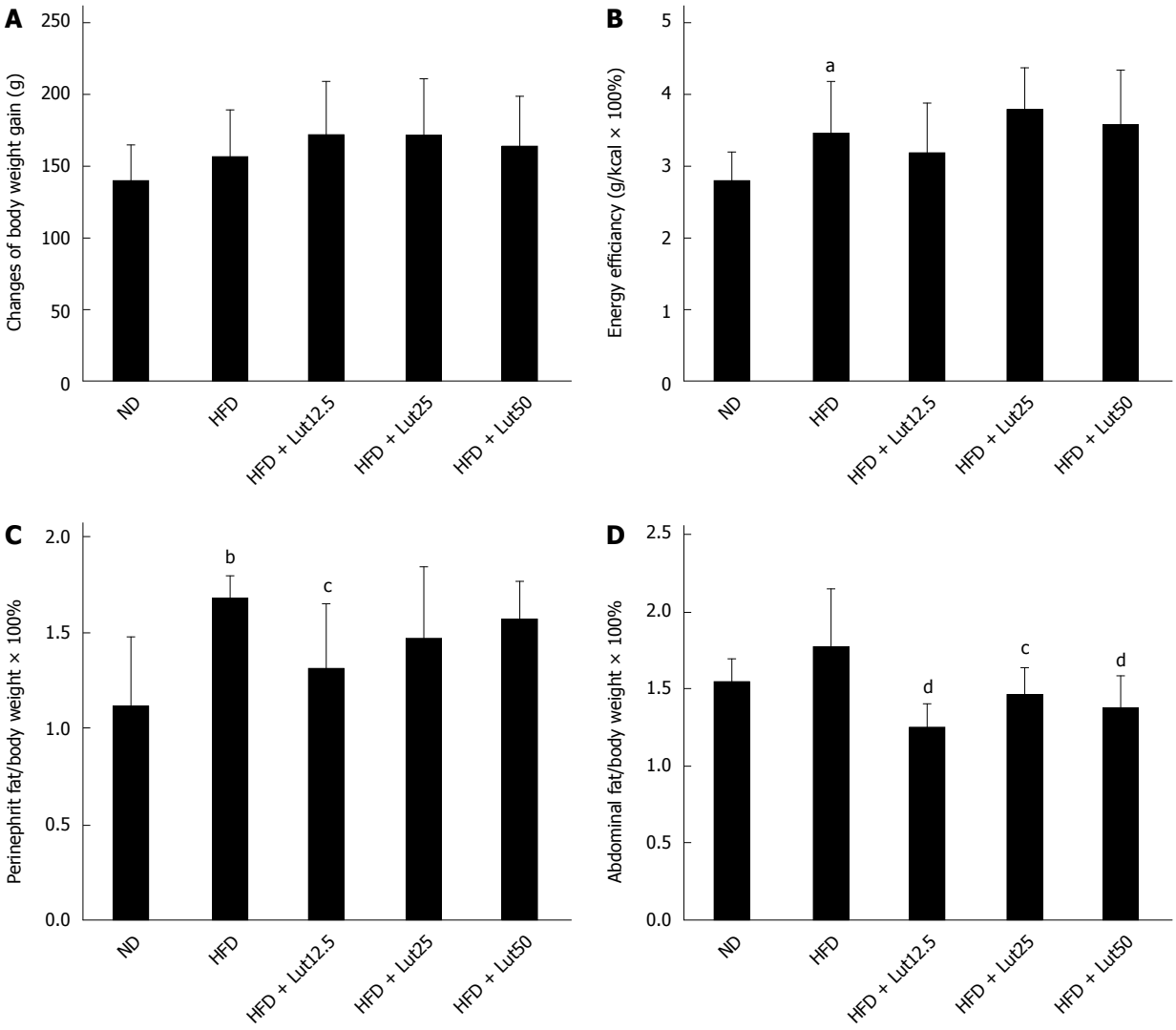


Figure 3 Effects of lutein on rats fed a high-fat diet. After being stratified into 4 groups based on total cholesterol, the rats were fed a high fat diet (HFD) plus 0, 12.5, 25, or 50 mg/kg body weight/d lutein for 45 d. Factors including body weight (A), energy efficiency (B), perinephric fat index (C), and abdominal fat index (D) were examined. Data are expressed as the mean ± SD (n = 8). $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* normal diet (ND) group, $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* HFD group.

Table 3 Effects of lutein on lipid metabolism in rats fed a high fat diet

Group	Serum (mmol/L)				Liver (μmol/g)	
	TC	TG	LDL-C	HDL-C	TC	TG
ND	1.01 ± 0.15	0.55 ± 0.16	0.184 ± 0.134	0.80 ± 0.09	0.93 ± 0.06	2.14 ± 0.44
HFD	1.88 ± 0.25^{b}	0.57 ± 0.17	0.566 ± 0.350^{a}	0.59 ± 0.15^{b}	1.65 ± 0.13^{b}	8.28 ± 1.40^{b}
HFD + Lut12.5	1.95 ± 0.25	0.45 ± 0.11	0.515 ± 0.487	0.88 ± 0.11^{d}	1.74 ± 0.10	7.09 ± 1.00^{c}
HFD + Lut25	1.53 ± 0.23^{d}	0.51 ± 0.12	0.305 ± 0.224	0.80 ± 0.17^{d}	1.66 ± 0.15	6.41 ± 0.97^{d}
HFD + Lut50	1.98 ± 0.24	0.45 ± 0.11	0.712 ± 0.342	0.70 ± 0.11	1.53 ± 0.08^{c}	7.27 ± 1.14

Values represent the mean ± SD of n = 8 rats/group. Rats in the normal diet (ND) group were supplied with a standard diet for 55 d. Rats in the high fat diet (HFD) group were fed a high fat diet for 10 d first, and then fed a high fat diet plus 0, 12.5, 25, or 50 mg/kg (body weight)/d lutein for 45 d. $^{b}P < 0.01$ *vs* ND group, $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* HFD group. TC: Total cholesterol; TG: Triglyceride; LDL-C: Low density lipoprotein-cholesterol; HDL-C: High density lipoprotein-cholesterol.

HFD feeding ($P < 0.05$; Figure 7). After being supplied with lutein for 45 d, mRNA and protein expression were up-regulated, significantly so for PI3K and GLUT2 ($P < 0.05$; Figure 7B and C), indicating increased insulin sensitivity.

Effects of lutein on protein content of hepatic PPAR-α and SIRT1 in rats fed a HFD

To further explore the underlying mechanisms of lutein in lipid metabolism, we detected the protein content of hepatic PPAR-α, a key factor in the regulation of lipid

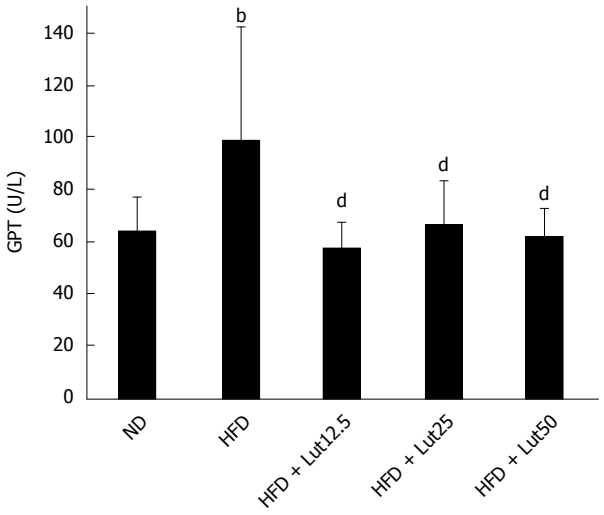


Figure 4 Lutein influences glutamic pyruvic transaminase in rats fed a high-fat diet. Rats were fed a high fat diet (HFD), except for the normal diet (ND) group, and lutein was administrated at doses of 12.5, 25, or 50 mg/kg body weight/d on the 10th day. Data are expressed as the mean ± SD (n = 8). ^{a}P < 0.05 *vs* the ND group, ^{c}P < 0.05, compared to the HFD group. ^{b}P < 0.01 *vs* ND group, ^{d}P < 0.01 *vs* HFD group.

metabolism[15]. As shown in Figure 8A, HFD feeding inhibited PPAR-α protein accumulation, however lutein supplementation reversed this phenotype, especially in the high-dose group (P < 0.05). Meanwhile, the protein levels of SIRT1, the upstream regulator of PPAR-α[16], decreased notably in the HFD group compared with the ND group, and lutein supplementation ameliorated the expression significantly in a dose-dependent pattern in the high-dose group (P < 0.05; Figure 8B).

DISCUSSION

In this study, we provided evidence that lutein supplementation could ameliorate insulin resistance and hepatic lipid accumulation. We also found that lutein supplementation augmented the mRNA and protein levels of key molecules related to insulin signaling which were suppressed by a HFD and the expression of PPAR-α, which is a key factor in the regulation of hepatic lipid metabolism. Furthermore, we found that lutein supplementation restored the expression of SIRT1, which regulates hepatic lipid metabolism and insulin signaling. All of these results suggest the beneficial effects of lutein on NAFLD.

According to the results of HOMA indexes, we found that lutein supplementation could improve insulin sensitivity. However, the process of insulin-regulated glucose homeostasis depends on glucose binding, as well as activating transmembrane insulin receptors and downstream targets[20]. The liver is one of the major target organs for insulin signaling[21,22], and some studies suggest that IRS2 can compensate IRS1 deficiency more effectively in liver and β-cells than in muscle or adipose tissues[23]. The liver is the main storage organ for carotenoids and controls the distribution of carotenoids to other tissues[24]. Therefore, we measured the mRNA and protein levels of IRS2, PI3K-P85, and GLUT2 in hepatic insulin signaling. As expected, the expression of these genes was inhibited in rats fed a HFD, as described in other studies[25-27]. However, lutein supplementation restored the insulin signaling pathway.

A large body of evidence supports a complex interaction between NAFLD and insulin resistance[28,29]. Some studies have suggested that abdominal adipose tissue has an important role in the development of insulin resistance[30]. Furthermore, visceral adipose tissue (VAT), a harmful fat deposition, has been considered to induce liver insulin resistance and further induce systemic insulin resistance[21,31]. According to our study, lutein supplementation for 45 d decreased serum TC, HDL-C, and perinephric and abdominal fat, as well as improve visceral fat deposition, without significant effects on body weight or energy efficiency. Moreover, lutein supplementation recovered liver function by decreasing hepatic TG, TC, and serum GPT levels effectively and improving lipid accumulation. These results suggest that lutein supplementation plays a potential role in preventing hepatic dyslipidemia and insulin resistance. However, the underlying mechanism is still unknown.

Some studies have demonstrated that PPAR-α plays an important role in the regulation of hepatic lipid metabolism[15,32] and that the inhibition of PPAR-α might induce hepatic steatosis[33]. Thus, we tested the protein level of PPAR-α and found that HFD feeding significantly inhibited the expression of PPAR-α, and that lutein supplementation reversed such inhibition effectively. Consistent with our results, some studies have also found that lutein may be an inducer of PPAR expression[34].

Meanwhile, SIRT1, a NAD^{+}-dependent deacetylase, has been reported to be the upstream regulator of PPAR-α[15], regulating lipid metabolism by activating PPAR-α[35]. However, some studies have suggested that SIRT1 is involved in regulating hepatic insulin signaling[36] and preventing insulin resistance[37]. When we tested the protein content of hepatic SIRT1, as expected, HFD feeding decreased the expression of SIRT1, and lutein supplementation increased SIRT1 expression in a dose-dependent manner.

In our study, the number of rats chosen for real-time polymerase chain reaction and Western blot analysis was somewhat limited, and so, in future studies, we would increase the sample size. In addition, the doses of lutein need more consideration in future studies.

In summary, our findings suggest that lutein supplementation could ameliorate hepatic lipid accumulation and insulin resistance induced by a HFD, possibly *via* the activation of the expression of SIRT1 and, subsequently, PPAR-α and other key factors in insulin signaling. These findings provide a new prospect for preventing NAFLD.

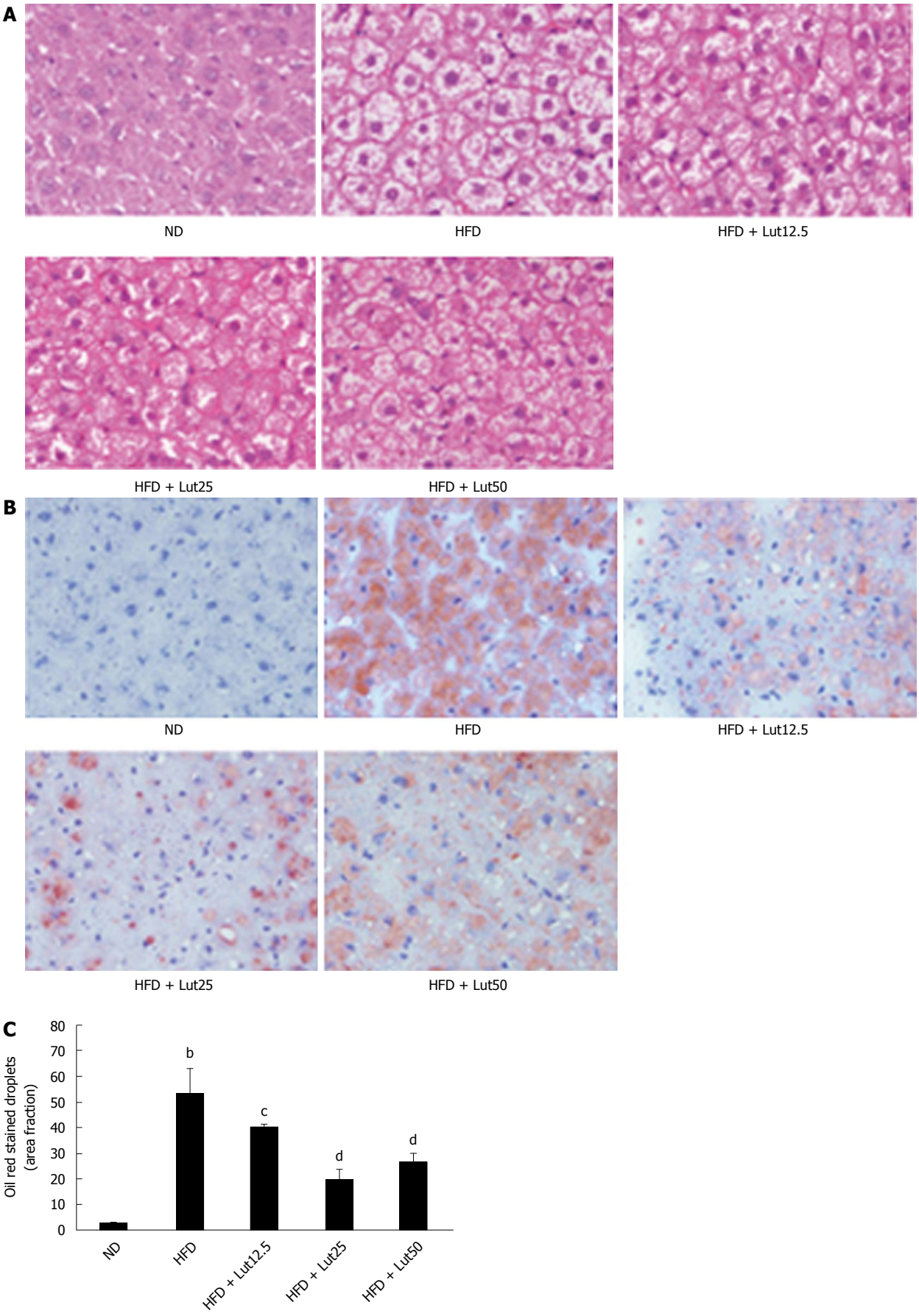


Figure 5 Lutein prevented hepatic lipid accumulation in rats fed with high-fat diet. Hematoxylin and eosin (H&E) stain (A) and Oil Red O stain (B) of liver sections are shown. Original magnification × 400. Quantitative analysis of hepatic fat accumulation is shown (C). Data are normalized to % of field area and represent the mean ± SD. $^{b}P < 0.01$ *vs* the normal diet (ND) group, $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* the high fat diet (HFD) group ($n = 3$).

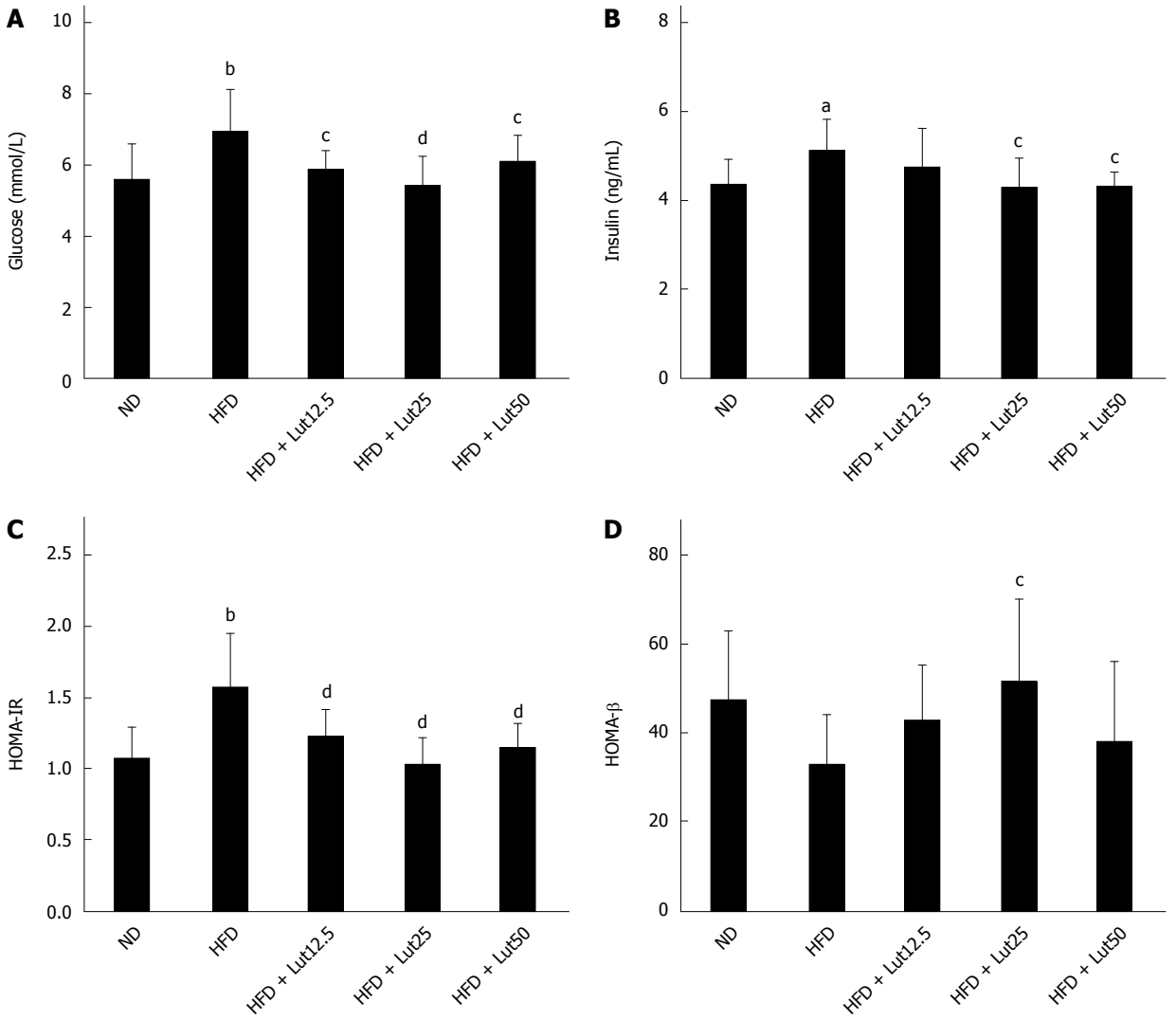
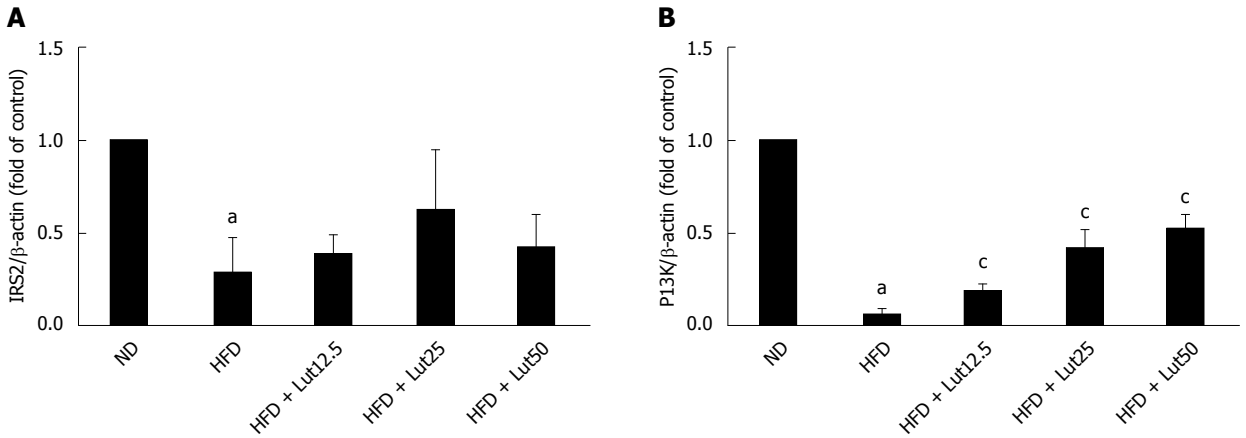


Figure 6 Lutein's effect on fasting glucose (A), fasting insulin (B), HOMA-IR (C), and HOMA-β (D) in rats fed a high-fat diet. After the 45-d lutein intervention, fasting glucose was tested as described and fasting insulin was measured using an insulin ELIZA kit following the manufacturer's instructions. HOMA-IR = FIN × FPG/22.5, HOMA-β = 20 × FIN/(FPG-3.5). [a]*P* < 0.05, [b]*P* < 0.01 *vs* the normal diet (ND) group, [c]*P* < 0.05, [d]*P* < 0.01 *vs* high-fat diet (HFD) group. Data are expressed as the mean ± SD (*n* = 8).



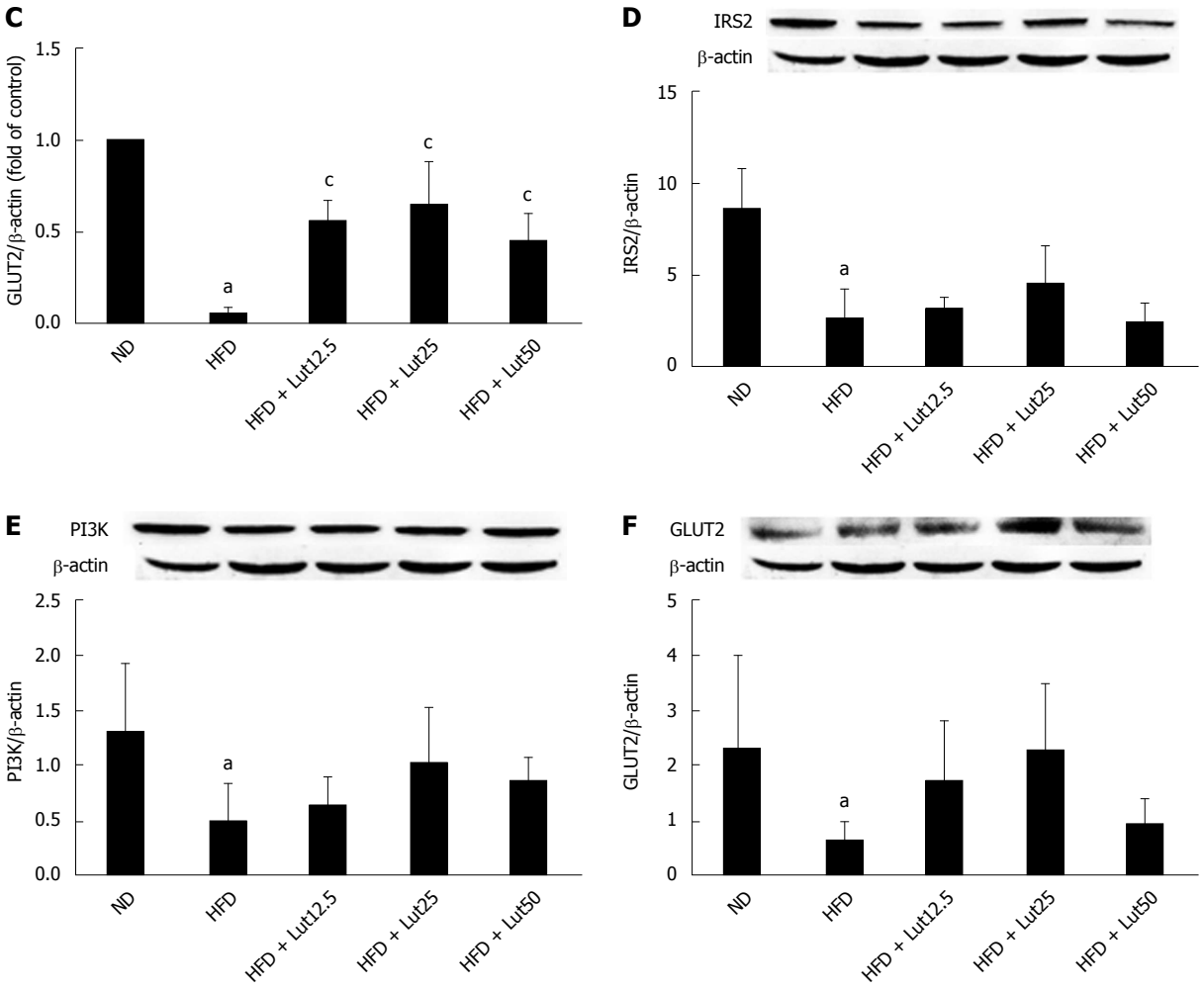


Figure 7 Lutein supplementation improved insulin signaling in rat liver. Effects of lutein on the mRNA expression of IRS2 (A), PI3K (B), and GLUT2 (C) in rat liver (n = 4). Total RNA was extracted from rat livers using TRIzol. IRS2, PI3K, and GLUT2 expression was analyzed by Real-Time RT-PCR. β-actin mRNA was quantified as an endogenous control. IRS2, PI3K, and GLUT2 are presented as fold changes relative to the control. Effect of lutein on the protein expression of hepatic IRS2 (D), PI3K (E), and GLUT2 (F) in rats (n = 3). After the rats were treated with lutein for 45 d, hepatic lysates were prepared and immunoblotted with corresponding antibodies. Blotting with anti β-actin was used as a protein loading control. Data are expressed as the mean ± SD. $^{a}P < 0.05$ *vs* normal diet (ND) group; $^{c}P < 0.05$ *vs* the high-fat diet (HFD) group.

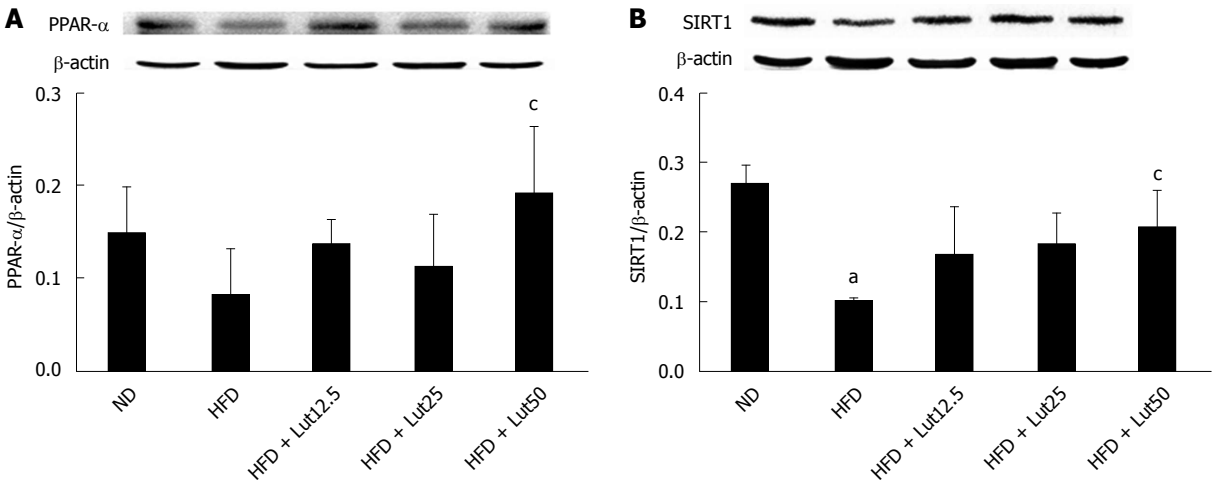


Figure 8 Lutein supplementation improved the expression of peroxisome proliferators activated receptor-α and sirtuin 1 in rat liver. After the rats were treated with lutein for 45 d, the hepatic lysates were prepared and immunoblotted with corresponding antibodies. Blotting with anti β-actin was used as a protein loading control. Data are expressed as the mean ± SD (n = 3). $^{a}P < 0.05$ *vs* normal diet (ND) group; $^{c}P < 0.05$ *vs* the high-fat diet (HFD) group.

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COMMENTS

Background

Non-alcoholic fatty liver disease (NAFLD) is the most common type of chronic liver diseases in the majority of developed countries. With the spread of the Western lifestyle in developing countries, NAFLD is beginning to affect more people. Therefore, it is important to find effective measures for the control of NAFLD. Lutein has been reported to have positive effects on lipid metabolism and insulin resistance, which are two important roles in the pathophysiology of NAFLD. However, there is only limited mechanistic research available regarding the effects of lutein on NAFLD or its risk factors.

Research frontiers

Lutein is one of the hundreds of known naturally oxygenated carotenoids and is abundantly present in vegetables, fruits, and egg yolks. Although, lutein has been found to play a key role in improving visual performance because of its strong blue light filtering ability, it has recently drawn increasing attention to its function in chronic diseases other than oculopathy. In NAFLD, the research hotspot is on ways to modulate lipid metabolism disturbance and insulin resistance.

Innovations and breakthroughs

This study revealed that lutein has positive effects on NAFLD *via* the modulation of hepatic lipid accumulation and insulin resistance, possibly *via* the activation of the expression of sirtuin 1 (SIRT1) and, subsequently, peroxisome proliferator activated receptor-α (PPAR-α). This study provides a new prospect for preventing NAFLD.

Applications

NAFLD is beginning to affect more and more people around the world, possibly due to changes in dietary habits and the increasing prevalence of sedentary lifestyles. Therefore, it is important to develop good dietary habits to treat NAFLD. The results from the present study suggest that lutein, which is abundant in vegetables, fruits, and egg yolks, has potential positive effects on NAFLD.

Terminology

NAFLD is a progressive pathological change in chronic liver disease caused by the disturbance of lipid metabolism together with insulin resistance. The major indexes of lipid metabolism are: total cholesterol, triglyceride, high density lipoprotein-cholesterol, and low density lipoprotein-cholesterol. Glutamic pyruvic transaminase plays an important role in liver function. The key factors in the hepatic insulin signaling pathway are insulin receptor substrate-2, phosphatidylinositol 3-kinase, and glucose transporter-2. PPAR-α is a key factor in the regulation of lipid metabolism. SIRT1 is the upstream regulator of PPAR-α.

Peer-review

The authors evaluated the role of lutein supplementation on hepatic fat content and insulin sensitivity in rats on a high fat diet. It is a well-designed and thorough study showing promising results.

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ORIGINAL ARTICLE

Basic Study

Extrahepatic portacaval shunt *via* a magnetic compression technique: A cadaveric feasibility study

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Author contributions: Yan XP and Liu WY contributed equally to this work; Yan XP and Lv Y designed the study; Yan XP, Lv Y and Ma J performed the research and acquired the data; Yan XP and Li JP analyzed the data; Yan XP, Liu WY and Li JP drafted the manuscript; Yan XP, Liu WY, Ma J and Li JP contributed significantly to the revision of the manuscript.

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Institutional review board statement: The entire study was carried out in strict accordance with protocols approved by the Xi'an Jiaotong University Biomedical Ethics Committee (Ethics Permit Number: 2014-0303).

Institutional animal care and use committee statement: This study does not involve any animal experiments.

Conflict-of-interest statement: The authors declare no conflict of interests.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at luyi169@126.com. Participants gave informed consent for data sharing.

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Abstract

AIM: To explore the anatomical feasibility of portacaval shunt using a magnetic compression technique (MCT) in cadavers.

METHODS: Computed tomography (CT) images of 30 portal hypertensive patients were obtained. The diameters of the portal vein (PV), the inferior vena cava (IVC), and distance between the two structures were measured. Similar measurements were performed on 20 adult corpses. The feasibility of portacaval shunt based on those measurements was analyzed. First stage of the extrahepatic portacaval shunt using MCT was performed on five cadavers. Specifically, the PV and IVC were exposed through an abdominal incision of the cadavers. The parent magnet was introduced from the femoral vein and was delivered into the IVC by an anchor wire and a 5F Cook catheter. The daughter magnet was introduced into the PV through the splenic vein using an

interventional guide wire. When the daughter magnet met the parent magnet, they automatically clipped together and the first stage of the portacaval shunt was set up.

RESULTS: The average diameters of the PV and the IVC measured from the 30 CT image were 14.39 ± 2.36 mm and 18.59 ± 4.97 mm, respectively, and the maximum and minimum distances between the PV and the IVC were 9.79 ± 4.56 mm and 9.50 ± 4.79 mm, respectively. From 20 cadavers, the average diameters of the PV and the IVC were 14.48 ± 1.47 mm and 24.71 ± 2.64 mm, and the maximum and minimum distances between the PV and the IVC were 10.14 ± 1.70 mm and 8.93 ± 1.17 mm, respectively. The distances between the PV and the IVC from both the CT images and the cadavers were within the effective length of portacaval anastomosis using MCT (30.30 ± 4.19 mm). The PV and IVC are in close proximity to each other with no intervening tissues or structures in between. Simulated surgeries of the first stage using MCT on five cadavers was successfully performed.

CONCLUSION: Anatomically, extrahepatic portacaval shunt employing MCT is highly feasible in humans.

Key words: Portal vein; Inferior vena cava; Portacaval shunt; Magnetic compression technique; Anatomy; Cadaver

Core tip: The portacaval shunt using magnetic compression technique (MCT) was first established by our group in a canine model. Here, we assessed its feasibility in humans by analyzing computed tomography images and the anatomical parameters of cadavers. The average diameters of the portal vein (PV), the inferior vena cava (IVC) and the distances between the PV and the IVC were all within the parameters that allowed the setup of portacaval shunt using MCT. Finally, first stage simulation of the portacaval shunt was successfully set up in five cadavers. Our results indicated that the portacaval shunt is highly feasible in humans.

Yan XP, Liu WY, Ma J, Li JP, Lv Y. Extrahepatic portacaval shunt *via* a magnetic compression technique: A cadaveric feasibility study. *World J Gastroenterol* 2015; 21(26): 8073-8080 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i26/8073.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i26.8073

INTRODUCTION

Surgical shunting is an important treatment option for portal hypertension[1]. The traditional, hand-sewn surgical shunt currently used is time-consuming, highly technical and subject to postoperative complications. Side-to-side portacaval shunt has a high incidence of hepatic encephalopathy[2,3]. H-graft portacaval shunt, as a limited portacaval shunt, can effectively reduce the incidence of hepatic encephalopathy[4]. However, it is also technically challenging and patients who undergo the procedure must be put on long-term use of anticoagulants.

The magnetic compression technique (MCT) exploits the attraction between magnets to pull together an area of ischemic necrosis and its surrounding tissue to promote healing. The major advantages of the MCT are that it is simple, minimally invasive and reliable. Currently, MCT is applied in esophageal atresia[5,6], biliary stenosis[7-12], vascular anastomosis[13-19] and gastro-intestinal anastomosis[20-27]. Previously, we applied Roux-en-Y choledochojejunostomy incorporating MCT for obstructive jaundice in a canine model[28]. We also confirmed that magnetic rings could be used for rapid vascular reconstruction in a canine liver transplantation model[19].

We first reported the establishment of a portacaval shunt using MCT in dogs, the significant advantage of which is that no stent or any other foreign device was installed[29]. In the present study, we assessed some anatomical parameters that might affect the establishment of extrahepatic portacaval shunt using MCT in humans, and performed five preliminary cadaveric surgeries.

MATERIALS AND METHODS

Ethical statement

The entire study was carried out in strict accordance with protocols approved by the Xi'an Jiaotong University Biomedical Ethics Committee (Ethics Permit Number: 2014-0303). The cadavers were obtained from the Department of Anatomy, Health Science Center, Xi'an Jiaotong University, Shaan Xi Province, China. Written consent was obtained from the immediate family members of the deceased for educational and scientific research purposes. The format of the consent form is in accordance with the guidelines of the China Organ Donation Administrative Center.

The Xi'an Jiaotong University Biomedical Ethics Committee approved the use of the computed tomography (CT) data collected from patients. The project was explained to the patients. The privacy of the patients in this study was strictly protected and only age and CT data essential for the study were collected.

Human cadaver and gross anatomy

Twenty adult cadavers (14 male, 6 female) embalmed with formaldehyde were obtained. Cadavers of people with a history of abdominal surgery; abdominal tumor that might change the anatomical location of portal vein (PV) or inferior vena cava (IVC); diseases of PV or IVC system; prolonged storage after death, or improper

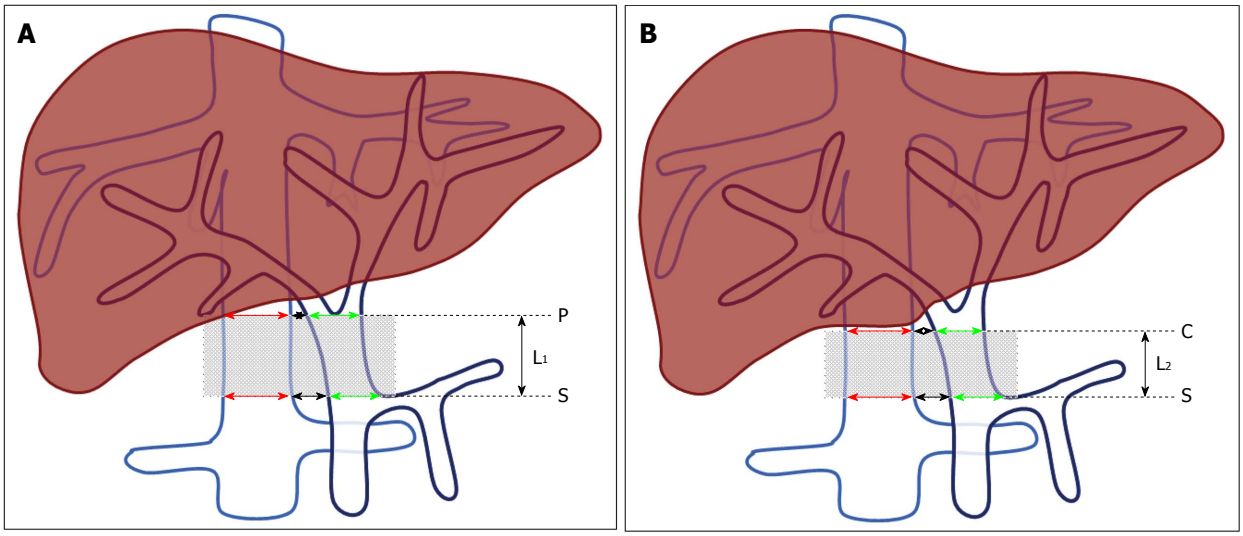

Figure 1 Effective length of the portacaval anastomosis using the magnetic compression technique. A: When the origin of the portal vein (PV) branch is below the lower edge of the caudate lobe, the effective length, L1, is the distance between the intersection of the splenic vein and the PV, here S, and the origin of the PV branching, P. B: When the origin of the PV branch is higher than the lower edge of the caudate lobe, the effective length, L2, is the distance between S and the lower edge of caudate lobe, C. The red arrow indicates the diameter of the inferior vena cava (IVC); the green arrow indicates the diameter of the PV; the black arrow indicates the distance between the PV and the IVC.

handling that might interfere with the measurements were excluded.

We first opened the abdomen of the cadaver and assessed the abdominal organs, and verified the absence of structural abnormalities or obvious lesions. We then incised the hepatoduodenal ligament, blunt dissected and observed the PV, IVC, common bile duct, and hepatic proper artery. The lower boundary of the effective area of portacaval anastomosis was defined as the plane of the splenic-mesenteric confluence (S, Figure 1). The upper boundary was defined as the plane of PV bifurcation (P, Figure 1A) or the inferior margin of the liver caudate lobe (C, Figure 1B). The effective length of the portacaval anastomosis was defined as the distance between S and P (L1, Figure 1A) or S and C (L2, Figure 1B). Twenty cadavers were measured.

CT images of patients with portal hypertension

From 2012 to 2013, 30 adult patients with portal hypertension underwent abdominal CT scans. All CT studies were performed with a 16-slice CT scanner (Somatom Sensation 16; Siemens, Germany). Patients with a history of abdominal surgery, portal venous system thrombosis, or other factors that might lead to distinct anatomical changes of the PV or IVC were excluded. The diameters of the PV and IVC at the position of the splenic-mesenteric confluence (S, Figure 1) and PV bifurcation (P, Figure 1A) or inferior margin of the liver caudate lobe (C, Figure 1B) were each measured as illustrated in Figure 1.

Magnetic device

Two neodymium (Nd-Fe-B) permanent magnets (parent and daughter) were custom-manufactured for vascular experimental use (Northwest Institute for Nonferrous Metal Research, Xi'an, China; Figure 2). The parent magnet was a semi-cylinder with round ends, 15 mm L × 4 mm W × 3 mm H (Figure 2A-C). The parent magnet was attached to a special anchor wire, which was modified from a 2-0 prolene wire (Ethicon; Johnson and Johnson, Somerville, NJ, United States) by cutting off the suture needles from its ends. The daughter magnet, without wire, has the same dimensions as the parent magnet but with a long-axis paralleled foramen (diameter, 1.2 mm) inserted through the center.

The parent magnet was introduced from the femoral vein and delivered into the IVC by an anchor wire and a 5F Cook catheter (Cook, Bloomington, IN, United States). An interventional guide wire was inserted through the foramen of the daughter magnet. The daughter magnet was introduced into the PV through the splenic vein using an interventional guide wire (0.035 in; TERUMO, Japan) and a 5F Cook catheter (Cook; Figure 2D).

The force of the parent and daughter magnet was 6.8 N with 0-mm intermagnet separation and magnetic density 2400 G. The weights of the parent and daughter magnet were 0.80 g and 0.75 g, respectively.

Shunt procedure

Shunt surgery was performed with reference to our previous procedure[29]. The PV and IVC were exposed through an abdominal incision, and the splenic vein and 15 mm of the right femoral vein were exposed. The prolene wire affixed to the parent magnet was inserted into a 5F Cook catheter, and the end of the prolene wire was tightly drawn to ensure that the parent magnet was fixed to the other end of the Cook catheter. An incision was made at the right femoral

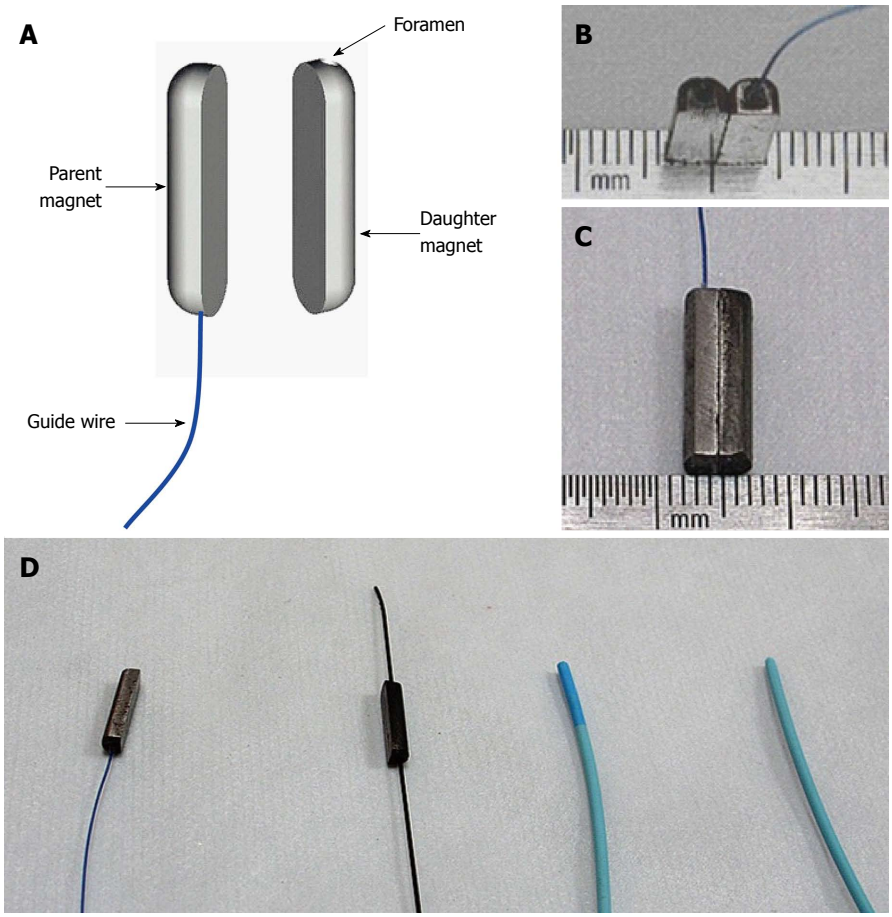

Figure 2 Magnetic compression system devices. A: Schematic of the parent and daughter magnets; B, C: The magnetic compression system devices; D: Guide wire and catheter used to complete the operation.

vein. Then, through the incision, the parent magnet was advanced to the target position on the IVC by the 5F Cook catheter.

A venotomy was performed on the splenic vein. An interventional guide wire was inserted through the hole of the daughter magnet. One end of the interventional guide wire was introduced into the PV through the incision on the splenic vein, and the other end was inserted into a 5F Cook catheter and extended the catheter. While the interventional guide wire was maintained in the PV, the daughter magnet, guided by the catheter, was moved to the target position of the PV through the incision of the splenic vein.

After the daughter magnet met the parent magnet, they automatically clipped together. The interventional guide wire and catheter were then withdrawn from the splenic vein and femoral vein. The first stage (steps 1-4) of the surgery was thus finished.

After a period of time (5-7 d in dogs), a RUPS-100 set with vascular sheath was navigated to the position of the magnet in the IVC through the femoral vein. Under X-ray guidance, the needle of the RUPS-100 set was advanced slowly and smoothly along the outline of the magnets and the magnets was detached from the vascular wall. The magnets were pulled out of the body by the wire fixed to the parent magnet. The portal-IVC shunt was set up (Figure 3).

In this study, only the first stage shunt procedures were performed on five cadavers.

Statistical analysis

The statistical methods of this study were reviewed by Qian Li from First Affiliated Hospital, Xi'an Jiaotong University. Data are expressed as mean ± SD. Gross anatomy and CT scan data were analyzed using an independent samples *t*-test. A *P*-value < 0.05 was considered statistically significant. Data were analyzed with SPSS 17.0 software.

RESULTS

In this cadaveric study, the IVC was at the right-posterior position of the PV; the hepatoduodenal ligament, a small amount of connective tissue and the retroperitoneum lay between them. The hepatic proper artery was located at the left-inferior side of the PV and the common bile duct was right anterior to the PV (Figure 4). There was no important structure in the effective area of the portacaval anastomosis.

From 30 CT imaging measurements, the average diameters of the PV and the IVC in portal hypertensive patients were 14.39 ± 2.36 mm and 18.59 ± 4.97 mm,

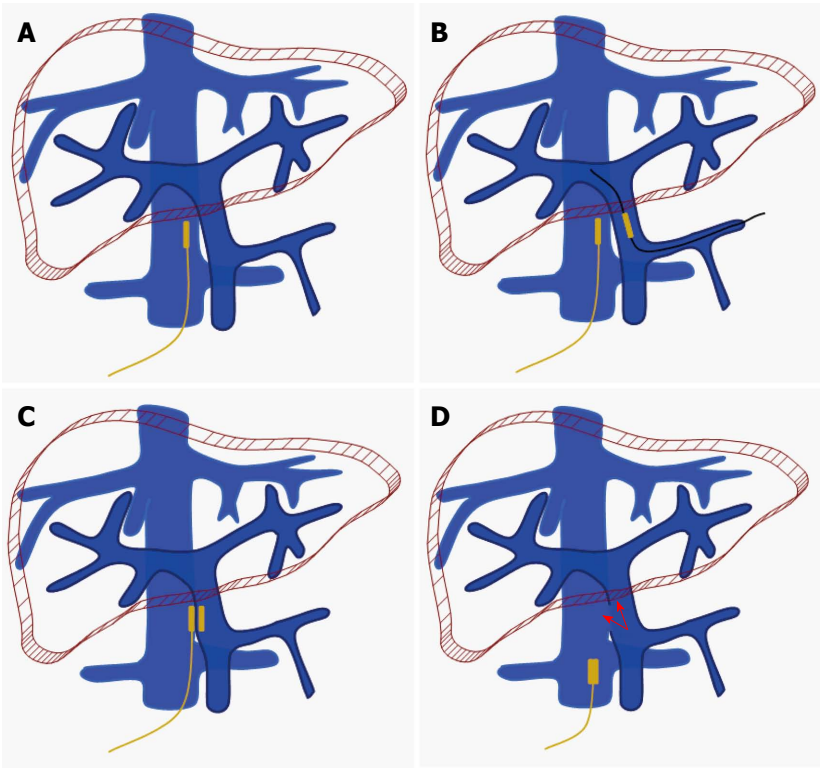

Figure 3 Surgical protocol and procedures. A: The parent magnet was guided to the target position of the inferior vena cava (IVC) by the wire fixed to one end and the catheter; B: The daughter magnet was advanced to the anastomosis position of the portal vein (PV) by the guide wire and catheter through the incision on the splenic vein; C: Position of the magnets and the wire after the first surgery; D: The magnets were pulled out of the body by the wire fixed to the parent magnet and the portal-IVC shunt was set up.

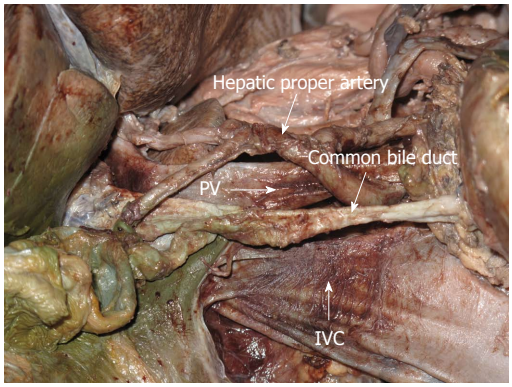

Figure 4 Anatomy of portacaval anastomosis in a cadaver. The right posterior wall of the portal vein (PV) faces the left anterior wall of the inferior postcava. The common bile duct is at the right anterior of the PV. There is no important tissue in the postcaval space.

Table 1 Parameters of the portal vein and the inferior vena cava from cadavers and computed tomography scan of portal hypertension patients

	CT scan[1]	Cadaver[2]	*P* value[3]
Male, *n*	17	14	
Female, *n*	13	6	
Age, yr	50.2 ± 11.0	53.9 ± 10.7	
Diameter of PV, mm	14.39 ± 2.36	14.48 ± 1.47	0.871
Diameter of IVC, mm	17.17 ± 5.94	24.71 ± 2.64	0.000
Interval space 1, mm[4]	9.50 ± 4.79	10.14 ± 1.70	0.509
Interval space 2, mm[5]	9.79 ± 4.56	8.93 ± 1.17	0.332
Effective length, mm	-	30.30 ± 4.19	-

[1]*n* = 30; [2]*n* = 20; [3]*P* value < 0.05 was considered significant; [4]at the plane of S; [5]at the plane of P or C. PV: Portal vein; IVC: Inferior vena cava; CT: Computed tomography.

respectively (Table 1). The maximum and minimum distances between the PV and the IVC were 9.79 ± 4.56 mm and 9.50 ± 4.79 mm, respectively (Table 1).

From 20 cadavers, the average diameters of the PV and IVC were 14.48 ± 1.47 mm and 24.71 ± 2.64 mm, respectively (Table 1). The maximum and minimum distances between the PV and the IVC were 10.14 ± 1.70 mm and 8.93 ± 1.17 mm , respectively (Table 1). The distances between the PV and the IVC from both the CT image analysis and that of the cadavers are within the effective length of portacaval anastomosis using MCT (30.30 ± 4.19 mm).

The average diameters of the IVC determined from CT image measurements and those from the cadavers were significantly different (P = 0.000). The cross sections of the IVCs were highly variable on CT images: some were oval rather than round shaped. In such cases, we measured the length and width of the oval-shaped cross section and took the average as the value of its diameter. However, this measurement might not be accurate and could account for the deviation between CT and cadaveric measurements. Nevertheless, despite the discrepancy, the value of the IVC diameter measured from CT images was still within the parameters that allow the performance of

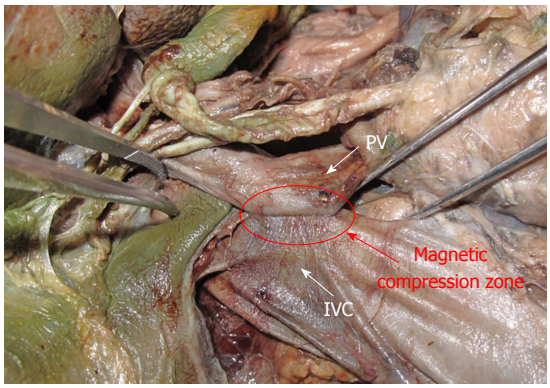

Figure 5 Compression of the walls of the portal vein and postcava implemented through the attraction of the parent and daughter magnets in a cadaver. The elliptical area circled in red indicates the effective zone of the compression. No other vessel or bile duct was compressed. PV: Portal vein; IVC: Inferior vena cava.

the operation. There were no significant differences between the CT images and cadavers with regard to the diameters of the PV or the distances between the PV and the IVC ($P > 0.005$). This suggested that measurements taken from CT images are mostly reliable and close to the anatomical measurements.

The operation utilizing MCT was performed on five adult cadavers successfully, without any unexpected events. When the parent and daughter magnets each reached their target positions, they automatically joined and there was no damage to the proper hepatic artery, common bile duct, or other important structures because of the compression. More importantly, the magnetism was maintained at the satisfactory force and overcame the tension of the blood vessels (Figure 5).

DISCUSSION

Although we previously verified the reliability, feasibility, safety and efficiency of MCT in dogs[29], three essential issues remain to be resolved before it can be applied in humans. Firstly, whether there are intervening structures between the PV and the IVC that will be compressed when the parent and daughter magnets join. Secondly, whether the magnetic force between the parent and daughter magnets is strong enough to pull the two structures together. Thirdly, whether human anatomical characteristics allow this operation. The present study addressed all three of these key issues.

Zirinsky *et al*[30] first proposed the concept of a portacaval space, defining it as the gap between the PV and the IVC. Many studies have shown that the caudate lobe of the liver is generally in the upper part of the portacaval space, and the portacaval node, the portacaval vessels, and the cystic duct are in the lower part of the portacaval space[31,32]. Our anatomical analysis of 20 adult cadavers indicated that these structures cannot interfere with the portacaval anastomosis and would not be affected by the procedure. The hepatic PV lies left-inferior to the IVC; thus, when the parent and daughter magnets join, the right-posterior wall of the hepatic PV and the left-anterior wall of the IVC are compressed. Our autopsies showed that the proper hepatic artery and the bile duct are located left-inferior and right-inferior of the hepatic PV, respectively. However, the simulation procedure on the corpses confirmed that the magnets hardly compress these two structures.

One prerequisite for the procedure is that the distance between the PV and the IVC is within the working range of the magnets. According to our previous studies, the maximum distance that will allow attraction between the magnets is 3.5 cm. However, the ideal interval in experimental dogs was < 1.6 cm, because of the tension of the hepatic PV and the IVC vessel. The mean minimum distance between the hepatic PV and the IVC, based on measurements in the 20 adult corpses, was 0.96 cm ± 0.17 cm. Therefore, we assumed that the parent and daughter magnets are also able overcome the resistance of the blood vessels in the human body. We concluded that the magnetic attraction is sufficient to pull the PV and the IVC together.

In our previous study in dogs[29], the designed magnet was 12 mm in length and 2.5 mm in width. In humans, the diameter of the transjugular intrahepatic portosystemic shunt (TIPS) stent and prosthetic H-graft portacaval shunt is from 8 to 10 mm[1,4]. In our current study, the dimension of the magnet used was 15 mm × 4 mm × 3 mm. Our anatomical analysis revealed that the dimension of the hepatic PV and the IVC is sufficient to accommodate this size. Therefore, we suggest that the optimum length of a magnet used for a human portacaval shunt should be around 10 to 15 mm.

To better visualize the progress of the surgery, we opened the cadavers' abdomen in this study. We reset the daughter magnet to the hepatic PV *via* the splenic vein. However, it is highly feasible clinically to implement minimally invasive surgery in humans by introducing the daughter magnet to the hepatic PV *via* the transjugular and transhepatic path.

Taken together, the results presented in the present study addressed the three essential issues regarding the feasibility of portacaval shunt with MCT in humans.

Based on our preliminary studies, the automatic assembly of the magnets was the critical problem. We emphasized the importance of a lack of previous abdominal surgeries, because postoperative adhesion might lead to extra resistance and impede attachment of the magnets. In the procedure, once the magnets had successfully clipped together, detachment was unavailable under interventional guidance; thus, it was crucial to hold still one magnet (the daughter one or the parent one) at the suitable compressed spot till the other one came for assembling. Also, during the

withdrawal of the magnets, it required the assistance of a RUPS-100 needle to detach the device and the risk of puncturing IVC should be avoided.

A limitation of this study is that we only performed the first stage of the entire procedure. This is because the second stage requires the formation of copious fibrous connective tissue around the anastomosis, which is impossible to simulate in cadavers. In addition, the timing of the second surgery is crucial to the success of MCT - if there is not enough time remold, hemorrhage will be inevitable during the second surgery. On the other hand, if the second surgery is performed after a prolonged interval after the first, the magnets will become embedded in the adhesive tissues and will be difficult to remove. Based on our experience, 7 d after the first surgery is the optimum working window for the second surgery in dogs. The optimum timeframe for portacaval remolding in humans requires further study.

In conclusion, through preliminary trials on cadavers we confirmed the anatomical feasibility of implementing extrahepatic portacaval shunt using magnetic compression in humans. However, this pioneering technique requires further clinical validation.

COMMENTS

Background

Magnetic compression techniques (MCTs) have been applied in alimentary anastomosis, such as gastroenterostomy and bilioenterostomy, as well as in the end-to-end or side-to side vascular anastomosis. The authors originally established the side-to-side portacaval shunt using magnetic compression and interventional techniques in dogs. They analyzed the anatomical data and conducted the simulated operation on cadavers, aiming to demonstrate the preliminarily feasibility of this procedure in humans.

Research frontiers

The MCT, which is easy, reliable, efficient and fast, has been intensively researched and applied in hollow organ anastomosis; however, no application in portacaval shunt establishment has been reported.

Innovations and breakthroughs

This study established an innovative side-to-side portacaval shunt combining magnetic compression and interventional techniques, and laid a foundation for the further clinical application by analyzing the anatomical relations and conducting a simulated procedure on corpses.

Applications

Utilizing the side-to-side portacaval shunt under magnetic compression and interventional techniques represents an alternative for the portal hypertension therapy, with the advantages such as minimal invasiveness, stable stoma and freedom from long-term implants.

Terminology

Magnetic compression is a novel procedure utilizing a magnetic force to perform sutureless anastomosis in hollow organs. Combined with endoscopic or interventional techniques, some conventional laparotomies may be solved using this simplified minimal invasive procedure.

Peer-review

The research group pioneered the animal experiment of portacaval shunt using a MCT. Based on a previous study, they further tested its feasibility for human application. The study was well designed and conducted with great invention.

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ORIGINAL ARTICLE

Basic Study

Ischemic preconditioning ameliorates intestinal injury induced by ischemia-reperfusion in rats

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Author contributions: Ji YY and Wang ZD contributed equally to this work; Ji YY and Wang ZD performed the research and wrote the paper; Ji YY, Wang ZD, Wang SF, Wang BT, Yang ZA and Zhou XR designed the research and analyzed the data; Lei NN and Yue WN contributed materials and methods.

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Institutional animal care and use committee statement: All animal experiments were carried out in accordance with the Animal Care Committee of Xi'an Jiaotong University, China.

Animal care and use statement: The animal protocol was designed to minimize pain or discomfort to the animals. The animals were acclimatized to laboratory conditions (23 ℃, 12h/12h light/dark cycle, 50% humidity, and *ad libitum* access to food and water) for two weeks prior to experimentation. All animals were euthanized by barbiturate overdose (150 mg/kg pentobarbital sodium, i.v.) for tissue collection.

Conflict-of-interest statement: The authors have no competing interests.

Data sharing statement: No additional data are available.

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Abstract

AIM: To evaluate preventative effects of ischemic preconditioning (IP) in a rat model of intestinal injury induced by ischemia-reperfusion (IR).

METHODS: Male Sprague-Dawley rats (250-300 g) were fasted for 24 h with free access to water prior to the operation. Eighteen rats were randomly divided into three experimental groups: S group (n = 6), rats were subjected to isolation of the superior mesenteric artery (SMA) for 40 min, then the abdomen was closed; IR

group (n = 6), rats were subjected to clamping the SMA 40 min, and the abdomen was closed followed by a 4-h reperfusion; IP group (n = 6) rats underwent three cycles of 5 min ischemia and 5 min reperfusion, then clamping of the SMA for 40 min, then the abdomen was closed and a 4-h reperfusion followed. All animals were euthanized by barbiturate overdose (150 mg/kg pentobarbital sodium, i.v.) for tissue collection, and the SMA was isolated *via* median abdominal incision. Intestinal histologic injury was observed. Malondialdehyde (MDA), myeloperoxidase (MPO) and tumor necrosis factor (TNF)-α concentrations in intestinal tissue were measured. Intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 expression, as well as nuclear factor (NF)-κB activity and expression in intestinal tissue were also determined.

RESULTS: Compared with the IR group, IP reduced IR-induced histologic injury of the intestine in rats (2.00 ± 0.71 *vs* 3.60 ± 0.84, $P < 0.05$). IP significantly inhibited the increase in MDA content (5.6 ± 0.15 μmol/L *vs* 6.84 ± 0.18 μmol/L, $P < 0.01$), MPO activity (0.13 ± 0.01 U/L *vs* 0.24 ± 0.01 U/L, $P < 0.01$), and TNF-α levels (7.79 ± 2.35 pg/mL *vs* 10.87 ± 2.48 pg/mL, $P < 0.05$) in the intestinal tissue of rats. IP also markedly ameliorated the increase in ICAM-1 (204.67 ± 53.27 *vs* 353.33 ± 45.19, $P < 0.05$) and VCAM-1 (256.67 ± 58.59 *vs* 377.33 ± 41.42, $P < 0.05$) protein expression in the intestinal tissues. Additionally, IP remarkably decreased NF-κB activity (0.48 ± 0.16 *vs* 0.76 ± 0.22, $P < 0.05$) and protein expression (320.23 ± 38.16 *vs* 520.76 ± 40.53, $P < 0.01$) in rat intestinal tissue.

CONCLUSION: IP may protect against IR-induced intestinal injury by attenuation of the neutrophil-endothelial adhesion cascade *via* reducing ICAM-1 and VCAM-1 expression and TNF-α-induced NF-κB signaling pathway activity.

Key words: Intercellular adhesion molecule; Ischemia-reperfusion; Ischemic preconditioning; Nuclear factor-κB; Tumor necrosis factor-α

Core tip: Although intestinal ischemic preconditioning (IP) triggers powerful protective effects, particularly in surgical ischemic operation and transplantation, mechanisms by which IP alleviates intestinal injury remain to be elucidated. The present study found that IP protects against intestinal ischemia-reperfusion-induced injury by reducing intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 expression, and tumor necrosis factor-α-induced nuclear factor-κB signaling pathway activity. The results suggest that intestinal IP may be clinically useful in the future to treat patients with intestinal ischemia-reperfusion-induced injury.

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INTRODUCTION

The intestine is more sensitive to the injury induced by ischemia-reperfusion (IR) than other internal organs[1]. Ischemia followed by reperfusion injury contributes to a complex series of events, including nitric oxide imbalance, neutrophil accumulation, cell apoptosis, and activation of inflammatory mediators, causing cellular and tissue damage[2,3]. Intestinal IR injury, which can been presented in several clinical settings such as intestinal obstruction, hemorrhagic shock, intestinal transplantation, and neonatal necrotizing enterocolitis, may result in local and remote organ damage including systemic inflammatory response syndromes and multiple organ dysfunction syndrome[4].

Various methods with protective effects, such as ischemic preconditioning (IP), pharmacologic treatment, and chemical agents, have been applied to decrease intestinal IR injury. IP refers to a phenomenon in which a tissue or organ is rendered resistant to the deleterious effects of prolonged IR by prior exposure to brief periods of vascular occlusion, displaying beneficial effects locally and systemically, reducing mucosal damage, neutrophil accumulation, cell apoptosis, and activation of inflammatory mediators. IP was first proposed as a possible therapeutic strategy in 1986 by Murry *et al*[5], who showed it reduced infarct size in rat heart. With respect to intestinal IP, Hotter *et al*[6] first reported the protection of IP from intestinal IR injury in 1996. Intestinal IP, repetitive brief periods of ischemia, provides a way of protecting intestinal tissue from damage inflicted by IR.

Intestinal IP has been widely investigated in recent years, ameliorating neutrophil sequestration, attenuating cell apoptosis, and decreasing inflammatory mediator release such as tumor necrosis factor (TNF)-α and interleukin-1[7-10]. However, the protective mechanisms of intestinal IP remain poorly understand. Therefore, the aim of this study was to determine the effects of IP in a rat model of intestinal IR by assessing histologic injury, oxygen-free radical injury [malondialdehyde (MDA)], neutrophil infiltration [myeloperoxidase (MPO)], inflammatory response (TNF-α), expression of endothelial cell adhesion molecules [intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1], and

nuclear factor (NF)-κB activity and expression.

MATERIALS AND METHODS

Animals

All animal experiments were carried out in accordance with the Animal Care Committee of Xi'an Jiaotong University, China. Eighteen male Sprague-Dawley rats (250-300 g) were purchased from the animal center of Xi'an Jiaotong University and housed under pathogen-free conditions. The animal protocol was designed to minimize pain or discomfort to the animals. The animals were acclimatized to laboratory conditions (23 ℃, 12 h/12 h light/dark cycle, 50% humidity, *ad libitum* access to food and water) for 2 wk prior to experimentation. Animals were fasted 24 h and with free access to water prior to the operation. All animals were euthanized by barbiturate overdose (150 mg/kg pentobarbital sodium, i.v.) for tissue collection following isolation of the superior mesenteric artery (SMA) *via* median abdominal incision, as previously described[4].

Experimental design

The study was reviewed and approved by the Institutional Review Board of Xi'an Jiaotong University. Animals were randomly assigned to one of three groups: S group (n = 6), rats were subjected to isolation of the SMA for 40 min, then the abdomen was closed; IR group (n = 6), rats were subjected to clamping of the SMA for 40 min, and the abdomen was closed followed by a 4-h reperfusion; IP group (n = 6), rats underwent three cycles of 5 min ischemia and 5 min reperfusion, then clamping of the SMA for 40 min, then the abdomen was closed, followed by a 4-h reperfusion, as previously described[4].

Assessment of MDA level in intestine

Intestinal tissues (1.0-cm segments from 5 cm of the terminal ileum) were harvested, frozen immediately, and stored at -80 ℃ until assessment. A homogenate of 10% (w/v) was prepared. MDA level in intestinal tissues was determined by an MDA detection kit (Nanjing JianCheng Bioengineering Institute, Nanjing, China) following the colorimetric method provided by the manufacturer. The optical densities were read using a spectrophotometer at an absorbance of 532 nm. The results are expressed as μmol/L. Each sample was analyzed in duplicate and the results were averaged.

Determination of MPO activity in intestine

At the end of experiments, a 1.0-cm segment of intestinal tissue (from 5 cm of the terminal ileum) was harvested from each rat, frozen immediately, and stored at -80 ℃ until assessment. MPO, a mark of neutrophil accumulation and activation, was tested using a commercial assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). In brief, the intestinal tissues were homogenized in 50 mmol/L potassium phosphate buffer containing 0.5% hexadecyltrimethyl ammonium bromide (pH 6). The intestinal homogenates were centrifuged at 12500 *g* at 4 ℃ for 10 min. The supernatants were collected and reacted with 0.167 g/L *o*-dianisidine dihydrochloride, following 0.0005% H_2O_2 in 50 mmol/L phosphate buffer. Finally, the absorbance was determined spectrophotometrically at 460 nm.

Detection of TNF-α levels in intestine

A 1.0-cm segment intestinal tissue (from 5 cm of the terminal ileum) was harvested from each rat and frozen. Frozen tissues were homogenized and centrifuged at 1500 *g* for 15 min. Supernatants were transferred into fresh tubes for detection of TNF-α, determined using ELISA kits (R&D Systems, Minneapolis, MN, United States) according to manufacturer's procedure. The absorbance was read at 450 nm by a microplate reader (Biotek Instruments, CA, United States). The results are expressed as pg/mL.

Histopathologic examination of intestinal injury

A 1.0-cm segment of intestinal tissue (from 5 cm of the terminal ileum) was harvested from each rat and fixed in 10% formaldehyde. The tissues were paraffin-embedded and then stained with hematoxylin and eosin for light microscopy. Intestinal mucosal damage was evaluated by three expert investigators blinded to the experimental groups using the criteria of Chiu *et al*[11]. A score scaled at 0 to 5 represents the severity: 0, normal mucosa villi; 1, development of subepithelial Gruenhagen's space at the tip of villus; 2, extension of the subepithelial space with moderate epithelial lifting; 3, large epithelial lifting, possibly with a few denuded villi; 4, denuded villi with lamina propria and exposed capillaries; 5, disintegration of the lamina propria, ulceration, and hemorrhage.

NF-κB activity assay

NF-κB (p65) DNA-binding activity in the intestinal homogenate was tested using a commercial kit. The assay uses a 96-well plate coated with oligonucleotides containing the NF-κB-binding site. Activated NF-κB binds to the oligonucleotides and is tested by a specific antibody. The amount of active NF-κB was determined using a microplate reader (Biotek Instruments).

Western blot analysis

Protein samples (20 μg) were loaded onto SDS-PAGE gels and transferred onto a polyvinylidene difluoride membrane pretreated with 100% methanol (Bio-Rad Laboratories, Hercules, CA, United States). The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween 20, and then rat monoclonal anti-ICAM-1 (1:500), VCAM-1 (1:500), and NF-κB (p65) (1:400) antibodies (Santa Cruz Biotechnology, Inc., Dallas, TX, United States) were added to the supernatant and the mixture was incubated on a rotating wheel at 4 ℃ overnight;

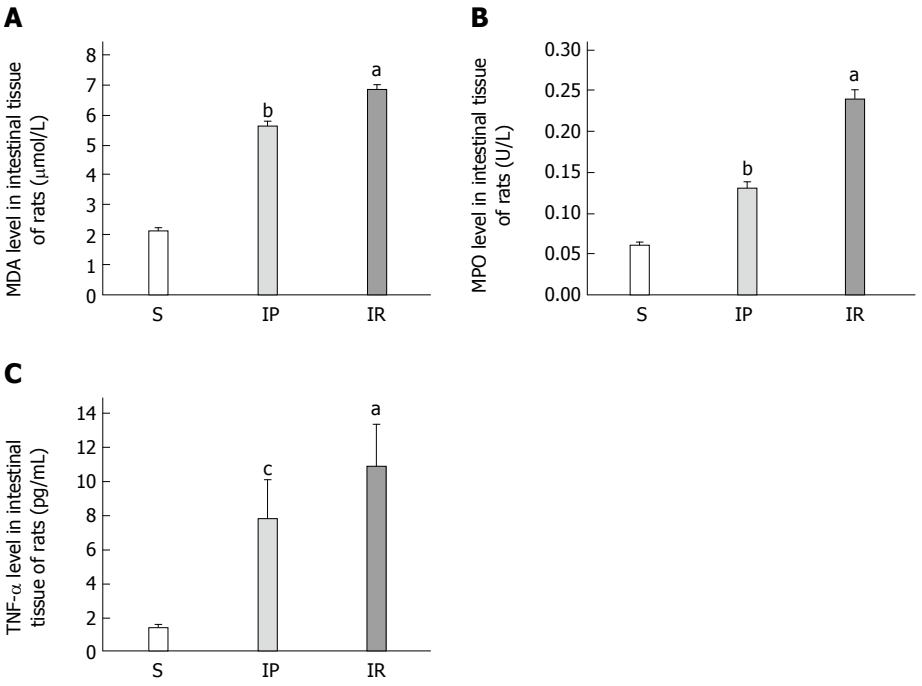

Figure 1 Intestinal tissue malondialdehyde, myeloperoxidase and tumor necrosis factor-α levels in rats. A: Malondialdehyde; B: Myeloperoxidase (MPO); and C: Tumor necrosis factor (TNF)-α levels in intestinal tissue. Data are presented as mean ± SD (n = 6). [a]P < 0.01 *vs* S group; [b]P < 0.01 *vs* IR group. S: Sham group; IP: Ischemic-preconditioning group; IR: Ischemia-reperfusion group.

β-actin (1:400) was used as a loading control. On the second day, membranes were washed with the same three times and incubated with a secondary antibody conjugated to horseradish peroxidase (1:2000; Santa Cruz Biotechnology, Inc.) for 1 h at room temperature, and the light signals were detected by X-ray film. Optical densities of the bands were scanned and quantified with the Syngene Gene Tools (Syngene Corp., Cambridge, United Kingdom). Three independent experiments were carried out to study protein expressions.

Statistical analysis

Data are expressed as mean ± SD, and were analyzed by analyses of variance followed by Tukey's *post hoc* tests (Prism 5; GraphPad Software, Inc., La Jolla, CA, United States). Differences were considered significant when P < 0.05.

RESULTS

Intestinal IP attenuates MDA level

We examined whether IP attenuates intestinal tissue injury in rats. In this study, we characterized intestinal tissue injury using MDA levels tested 4 h after intestinal IP or IR. As shown in Figure 1A, intestinal tissue MDA levels in the IR group were significantly increased compared to the S group (6.84 ± 0.18 μmol/L *vs* 2.13 ± 0.11 μmol/L, P < 0.01). IP significantly decreased MDA levels compared to the IR group (5.6 ± 0.15 μmol/L *vs* 6.84 ± 0.18 μmol/L, P < 0.01).

Intestinal IP decreases MPO level

We next evaluated whether IP decreases aggregation and adhesion of neutrophils in intestinal tissue. In this study, we tested intestinal MPO as marker of aggregation and adhesion of neutrophils. As shown in Figure 1B, MPO levels in the IR group were significantly increased compared to the S group (0.24 ± 0.01 U/L *vs* 0.06 ± 0.00 U/L, P < 0.01). IP significantly decreased MPO levels compared to the IR group (0.13 ± 0.01 U/L *vs* 0.24 ± 0.01 U/L, P < 0.01).

Intestinal IP suppresses TNF-α levels

We then measured whether IP suppresses levels of proinflammatory molecules in intestinal tissue by measuring TNF-α. As shown in Figure 1C, intestinal tissue TNF-α levels in the IR group were significantly increased compared to the S group (10.87 ± 2.48 pg/mL *vs* 1.37 ± 0.21 pg/mL, P < 0.01). IP significantly decreased TNF-α levels compared to the IR group (7.79 ± 2.35 pg/mL *vs* 10.87 ± 2.48 pg/mL, P < 0.05).

Intestinal IP attenuates intestinal tissue injury

We next investigated whether IP attenuates intestinal tissue injury assessed using the criteria of Chiu *et al*[11]. As shown in Figure 2, intestinal tissue injury in the IR group was significantly increased compared to the S group (3.60 ± 0.84 *vs* 0.40 ± 0.55, P < 0.01). IP significantly decreased intestinal tissue injury

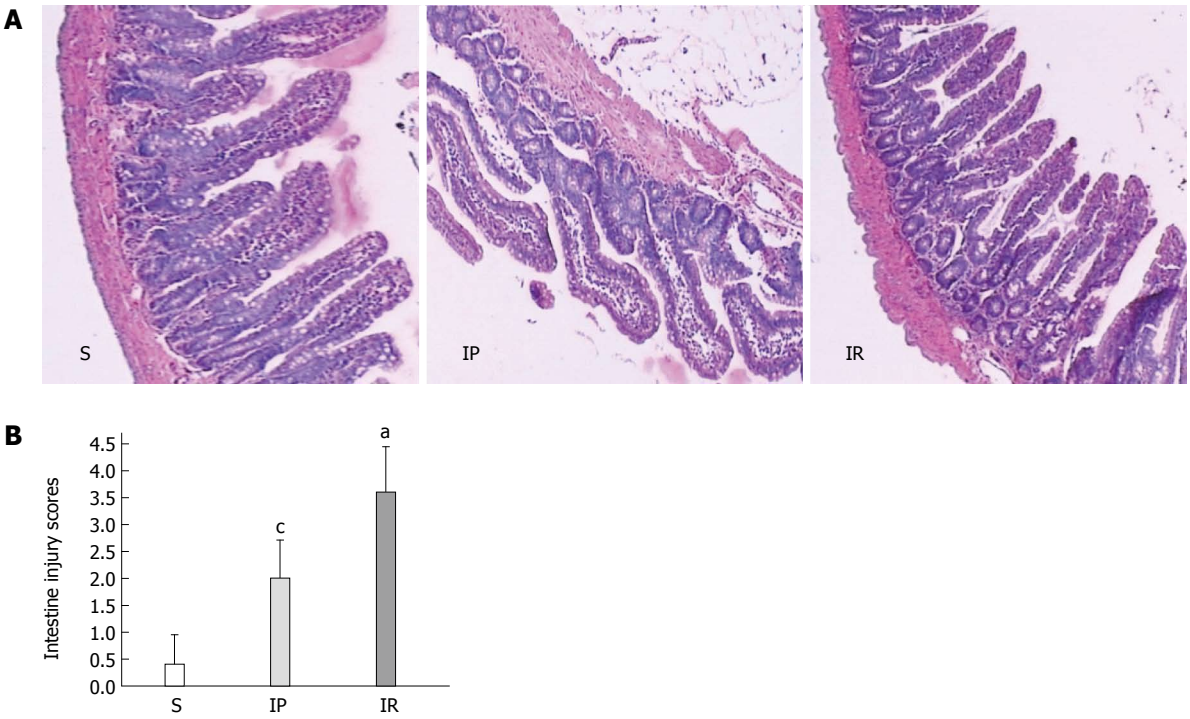

Figure 2 Morphologic changes in rat intestinal tissue. A: Representative morphologic pictures of intestinal tissues (hematoxylin-eosin staining; × 400 magnification); B: Intestinal injury scores in rats. Data are presented as mean ± SD (n = 6). $^{a}P < 0.01$ *vs* S group; $^{c}P < 0.05$ *vs* IR group. S: Sham group; IP: Ischemic-preconditioning group; IR: Ischemia-reperfusion group.

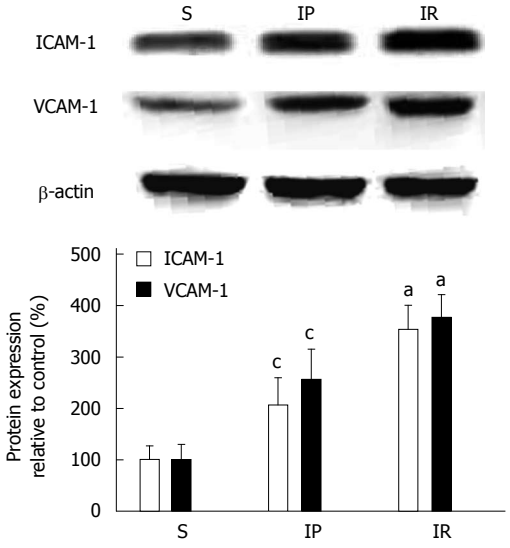

Figure 3 Protein expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in rat intestinal tissue. Protein expression was measured by Western blot. Data are presented as mean ± SD (n = 6). $^{a}P < 0.01$ *vs* S group; $^{c}P < 0.05$ *vs* IR group. S: Sham group; IP: Ischemic-preconditioning group; IR: Ischemia-reperfusion group; ICAM: Intercellular adhesion molecule; VCAM: Vascular cell adhesion molecule.

compared to the IR group (2.00 ± 0.71 *vs* 3.60 ± 0.84, $P < 0.05$).

Intestinal IP reduces ICAM-1 and VCAM-1 expression

We next determined whether IP reduces the protein expression of intercellular adhesion molecules in intestinal tissue. As shown in Figure 3, ICAM-1 expression in the IR group was significantly increased compared to the S group (353.33 ± 45.19 *vs* 100.00 ± 25.00, $P < 0.01$). IP significantly decreased ICAM-1 protein expression compared to the IR group (204.67 ± 53.27 *vs* 353.33 ± 45.19, $P < 0.05$). Intestinal tissue VCAM-1 protein expression in the IR group was significantly increased compared to the S group (377.33 ± 41.42 *vs* 107.00 ± 15.72, $P < 0.01$). IP significantly decreased the expression of VCAM-1 compared to the IR group (256.67 ± 58.59 *vs* 377.33 ± 41.42, $P < 0.05$).

Intestinal IP attenuates NF-κB activity and protein expression

We next tested whether IP reduces the translocation of nuclear factors in intestinal tissue by measuring intestinal NF-κB activity and protein expression. As shown in Figure 4A, NF-κB activity in the IR group was significantly enhanced compared to the S group (0.76 ± 0.22 *vs* 0.26 ± 0.10, $P < 0.01$). IP dramatically decreased intestinal tissue NF-κB activity compared to the IR group (0.48 ± 0.16 *vs* 0.76 ± 0.22, $P < 0.05$). As shown in Figure 4B, intestinal tissue NF-κB protein expression in the IR group was significantly increased compared to the S group (320.23 ± 38.16 *vs* 100.03 ± 20.00, $P < 0.01$). IP dramatically decreased NF-κB expression compared to the IR group (320.23 ± 38.16

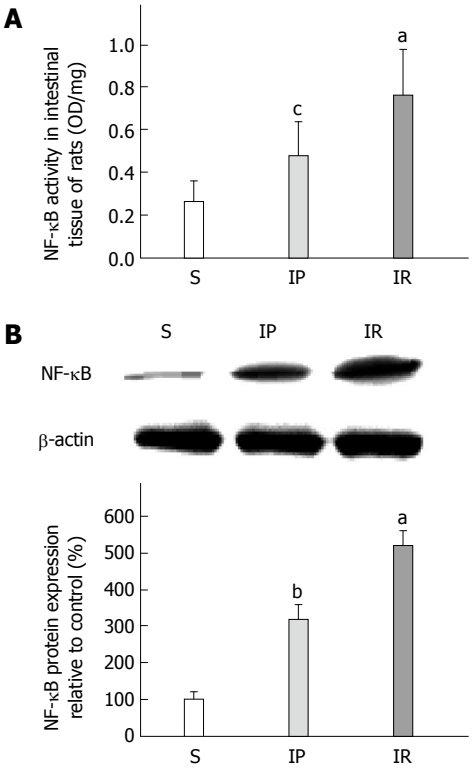

Figure 4 Nuclear factor-κB activity and protein expression in rat intestinal tissue. A: Nuclear factor (NF)-κB activity was tested by a commercial kit; B: NF-κB protein expression was analyzed by Western blot. Data are presented as mean ± SD (n = 6). $^{a}P < 0.01$ *vs* S group; $^{b}P < 0.01$, $^{c}P < 0.05$ *vs* IR group. S: Sham group; IP: Ischemic-preconditioning group; IR: Ischemia-reperfusion group.

vs 520.76 ± 40.53, $P < 0.01$).

DISCUSSION

Prolonged IR rapidly causes substantial and irreversible intestinal tissue damage. Although restoration of blood flow to intestinal ischemic tissue is critical for tissue salvage, reperfusion also introduces inflammatory changes that exacerbate injury[12]. IP is a simple procedure conferring cytoprotection in critical organs that has clinical applications[13,14]. Although intestinal IP triggers powerful protective effects mechanisms by which it alleviates intestinal injury remain to be elucidated.

Hotter *et al*[6] demonstrated the protective benefits of IP against intestinal IR injury. IP also attenuates intestinal IR injury in a canine bowel transplantation model by decreasing oxidative stress[15]. Moreover, IP protects against intestinal IR injury *via* the mast cell degranulation-mediated release of mast cell carboxypeptidase A[1]. Furthermore, IP attenuates intestinal IR injury *via* actions in several signaling pathways, including suppression of heme oxygenase[16] and modulation of the arachidonic acid cascade[17].

Our previous study showed that intestinal IP attenuates the capacity of antioxygen-free radicals, inhibits the release of proinflammatory cytokines, and alleviates apoptosis in IR-caused lung injury in rats[4]. As serum MDA, MPO, and TNF-α levels in rats were tested, we further examined the levels of these proteins in intestinal tissue. The results presented here show that IP reduces intestinal injury induced by IR, observed as attenuated histologic injury and MDA, MPO, and TNF-α levels in the intestine, and suppressed expression of ICAM-1 and VCAM-1, along with reduced activity and expression of NF-κB activity.

Neutrophils are a critical component of the inflammatory response that characterizes intestinal IR[18,19]. Activated neutrophils, which infiltrate the intestine during IR, produce inflammatory mediators, including TNF-α, ICAM-1, and VCAM-1, in the development of intestinal IR by releasing neutrophil proteases and reactive oxygen species, and sequestering polymorphonuclear neutrophils (PMNs) into ischemic intestinal tissue[20-22].

Depletion or inhibition of PMNs decreases intestinal tissue injury in IR[23]. PMNs can aggregate together leading to a "no flow" phenomenon in ischemic tissue after reperfusion[10]. Moreover, PMNs could contribute to vasoconstriction and decreased blood flow *via* the release of potent cytotoxic cytokines[24]. In the present study, intestinal IR significantly induced infiltration of neutrophils or PMNs, as observed as and increased MPO level. Intestinal IP effectively decreased infiltration of neutrophils or PMNs, shown by decreased MPO levels. This suggests that IP attenuates infiltration of neutrophils or PMNs in intestinal IR induced injury.

TNF-α is a cell-signaling protein associated with systemic inflammation and is one of the cytokines that make up the acute-phase reaction. It is produced mainly by activated macrophages, neutrophils, and natural killer cells. The primary role of TNF-α is in the regulation of immune cells, and disruption of its production has been implicated in IR[25,26]. Moreover, TNF-α is a potent chemoattractant for neutrophils, and promotes the expression of adhesion molecules on endothelial cells, helping neutrophils migrate into tissue[27].

Intestinal injury induced by IR is thought to result from intestine-derived TNF-α. Moreover, TNF-α could upregulate neutrophil adhesion molecules in the intestine, particularly ICAM-1, which then plays a vital role in the influx of neutrophils in intestinal tissue[28,29]. In this study, intestinal IR induced TNF-α production, as observed as increased TNF-α levels, which were significantly reduced by intestinal IP.

Accumulating evidence demonstrates that leukocyte-endothelial adhesion is mediated by cell adhesion molecules on vascular endothelial cells and counterpart ligands on leukocytes, especially in IR[30,31]. ICAM-1 and VCAM-1 expressed on these cells are central for the adherence of neutrophils and monocytes to the endothelium[32]. Their expression is also induced by proinflammatory stimuli such as TNF-α. After intestinal IR, increased TNF-α levels in the intestinal

tissue likely contributed to a mediator cascade, leading to neutrophil infiltration and the observed increases in ICAM-1 and VCAM-1 expression. Moreover, intestinal IP significantly decreased their expression. These data indicate that IP inhibits TNF-α production, and reduces ICAM-1 and VCAM-1 expression in an intestinal IR-induced acute inflammation reaction.

NF-κB is a critical transcription factor and is activated in intestinal IR, playing a key role in inflammatory responses[32]. Activation of NF-κB induces expression of multiple inflammation-related products, such as chemokines, cytokines, and adhesion molecules. Increased concentrations of these inflammatory proteins may result in intestinal injury. In general, NF-κB is composed of two polypeptides, a 50-kDa peptide (p50) and a 65-kDa peptide (p65). NF-κB is activated by a variety of signals associated with the etiology and pathophysiology of inflammation, including intracellular and/or extracellular stimuli such as TNF-α. Importantly, nuclear translocation of NF-κB (p65) allows it to bind to target promoter regions, including binding sites within the promoters for ICAM-1 and VCAM-1[33]. The data presented here indicate that intestinal IP significantly reduces TNF-α-induced activation and nuclear translocation of NF-κB as a molecular mechanism for protection against intestinal IR-induced injury.

The clinical application of IP involves a simple, safe, and tolerable procedure, with wide-ranging immunomodulatory effects. Recently, a randomized controlled trial showed that remote IP powerfully counteracts the injury to the intestinal mucosa caused by a period of ischemia and subsequent reperfusion during elective open infrarenal abdominal aortic aneurysm repair[13]. Although intestinal protection with IP is a reproducible and powerful phenomenon, it has not been readily translated to routine clinical use. Therefore, more clinical trials are needed to further investigate intestinal IP use during gastrointestinal surgeries.

Taken together, IP protects against intestinal IR-induced injury potentially by attenuating a neutrophil-endothelial adhesion cascade. The mechanism involves reducing ICAM-1 and VCAM-1 expression and TNF-α-induced NF-κB signaling pathway activity. The present study provides an insight into the cytoprotection of intestinal IP-reduced IR. These findings further support our contention that intestinal IP may be clinically useful in the future to treat patients at risk for intestinal IR-induced injury.

COMMENTS

Background

Intestinal ischemic preconditioning (IP) has been widely investigated in recent years. Prolonged ischemia-reperfusion (IR) induces morphologic changes in the intestine, attenuating cell apoptosis, ameliorating neutrophil sequestration, and decreasing inflammatory mediator release, such as of tumor necrosis factor (TNF)-α and interleukin-1. However, the protective mechanisms of intestinal IP remain poorly understand.

Research frontiers

Although intestinal IP triggers a powerful protective effect, particularly in surgical ischemic operation and transplantation, mechanisms by which IP alleviates intestinal injury remain to be elucidated. The current research hotspot is to determine the effects of IP in a rat model of intestinal IR by analyzing data such as histologic injury, oxygen-free radical injury, neutrophil infiltration, levels of TNF-α, endothelial cell adhesion molecules expression, and nuclear factor (NF)-κB activity and expression.

Innovations and breakthroughs

IP protects against intestinal IR-induced injury potentially by attenuating the neutrophil-endothelial adhesion cascade *via* reducing expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 and TNF-α-induced NF-κB signaling pathway activity. The present study provides an insight into the cytoprotection of intestinal IP-reduced IR.

Applications

The study results suggest that intestinal IP may be clinically useful in the future to treat patients at risk for intestinal IR-induced injury.

Terminology

IR is a process of ischemia followed by reperfusion injury, which contributes to a complex series of events, including nitric oxide imbalance, neutrophil accumulation, cell apoptosis, and activation of inflammatory mediators, thus causing cellular and tissue damage. IP refers to a phenomenon in which a tissue or organ is rendered resistant to these deleterious effects by prior exposure to brief periods of vascular occlusion.

Peer-review

Intestinal IP has been widely investigated in recent years, however, the protective mechanisms of intestinal IP remain poorly understand. The results of this well-designed study suggest that IP protects against IR-induced intestinal injury by attenuating of neutrophil-endothelial adhesion cascade *via* reducing expression of endothelial cell adhesion molecules (intercellular adhesion molecule-1 and vascular cell adhesion molecule-1) and TNF-α-induced NF-κB signaling pathway activity.

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ORIGINAL ARTICLE

Basic Study

Liuweiwuling tablets attenuate acetaminophen-induced acute liver injury and promote liver regeneration in mice

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Author contributions: Lei YC designed this research; Lei YC, Li W and Luo P performed this research and analyzed the data; and Lei YC wrote the paper.

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Abstract

AIM: To explore the mechanism of protection against acetaminophen-induced acute liver injury by Liuweiwuling tablets.

METHODS: Intraperitoneal injections of acetaminophen (250 mg/kg) were used to induce acute liver injury in male C57BL/6 mice. A total of 24 healthy mice were randomly assigned to two groups: an acute liver injury group (control group) and a Liuweiwuling tablet group. Mice were given Liuweiwuling tablets or a vehicle (PBS) orally prior to the administration of acetaminophen. Serum alanine aminotransferase (ALT) and aspartate aminotransaminase (AST) levels were measured at different time points within one week, and pathological examinations of liver tissues were performed 36 h after induction of acute liver injury. Serum inflammatory cytokines, such as high mobility group box protein B1 (HMGB1), tumor necrosis factor (TNF)-α and interleukin IL-1β, were detected using an ELISA method according to the manufacturer's instructions. Hepatic morphological changes at 36 h were assessed by hematoxylin and eosin staining. Expression of proliferating cell nuclear antigen (PCNA) in liver tissue was determined by Western blot analysis. The mRNA levels of hepatocyte proliferation markers (PCNA, CyclinD1 and p21) were detected by real-time quantitative reverse transcription-polymerase chain reaction.

RESULTS: The levels of ALT/AST in the Liuweiwuling tablet group were decreased significantly at 6, 12 and 24 h compared to that of the control group (654.38 ± 120.87 *vs* 1566.17 ± 421.64, 1154.18 ± 477.72 *vs* 4654.84 ± 913.71 and 935.13 ± 252.34 *vs* 4553.75 ± 727.37, $P < 0.01$). Serum HMGB1 levels at 6 and 12 h for the Liuweiwuling tablet group were significantly lower than those of the control group (23.49 ± 3.89 *vs*

58.6 ± 3.65, 61.62 ± 13.07 *vs* 27.32 ± 5.97, $P < 0.01$). Furthermore, serum TNF-α and IL-1β levels at 12 h in the Liuweiwuling tablet group were also significantly lower than those of the control group (299.35 ± 50.61 *vs* 439.03 ± 63.59, 57.42 ± 12.98 *vs* 160.07 ± 49.87, $P < 0.01$). Centrilobular necrosis was evident in liver tissue of mice with acetaminophen-induced acute liver injury, but was almost abolished in the Liuweiwuling tablet group. The expression levels of PCNA and CyclinD1 were up-regulated in liver tissue in the Liuweiwuling tablet group (321.08 ± 32.87 *vs* 157.91 ± 21.52, 196.37 ± 25.39 *vs* 68.72 ± 11.27, $P < 0.01$); however, expression of p21 in liver tissue was down-regulated compared to that of the control group (40.26 ± 9.97 *vs* 138.24 ± 13.66, $P < 0.01$).

CONCLUSION: Liuweiwuling tablets can attenuate acute liver injury by decreasing inflammatory cytokine (HMGB1, TNF-α and IL-1β) levels and promoting liver regeneration.

Key words: Acute liver injury; Acetaminophen; Liuweiwuling tablets; Inflammatory cytokine; Liver regeneration

Core tip: Clinical studies have shown that Liuweiwuling tablets are effective against a variety of liver injuries; however, its mechanism has not been established, especially for drug-induced liver injury. In this study, we found that Liuweiwuling tablets can attenuate acetaminophen-induced acute liver injury by decreasing inflammatory cytokine levels and promoting liver regeneration. Our results provide direct evidence for the effective therapy of liver damage with Liuweiwuling tablets and their value in clinical applications. Moreover, this is the first report showing that Liuweiwuling tablets can promote liver regeneration.

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INTRODUCTION

China's ageing population is growing, and consequently, the medical demand is increasing. The irrational use of medicines and health products (including traditional Chinese medicine) and the incidence of drug-induced liver injury are also increasing. Therefore, it is important that better treatments are found to combat this disease. Clinical studies have demonstrated that Liuweiwuling tablets are effective against a variety of acute or chronic liver injuries that are caused by drugs, viruses and alcohol. Furthermore, they are also effective in preventing the progression of hepatic fibrosis and cirrhosis, and when combined with antivirals, may produce improved clinical effects[1-4]. However, the mechanism of the effect of Liuweiwuling tablets in the treatment of liver injury has not been established, especially for drug-induced liver injury. Research has shown that Liuweiwuling tablets can decrease alkaline phosphatase levels in mice with acetaminophen (APAP)-induced liver injury. The purpose of this study was to provide a better understanding of the mechanism of Liuweiwuling tablets in protecting hepatocytes from liver injury. By using an APAP-induced liver injury mouse model, we showed that Liuweiwuling tablets can protect hepatocytes from liver injury by decreasing the release of inflammatory cytokines, such as high mobility group box protein B1 (HMGB1), tumor necrosis factor (TNF)-α and interleukin IL-1β, and promoting liver regeneration.

MATERIALS AND METHODS

Materials

Six- to eight-week-old male C57BL/6 mice (weighing 20 ± 0.5 g) were purchased from Hunan Slack King of Laboratory Animal Co. Ltd (Changsha, China). The mice were maintained in a specific pathogen-free facility under controlled laboratory conditions (23 ℃, 12 h light/12 h dark cycle, 50% humidity, *ad libitum* access to food and water) for two weeks prior to experimentation and were treated humanely. All animal-related procedures were performed in the animal experiment center of Nanchang University and approved by the animal care and use committee of the Zhejiang Hospital. Animal breeding and processing were all in strict accordance with the laboratory animal breeding and user guide issued by the National Institutes of Health (NIH). Intragastric gavage was performed on conscious animals using straight gavage needles that were appropriate for the animal size (20 g body weight: 22 gauge, 1 inch length, 1.25 mm ball diameter). Reagents used included APAP (Sigma, United States), Trizol reagent (Invitrogen, United States) and rabbit anti-mouse PCNA polyclonal antibody (ABGENT, United States).

Animal models and grouping

A total of 24 male C57BL/6 mice were subjected to 12 h of fasting, but were permitted to drink water before the trial commenced. The mice were randomly assigned to two groups, an acute liver injury group (control group) and a Liuweiwuling tablet group. Each group consisted of 12 mice. The acute liver injury group was administered APAP 250 mg/kg, by an intraperitoneal injection, and the Liuweiwuling tablet group was given Liuweiwuling tablets (10.0 g/kg, 2 times per day by lavage) three days before the intraperitoneal injections of APAP. The acute liver injury

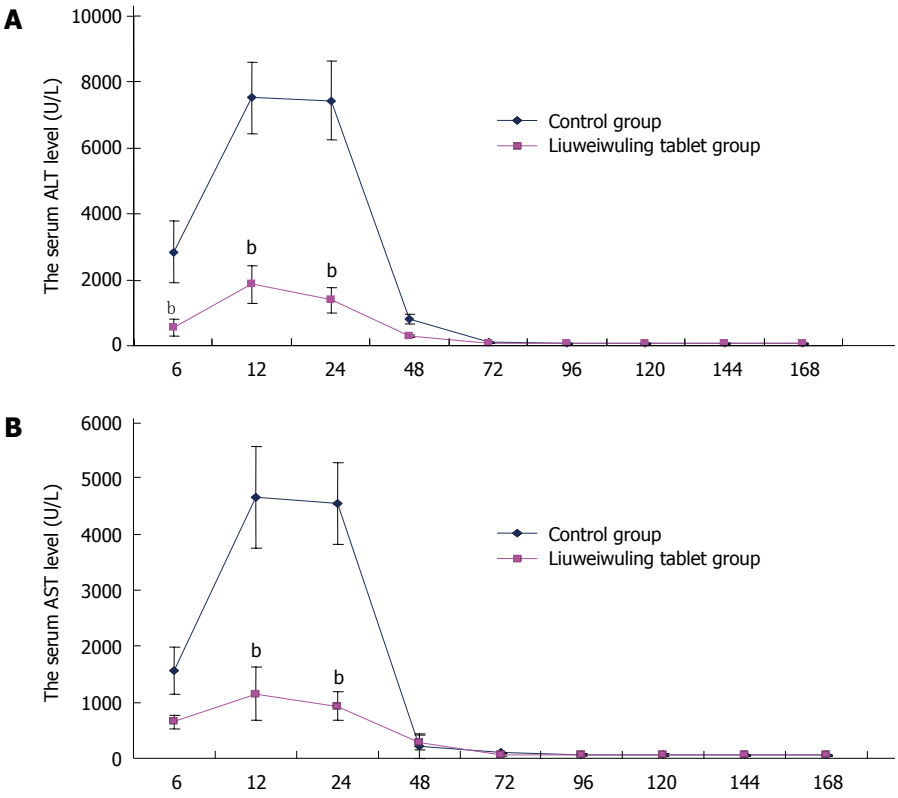

Figure 1 Liuweiwuling tablets significantly decrease serum alanine aminotransferase/aspartate aminotransaminase levels in mice with acetaminophen-induced acute liver injury. A: The levels of alanine aminotransferase (ALT) in the Liuweiwuling tablet group were decreased significantly at 6, 12 and 24 h compared with the control group (t = 8.20, 15.89 and 16.76, respectively; $^{b}P < 0.01$ *vs* control); B: The levels of aspartate aminotransaminase (AST) in the Liuweiwuling tablet group were also decreased significantly at 6, 12 and 24 h compared with the control group (t = 7.09, 11.75 and 16.28, respectively; $^{b}P < 0.01$ *vs* control). The above experiments were repeated more than 3 times.

group was given an equivalent amount of PBS by lavage in a corresponding time frame.

Specimen collection

Mice were anesthetized thoroughly with ether, and orbital blood was collected at 6, 12, 24, and 48 h and at one week after intraperitoneal injections of APAP. Serum was collected and stored at -80 ℃. Some animals were sacrificed and their liver tissues used for reverse transcription-polymerase chain reaction (RT-PCR) and immunoblotting assays at 36 and 48 h. The specimens were fixed with neutral buffered 10% formalin and subjected to hematoxylin and eosin (HE) staining and immunohistochemical detection. All animals were euthanized by a barbiturate overdose (intravenous injection, 150 mg/kg pentobarbital sodium) for tissue collection.

Serum biochemical and cytokine detection

Alanine aminotransferase (ALT) and aspartate aminotransaminase (AST) levels were measured with an automatic biochemical analyzer, while HMGB1, TNF-α and IL-1β were determined using an ELISA according to the manufacturer's instructions. Results were calculated based on a standard curve.

RNA extraction and quantitative RT-PCR

Hepatic tissue mRNA expression levels were detected by quantitative RT-PCR among those mice with acute liver failure at 36 h. Total RNA extraction was conducted using Trizol reagent (Invitrogen, United States) in accordance with the instructions provided with the reagent. RT-PCR primer sequences were as follows: PCNA: forward, 5′-AGCCACATTGGAGATGCTGTAGCCGTATTCA-3′, reverse, 5′-AAGTTCCCATTGCCAAGCTCTCC-3′; CyclinD1: forward, 5′-GCTGCAAATGGAACTGCTTCTGGT-3′, reverse, 5′-TACCATGGAGGGTGGGTTGGAAAT-3′; GAPDH, forward, 5′-GTTGTCTCCTGCGACTTCA-3′, reverse, 5′-GGTGGTCCAGGGTTTCTTA-3′. A real-time quantitative PCR detection system (produced by Roche) was used to construct a standard curve. Results were standardized using GAPDH, and relative amounts were then calculated according to the $2^{\Delta\Delta CT}$ method[5].

Western blot analysis

Hepatic tissue (0.5 g) was ground to provide a 1 mL homogenate (including a protease inhibitor cocktail), which was diluted 20-fold with the RIPA efficient cracking liquid and then transferred to a nitrocellulose membrane after polyacrylamide gel electrophoresis. After blocking overnight with 10% BSA at 4 ℃, the

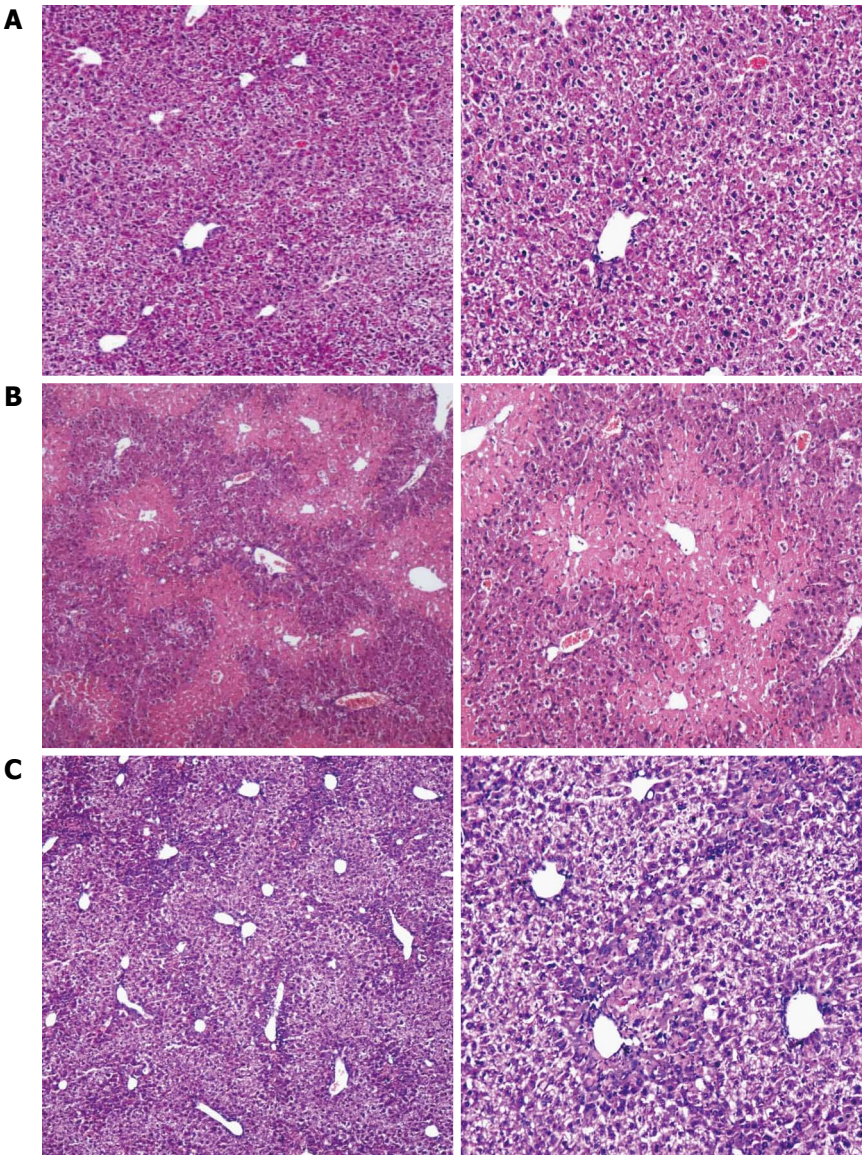

Figure 2 Influence of Liuweiwuling tablets on the central necrosis of hepatic lobules in mice with acute liver injury (magnification, × 10 and × 100, respectively). A: Normal mice; B: Evident necrosis in the central hepatic lobule in the acute liver injury group; C: Slight necrosis in liver tissue in the Liuweiwuling tablet group.

membrane was incubated with primary antibodies for 2 h at 37 ℃, then with sheep anti-rabbit IgG antibody conjugated with horseradish peroxidase for 0.5 h at 37 ℃. Finally, the target proteins were detected using a chemiluminescence method, and analysis of the grey levels was performed using the Quantity One software.

Statistical analysis

All data were statistically analyzed using the SPSS 18.0 software. Data are expressed as the mean ± SD, and the comparisons between the two groups were conducted by an independent sample *t*-test. A *P*-value less than 0.05 was considered significantly different.

RESULTS

Liuweiwuling tablets decrease serum ALT/AST levels in mice with APAP-induced acute liver injury

Serum levels of ALT/AST in mice with APAP-induced acute liver injury began to increase at 6 h, reached a peak at 12 h and then recovered gradually at 48 h. The levels of ALT/AST in the Liuweiwuling tablets group were decreased significantly at 6, 12 and 24 h compared to those in the control group ($P < 0.01$)

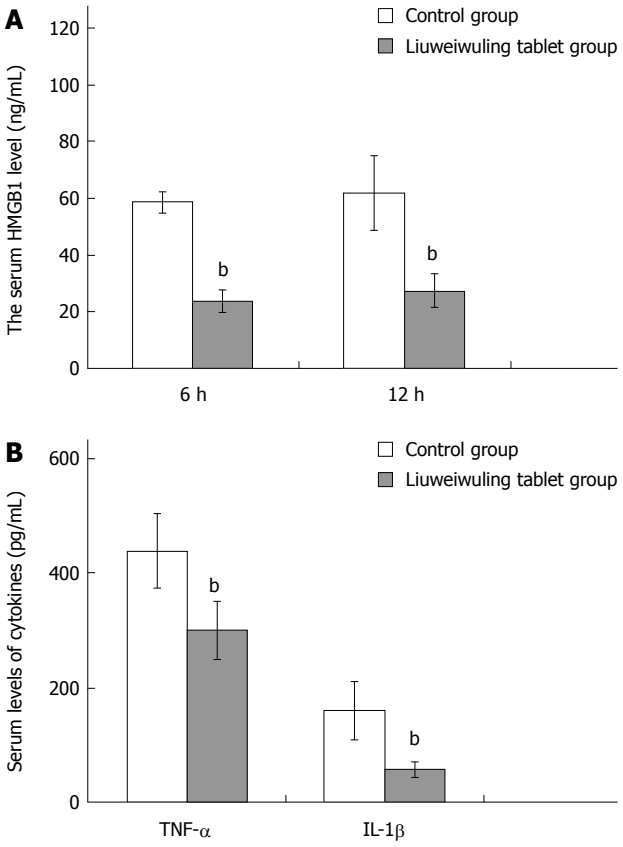

Figure 3 Liuweiwuling tablets decrease the level of high mobility group box protein B1, tumor necrosis factor-α and interleukin-1β in mice with acetaminophen-induced acute liver injury. A: There is a significant difference between the Liuweiwuling tablet group and the control group in HMGB1 levels at 6 and 12 h (t = 22.82 and 8.29, respectively; $^{b}P < 0.01$ *vs* control); B: The levels of TNF-α and IL-1β between the two groups were also significantly different at 12 h (t = 5.99 and 6.88, respectively; $^{b}P < 0.01$ *vs* control). The above experiments were repeated more than 3 times.

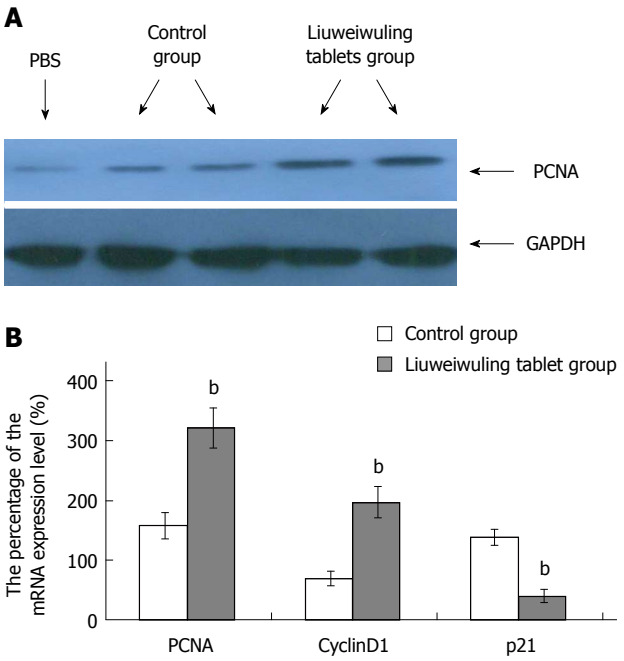

Figure 4 Liuweiwuling tablets accelerate liver regeneration in mice with acetaminophen-induced acute liver injury. A: Immunoblotting assays were used to detect the expression of PCNA in different processes; B: Real-time quantitative reverse transcription-polymerase chain reaction detection was used to detect mRNA expression. The levels of PCNA, CyclinD1 and p21 between the two groups were significantly different at 36 h (t = 14.37, 15.91 and 20.04, respectively; $^{b}P < 0.01$ *vs* control). The above experiments were repeated more than 3 times and the results are expressed as percentages compared with the normal group.

(Figure 1A and B).

Liuweiwuling tablets decrease the central necrosis of hepatic lobules in mice with APAP-induced acute liver injury

Compared to the normal mice, the extent of the central necrosis of hepatic lobules in the acute liver injury group was greater, as a significant amount of necrosis was observed (Figure 2A and B), while less necrosis was observed in the liver tissue of the Liuweiwuling tablet group (Figure 2C).

Liuweiwuling tablets decrease the levels of HMGB1, TNF-α and IL-1β in mice with APAP-induced acute liver injury

Recently, HMGB1 has been shown to play an important role in the acute inflammation of the liver. Serum levels of HMGB1 in mice with APAP-induced acute liver injury began to increase at 6 h, reached a peak at 12 h and then declined at 24 h. Serum levels of HMGB1 at 6 and 12 h in the Liuweiwuling tablet group were all significantly lower than those in the control group (P < 0.01) (Figure 3A). In addition, the levels of TNF-α and IL-1β in the Liuweiwuling tablet group at 12 h were also significantly lower than those in the control group (P < 0.01) (Figure 3B).

Liuweiwuling tablets accelerate liver regeneration in mice with APAP-induced acute liver injury

To observe the influence of Liuweiwuling tablets on liver regeneration in mice with APAP-induced acute liver injury, immunoblotting assays were used to detect the expression of PCNA. The results demonstrated that the expression of PCNA in the Liuweiwuling tablet group was increased at 48 h compared to the control group (Figure 4A). The real-time quantitative RT-PCR detection of liver tissue mRNA expression levels showed that the levels of PCNA and CyclinD1 that promote liver cell regeneration in the Liuweiwuling tablet group were up-regulated 3.4-fold and 2.1-fold, respectively, while the level of p21 that inhibits liver cell regeneration was down-regulated 2.9-fold. These differences were significant when compared with the control group (P < 0.01) (Figure 4B).

DISCUSSION

The acute liver injury model produced by intraperitoneal injection of APAP in mice is a good simulation of drug-induced liver injury and is presently a mature model[6-8]. This study shows that male mice

(C57BL/6) at the age of 6-8 wk and with weights of 20 ± 0.5 g are more ideal compared with female C57BL/6 and BALβ/c mice. Under the experimental conditions of this study, serum ALT/AST levels in mice with APAP-induced acute liver injury began to increase at 6 h, reached a peak at 12-24 h, and then recovered gradually at 48 h. HE staining at 36 h indicated necrosis in the central hepatic lobule in the acute liver injury group, which is in accordance with domestic and foreign research[9-11].

Recent studies indicate that HMGB1 plays a very important role in the inflammatory response of acute liver injury[12-16]. In this study, serum HMGB1 levels in mice with APAP-induced acute liver injury began to increase at 6 h, reached a peak at 12 h and then declined at 24 h. The serum HMGB1 levels at 6 and 12 h in the Liuweiwuling tablet group were all significantly lower than those of the control group. As observed with the HMGB1 levels, the levels of TNF-α, IL-1β and IL-6 in the Liuweiwuling tablet group were all significantly lower than those of the control group. The inflammatory factor HMGB1 can be released by stimulation of activated monocytes, macrophages, neutrophils and endothelial cells and can be passively released by necrotic cells[17-20]. HMGB1 can directly promote the release of inflammatory factors such as TNF-α and IL-1β. Furthermore, it can also promote the production of chemotactic factors that are released by peripheral blood mononuclear cells[21-23], increase the number of inflammatory cells and amplify the inflammatory response to aggravate liver damage. Recent studies indicate that TNF-α and IL-1β play an important role in APAP-induced acute liver injury[24-27]. Therapy with Liuweiwuling tablets inhibits the inflammatory response and liver tissue damage by decreasing the levels of HMGB1 and inflammatory factors TNF-α and IL-1β, leading to a decline of ALT/AST levels and an alleviation of liver inflammatory necrosis.

Modern pharmacological studies have shown that Schisandra chinensis, which is an ingredient in the Liuweiwuling tablet, is effective in reducing enzymes and in protecting the liver from chronic liver injury; fructus ligustri lucidi, forsythia and endives have a positive effect on reducing heat and removing toxicity for the liver; rhizoma zedoariae can desilt the extravasated blood and promote microcirculation; and ganoderma lucidum spore powder can enhance immunity and promote tissue repair. Therefore, we speculate that Liuweiwuling tablets can promote the regeneration of liver cells in acute liver injury. Previous studies have indicated that PCNA and CyclinD1 play important roles in liver cell regeneration; however, p21 inhibits liver cell regeneration[28-30]. This study shows that the expression of PCNA in the Liuweiwuling tablet group was increased at 48 h compared to a control group and that the levels of PCNA and CyclinD1 that promote liver cell regeneration in the Liuweiwuling tablet group were up-regulated 3.4-fold and 2.1-fold respectively, while the level of p21 that inhibits liver cell regeneration was down-regulated 2.9-fold. All of the above findings suggest that Liuweiwuling tablets can accelerate the regeneration of liver cells.

In conclusion, Liuweiwuling tablets can decrease the levels of ALT/AST and inflammatory factors (such as HMGB1, TNF-α and IL-1β) and promote liver regeneration. This may be one of the mechanisms involved in the treatment of acute liver damage. The above results regarding the effect of Liuweiwuling tablets on liver damage provide a new theoretical understanding and have important theoretical and application values.

COMMENTS

Background

Drug-induced liver injury is gradually increasing, and clinical studies have shown that Liuweiwuling tablets are effective against a variety of liver injuries caused by viruses, drugs and alcohol. However, the mechanism for the effectiveness of the treatment of liver injury has not been established, especially for drug-induced liver injury.

Research frontiers

Existing research shows that Liuweiwuling tablets can decrease the alkaline phosphatase levels in mice with acetaminophen-induced liver injury.

Innovations and breakthroughs

Liuweiwuling tablets can attenuate acetaminophen (APAP)-induced acute liver injury by decreasing inflammatory cytokine levels and promoting liver regeneration. Moreover, this is the first report showing that Liuweiwuling tablets can promote liver regeneration.

Applications

The present results provide direct evidence for the effective therapy of liver damage with Liuweiwuling tablets and have value in clinical applications.

Peer-review

Previous studies have shown that Liuweiwuling tablets can be effective against a variety of liver injuries, but the mechanism is unknown. In this study, the authors found that Liuweiwuling tablets can decrease the extent of APAP-induced acute liver injury by decreasing the levels of inflammatory cytokines (high mobility group box protein B1, tumor necrosis factor-α and interleukin-1β) and by promoting liver cell regeneration. This is the first report that provides direct evidence for the effective therapy of liver damage with Liuweiwuling tablets and value in clinical applications.

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ORIGINAL ARTICLE

Case Control Study

Increased serum soluble lectin-like oxidized low-density lipoprotein receptor-1 levels in patients with biopsy-proven nonalcoholic fatty liver disease

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Institutional review board statement: The study was reviewed and approved by Istanbul Medeniyet University Goztepe Education and Research Hospital Institutional Review Board (document No.: 8-B/28.12.2010) and written informed consent was obtained from all participants.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

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Abstract

AIM: To analyze the relationship between the serum lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) levels and clinical and histopathological features of biopsy-confirmed nonalcoholic fatty liver disease (NAFLD) patients.

METHODS: Fifty-three consecutive, biopsy-proven NAFLD patients (31 males and 22 females, mean age 42.5 ± 9.6 years) and 26 age- and gender-matched, healthy controls (14 males and 12 females, mean age 39 ± 10.7 years) were included. The patients

with NAFLD were consecutive patients who had been admitted to the hepatology outpatient clinic within the last year and had been diagnosed with NAFLD as the result of liver biopsy. The healthy controls were individuals who attended the outpatient clinic for routine health control and had no known chronic illnesses. The histological evaluation was conducted according to the NAFLD activity scoring system recommended by The National Institute of Diabetes and Digestive and Kidney Diseases Nonalcoholic Steatohepatitis Clinical Research Network. The serum LOX-1 levels were measured using an ELISA kit (Life Science Inc. USCN. Wuhan, Catalog No. E1859Hu) in both patients and healthy controls. A receiver operating characteristic (ROC) curve analysis was used to identify the optimal cutoff value of LOX-1 and thereby distinguish between patients with nonalcoholic steatohepatitis (NASH) and healthy controls. A *P*-value < 0.05 was considered statistically significant.

RESULTS: NAFLD and healthy control groups were similar in terms of age and sex. NAFLD patients consisted of 8 patients with simple steatosis (15%), 27 with borderline NASH (51%) and 18 with definitive NASH (34%). Metabolic syndrome was found in 62.2% of the patients with NAFLD. The mean serum LOX-1 level in biopsy-proven NAFLD patients was 8.49 ± 6.43 ng/mL compared to 4.08 ± 4.32 ng/mL in healthy controls ($P = 0.001$). The LOX-1 levels were significantly different between controls, simple steatosis and NASH (borderline+definite) cases (4.08 ± 4.32 ng/mL, 6.1 ± 6.16 ng/mL, 8.92 ± 6.45 ng/mL, respectively, $P = 0.004$). When the cut-off value for the serum LOX-1 level was set at 5.35 ng/mL, and a ROC curve analysis was performed to distinguish between steatohepatitis patients and controls; the sensitivity and specificity of the serum LOX-1 level were 69.8% and 69.2%, respectively.

CONCLUSION: The serum LOX-1 levels were significantly higher in NAFLD patients than in healthy controls. Additionally, the serum LOX-1 levels could differentiate between steatohepatitis patients and healthy controls.

Key words: Insulin resistance; Liver fibrosis; Metabolic syndrome; Nonalcoholic fatty liver disease; Steatohepatitis

Core tip: Lipoprotein receptor-1 (LOX-1) is a biomarker that has been demonstrated to be related to atherosclerosis, insulin resistance, obesity and diabetes. Nonalcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic syndrome. To date, no studies have investigated the association between serum LOX-1 and liver inflammation in biopsy-proven NAFLD patients. In this study, we have shown that the serum LOX-1 levels are correlated with the NAFLD histology scores, which might decrease the need for performing liver biopsy in NAFLD patients.

Ozturk O, Colak Y, Senates E, Yilmaz Y, Ulasoglu C, Doganay L, Ozkanli S, Oltulu YM, Coskunpinar E, Tuncer I. Increased serum soluble lectin-like oxidized low-density lipoprotein receptor-1 levels in patients with biopsy-proven nonalcoholic fatty liver disease. *World J Gastroenterol* 2015; 21(26): 8096-8102 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i26/8096.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i26.8096

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide, and its prevalence is continuously increasing[1,2]. The disease may present in different clinical forms. Though simple steatosis usually has a benign course, nonalcoholic steatohepatitis (NASH) may progress to liver fibrosis, cirrhosis and hepatocellular carcinoma due to ongoing necroinflammation[3,4].

Currently, liver biopsy is the gold standard for diagnosing NAFLD and NASH and for evaluating liver fibrosis[5]. However, liver biopsy is an invasive technique that is associated with several complications. Therefore, alternative non-invasive methods are under investigation for diagnosing this common disease[6].

Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is a protein coded by the oxidized low density lipoprotein (oxLDL) receptor-1 gene, and it is expressed by endothelial cells, vascular smooth muscle cells, macrophages, and adipocytes[7,8]. LOX-1 is a membrane glycoprotein that binds, internalizes, and degrades oxLDL. LOX-1 is activated by oxLDL and leads to endothelial dysfunction, apoptosis and atherosclerotic process via intracellular signal transduction. Additionally, LOX-1 has multiligand receptor features, and the defined ligands of LOX-1 are aged red blood cells, apoptotic cells, activated platelets, leukocytes, bacteria, phosphatidyl serine, advanced glycation endproducts, C reactive protein, and heat shock protein 70[9].

In addition to its role in the process of atherosclerotic, LOX-1 is active in inflammatory processes. Proinflammatory cytokines that are shown to upregulate LOX-1, such as transforming growth factor-beta, interleukin-6, interleukin-1α, interleukin-1β, and tumor necrosis factor-α, are also involved in the pathogenesis of NAFLD[9,10]. Furthermore, recent studies have reported an increase in the LOX-1 levels in diabetes mellitus, metabolic syndrome, and coronary artery disease[11,12].

In this study, we investigated the relationship between LOX-1 and histopathological changes and inflammation as well as clinical and biochemical parameters in liver biopsy-proven NAFLD (simple steatosis

and borderline and definitive NASH) patients. To address these questions, we evaluated the extracellular soluble component of LOX-1 in NAFLD cases and healthy controls.

MATERIALS AND METHODS

Study subjects

A total of 53 patients diagnosed with NAFLD (31 males and 22 females, mean age 42.5 ± 9.6 years) and 26 healthy control subjects (14 males and 12 females, mean age 39 ± 10.7 years) were included in the study. The patients with NAFLD were consecutive patients who had been admitted to the hepatology outpatient clinic within the last year and had been diagnosed with NAFLD based on liver biopsy. The healthy controls were individuals who attended the outpatient clinic for routine health control and had no known chronic illnesses. All participants in the study were recruited from Department of Gastroenterology of Istanbul Medeniyet University Göztepe Education and Research Hospital. To avoid selection bias for recruiting the patients and controls, all participants had to be residents of Istanbul for a minimum of 5 years as a prerequisite. The study was reviewed and approved by Istanbul Medeniyet University Goztepe Education and Research Hospital Institutional Review Board (document No. 8-B/28.12.2010) and written informed consent was obtained from all participants.

All NAFLD patients had elevated serum ALT levels for at least 6 mo, and none of the patients had alcohol consumption greater than 20 g/d. All patients were negative for viral hepatitis serology. Hemochromatosis, Wilson's disease, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis and alpha-1 antitrypsin deficiency were ruled out. All tests/procedures for the aforementioned excluded conditions were performed for research. Patients with biliary strictures and malignancies were excluded. Additionally, the patients had no history of using hepatotoxic medicines, such as estrogens, amiodarone, steroids, tamoxifen, methotrexate, valproic acid and herbal drugs. The data regarding the hepatotoxic drug history were obtained from both the patient's medical records and their interviews. The data for the hepatotoxic drug history were obtained by interview for controls. All serological and biochemical tests of the participants were performed in the same laboratory, the Central laboratory of Göztepe Education and Research Hospital. All individuals in the control group were healthy on physical examination and had normal liver parenchyma in the sonographic liver examination. Their serological and biochemical parameters were all in the normal ranges.

Clinical and laboratory evaluations

Physical examinations, anthropometric and biochemical measurements, and body mass index (BMI) calculations were performed in all study participants. Blood pressure measurements were performed in a quiet room with a sphygmomanometer after 10 min of resting. After a 12-h overnight fasting period, blood samples, both from patients with NAFLD and controls, from the antecubital veins were collected between 8:00-9:00 am. After 2 h, which allowed the blood to clot, at room temperature, the samples were centrifuged at 1000 G for 20 min. The sera obtained from the cases were stored at -80 ℃ until further analysis.

Diabetes mellitus was diagnosed according to the American Diabetes Association criteria[13]. Metabolic syndrome was diagnosed using the Adult Treatment Panel Ⅲ criteria[14]. Homeostasis Model of Assessment - Insulin Resistance (HOMA - IR) was calculated using the following equation: insulin resistance (IR) = fasting plasma glucose (mmol/L) × fasting plasma insulin (mU/L)/22.5 (IR was accepted as normal if IR < 2.5 and present if insulin resistance IR ⩾ 2.5).

Duplicate measurements of the LOX-1 serum levels were performed using an ELISA kit (Life Science Inc. USCN. Wuhan, Catalog No. E1859Hu) according to the manufacturer's instructions. The standard curve concentrations used for the ELISA were 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL, 0.312 ng/mL, and 0.156 ng/mL. The minimum detectable level of human LOX-1 is less than 0.03 ng/mL.

Histological analysis

A liver biopsy was performed under local anesthesia using a Hepafix 16-gauge needle (Braun Melsungen AG, Melsungen, Germany) with ultrasound guidance. All biopsy samples were fixed with 10% formaldehyde and then embedded in paraffin blocks. The liver specimens were stained with hematoxylin-eosin, Masson's trichrome and reticulin silver stains. An experienced hepatopathologist scored and evaluated the tissue specimens. The pathologist was blinded to all patient data. The histological evaluation was conducted according to the NAFLD activity scoring system (NAS) recommended by The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) NASH Clinical Research Network[15]. In this scoring system, hepatic steatosis was graded from 1 to 3 according to the steatosis ratio, with 5%-33%, 33%-66% and > 66% representing scores of 1, 2 and 3, respectively. Lobular inflammation was defined as an overall assessment of all inflammation; no foci was scored as 0, < 2 foci per × 200 field scored as 1, two-four foci per × 200 field scored as 2, and more than 4 foci per × 200 field scored as 3. Ballooning scoring is defined as a score of 0 if there is no ballooning of hepatocytes, 1 if there are few ballooning hepatocytes, and 2 if there are numerous ballooning hepatocytes. Fibrosis was staged as follows: stage 0, no liver fibrosis; stage 1, perisinusoidal or periportal fibrosis; stage 2, perisinusoidal and portal/periportal fibrosis; stage 3, bridging fibrosis and stage 4, cirrhosis.

Table 1 Clinical and biochemical characteristics of healthy controls and patients with nonalcoholic fatty liver disease[1] *n* (%)

Characteristic	Control (*n* = 26)	NAFLD (*n* = 53)	Simple steatosis (*n* = 8)	NASH (borderline + definite) (*n* = 45)	*P* value[2]	*P* value[3]
Gender (males/females)	14/12	31/22	4/4	27/18	0.690	0.800
Age (yr)	39 ± 10.7	42.5 ± 9.6	42.8 ± 13.2	4 1 ± 8.6	0.190	0.310
BMI (kg/m²)	28.7 ± 5.1	31.6 ± 5.3	29.9 ± 4.31	32.07 ± 5.5	0.030	0.040
Smoking	5 (19.2)	11 (20.7)	3 (37.5)	8 (17.7)	0.900	0.080
Waist circumference (cm)	85.2 ± 7.3	102.2 ± 9.1	100.2 ± 9.9	102.6 ± 9	0.002	0.002
Diabetes Mellitus	No	11 (20.8)	3 (37.5)	8 (17.7)	0.012	0.050
Metabolic syndrome	No	33 (62.2)	3 (37.5)	30 (66.6)	< 0.001	< 0.001
Hypertension	No	12 (22.6)	3 (37.5)	9 (20)	0.008	0.010
Systolic blood pressure (mmHg)	116 ± 17	121 ± 16	115 ± 15	122 ± 17	0.310	0.270
Diastolic blood pressure (mmHg)	74 ± 12	82 ± 10	79 ± 8	82 ± 10	0.006	0.010
ESR median (min-max, mm/h)	21 (6-38)	11.5 (3-40)	16 (4-40)	11 (3-38)	0.001	0.110
CRP median (min-max, mg/L)	0.4 (0.2-1.5)	0.4 (0.1-2)	0.5 (0.1-1.4)	0.4 (0.1-2)	0.720	0.670
Hemoglobin A1c (%)	5.7 ± 0.3	5.9 ± 0.9	5.5 ± 0.4	5.9 ± 0.9	0.730	0.310
HOMA-IR median (min-max)	2 (0.6-3.2)	2.5 (0.3-11.8)	1.6 (0.3-4.2)	2.7 (0.5 ± 11.8)	0.001	0.010
AST median (min-max, U/L)	19 (9-60)	35 (16-147)	36 (19-91)	35 (16-147)	< 0.001	< 0.001
ALT median(min-max, U/L)	18 (5-50)	52 (17-196)	43 (18-97)	53 (17-196)	< 0.001	< 0.001
Total cholesterol (mmol/L)	5.15 ± 1	5.43 ± 1.39	5.46 ± 1.13	5.43 ± 1.45	0.450	0.670
HDL-cholesterol (mmol/L)	1.21 ± 0.2	1.19 ± 0.2	1.26 ± 0.2	1.16 ± 0.2	0.290	0.310
LDL-cholesterol (mmol/L)	3.52 ± 0.8	3.67 ± 0.9	3.67 ± 0.8	3.67 ± 0.9	0.520	0.770
Triglycerides (mmol/L)	1.4 ± 0.6	2.5 ± 2.12	2.2 ± 1.08	2.5 ± 2.27	0.005	0.420
Ferritin median (min-max, pmol/L)	20.7 (2.8-101)	91 (6.5-326)	141.5 (7.8-300)	88.5 (6.5-326)	< 0.001	< 0.001
LOX-1 (ng/mL)	4.08 ± 4.32	8.49 ± 6.43	6.1 ± 6.16	8.92 ± 6.45	0.001	0.004

[1]The patients with NAFLD and controls was similar according to the socioeconomic status, racial and ethnic background, and, dietary and/or physical activity habits, religion; [2]*P* value: for comparison of control and NAFLD (*t* test for continuos variables and χ^2 test for categorical, as variables); [3]*P* value: for comparison of control, simple steatosis, and NASH (One-way ANOVA for continues variables and χ^2 test for categorical variables). Normal values in laboratory tests: ESR: Erythrocyte Sedimentation Rate (0-20 mm/h); CRP: C-reactive protein (< 8 mg/L); HbA1c (4.3-5.8 proportion of total hemoglobin); total cholesterol (2.6-5.2 mmol/L); triglyceride (0.7-1.7 mmol/L); LDL cholesterol (1-3.37 mg/dL); HDL cholesterol (> 0.9 mmol/L); AST (5-32 U/L); ALT (5-38 U/L); ferritin (54-755 μg/L in males and 25-755 μg/L in females); BMI (body mass index) (18-25 kg/m²); LOX-1: Lectin-like oxidized low-density lipoprotein receptor-1; HOMA-IR and metabolic syndrome are described in the text, Diabetes Mellitus was diagnosed according to ADA 2010 criteria, NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis.

Histologically, the total NAS scores were calculated as a sum of the steatosis (1-3), lobular inflammation (0-3) and ballooning (0-2) scores. Based on this scoring system, patients with a total NAS score of 0-2 were diagnosed with simple steatosis, 3-4 borderline NASH, and 5 or greater definitive NASH[15].

Statistical analysis

StatMate 2.0 (GraphPad Inc., San Diego, CA, United States) was used for the power calculation of the study. The data were analyzed using SPSS 16.0 (IL United States SPSS Inc., Chicago, IL, United States). Normally distributed continuous variables are presented as the mean ± SD. Student's *t*-test was used to evaluate the difference between the independent groups. Differences in the levels of LOX-1 among the NAFLD subgroups (simple steatosis and borderline NASH+definitive NASH) and control group were determined by one-way analysis of variance (ANOVA) followed by a Bonferroni multiple-comparison post hoc test. Categorical data were analyzed using the χ^2 test. A Spearman rank correlation was used to examine the relationship between variables. A receiver operating characteristic (ROC) curve analysis was used to identify the optimal cutoff value of LOX-1 for distinguishing between patients with NASH and healthy controls. A *P*-value < 0.05 was considered statistically significant. The statistical methods of this study were reviewed by Recep Minga, biomedical statistician of İkon Research and Consultancy co.

RESULTS

The clinical and biochemical characteristics of the healthy controls and NAFLD patients are presented in Table 1. The age and gender distribution were similar in both groups. The following characteristics were significantly higher in the NAFLD patients: waist circumference, diastolic blood pressure, HOMA-IR, triglyceride level and transaminase and ferritin levels. Eight patients (15%) had simple steatosis, whereas 27 (51%) had borderline NASH and 18 (34%) were diagnosed with definite NASH. Metabolic syndrome was found in 62.2% of the patients with NAFLD.

The LOX-1 level was significantly higher in the NAFLD group compared to healthy controls (8.49 ± 6.43 ng/mL *vs* 4.08 ± 4.32 ng/mL, respectively, *P* = 0.001). The LOX-1 levels were significantly different between the control, simple steatosis and NASH (borderline + definite) cases (4.08 ± 4.32 ng/mL, 6.1 ± 6.16 ng/mL, 8.92 ± 6.45 ng/mL, respectively, *P* = 0.004). The distribution of the serum LOX-1 levels in

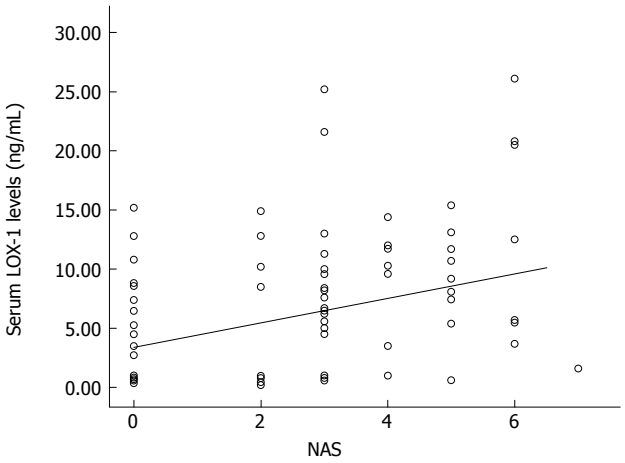

Figure 1 Distribution of serum levels of lectin-like oxidized low density lipoprotein receptor-1 in healthy controls and patients according to non-alcoholic fatty liver disease Activity Score[△]. The LOX-1 levels were significantly different between controls, simple steatosis and NASH (borderline + definite) cases (4.08 ± 4.32 ng/mL, 6.1 ± 6.16 ng/mL, 8.92 ± 6.45 ng/mL, respectively, P = 0.004). LOX-1: Lectin-like oxidized low density lipoprotein receptor-1; NAS: Non-alcoholic fatty liver disease Activity Score; [△]: All healthy controls were considered to have 0 point in NAS.

the study subgroups (controls and patients) is shown in a scatterplot figure (Figure 1). In addition, the LOX-1 serum levels were significantly higher in the NASH group than in the healthy subjects (8.92 ± 6.45 ng/mL *vs* 4.08 ± 4.32 ng/mL, P = 0.003). The LOX-1 levels were not significantly different in the NAFLD subgroups based on histological data. As a result, multiple linear regression analysis was not performed. In the NAFLD group, cases with (33) or without (20) metabolic syndrome had no significant difference in LOX-1 (7.27 ± 5.32 ng/mL *vs* 10.63 ± 7.84 ng/mL, respectively, P = 0.849).

The LOX-1 cut-off value that was used to distinguish healthy controls and NASH (borderline and definite) was 5.35 ng/mL. The area under ROC (AUROC) according to this cut-off level was 72.5% (SE = 0.06, Mann Whitney U-test, P = 0.001). With this cut-off value, the LOX-1 measurement could distinguish NASH patients from healthy controls with a sensitivity of 69.8%, specificity of 69.2%, negative predictive value of 69.6%, and positive predictive value of 69.4%.

DISCUSSION

In this study, we demonstrated, for the first time, a significant difference in the LOX-1 levels between biopsy-proven NAFLD and healthy controls. The LOX-1 levels were significantly higher in NASH patients compared with healthy controls. Therefore, LOX-1 may distinguish NASH cases from healthy subjects. There was no difference between the LOX-1 levels in the simple steatosis subgroup and the healthy controls or NASH cases.

Previous studies have reported that diabetes, obesity, and hypertension cases had high serum LOX-1 levels[9,16,17]. The elevated LOX-1 levels in NAFLD patients are in parallel with these metabolic disorders. This result may be due to insulin resistance, which is an underlying common pathophysiologic mechanism of these disorders[16,18].

In this study, the LOX-1 levels in patients with NAFLD were higher than healthy controls. Although there is a gradual increase in the progression from healthy control to simple steatosis and then to NASH (borderline + definite) subgroup cases, the only significant difference was obtained between controls and NASH patients. However, we did not find any significant difference between simple steatosis and the NASH subgroups. Lubrano *et al*[19] reported that the serum LOX-1 levels were correlated with other inflammatory markers and with the severity of coronary artery disease. Additionally, in an endothelial dysfunction study, Sakurai *et al*[20] showed that the LOX-1 level is linearly correlated with increasing oxidative stress.

The high LOX-1 levels in coronary artery disease, endothelial dysfunction and NAFLD patients suggest that inflammatory processes and oxidative stress are common pathophysiologic mechanisms. In addition, coronary artery disease and endothelial dysfunction were present in NAFLD patients[21-23]. One important result of this study is the identification of high LOX-1 levels in patients with steatohepatitis.

Liver biopsy is still the gold standard method for establishing NASH, which is the progressive form of NAFLD. However, due to several complications of this invasive procedure, a number of alternative methods and non-invasive diagnostic modalities are in development[15,24,25]. Increased LOX-1 serum levels in patients with NASH may simplify the selection of cases for differentiating between NAFLD subgroups prior to liver biopsy. However, these data must be confirmed by studies with large patient samples.

There is no relationship between the LOX-1 level and degree of fibrosis in our study. Kelly *et al*[26] reported a relationship between the LOX-1 level and renal function and fibrosis in obese and diabetic rats. Injections of anti-LOX-1 antibodies into the rats improved renal function and reduced fibrosis. In a study examining the relationship between LOX-1 and angiotensin Ⅱ (which has roles in fibrotic processes) in human coronary artery endothelial cell culture, the activation of angiotensin Ⅱ type 1 receptors was shown to increase the LOX-1 levels[27,28]. The angiotensin Ⅱ type 1 receptor is also involved in the development of liver fibrosis[29]. Although these findings show a relationship between LOX-1 and kidney fibrosis, they do not suggest a causal relationship with liver fibrosis. There is a need for large-scale studies on LOX-1 expression at the cellular level to determine the role of LOX-1 in the pathogenesis of fibrosis. Based on our data, we concluded that in the natural course of NAFLD, inflammation is associated with elevated LOX-1 levels but that fibrosis is not.

There are some limitations to this study. The first limitation is the small number of cases. Therefore, the results must be verified in additional large-scale studies. Second, the study is a cross-sectional, case-control study; therefore, it does not provide information on the pathophysiologic and causal relationship for the disease course. The measurement of only the serum LOX-1 levels and not the liver tissue levels is also a limitation. Additionally, there is no evidence available at the tissue level in patients with NAFLD. Fourth, the patients enrolled in this study were only of Turkish descent; therefore, additional research is needed to assess the role of LOX-1 in different ethnic populations. Finally, the measured and unmeasured differences between the studied groups could have accounted for the findings.

In conclusion, this study showed an association, but not casuality, between serum LOX-1 levels and both NAFLD and NASH. As a result, the serum LOX-1 levels may be a useful marker for differentiating patients with NAFLD and NASH from healthy individuals, but our results must be verified in large-scale randomized trials.

COMMENTS

Background

Nonalcoholic fatty liver disease (NAFLD) is a leading cause of chronic liver disease. It is the hepatic manifestation of metabolic syndrome. Sensitive non-invasive test is needed to diagnose patients.

Research frontiers

Serum Lipoprotein receptor-1 (LOX-1) is a novel biomarker of atherosclerosis and associated to diabetes, hypertension and metabolic syndrome. As NAFLD is related to these entities, LOX-1 might have a role in NAFLD pathogenesis.

Innovations and breakthroughs

Serum LOX-1 levels are increased in correlation with NAFLD activity scores and might improve to differentiate healthy people from definite nonalcoholic steatohepatitis (NASH).

Applications

Histopathological evaluation of the liver is needed for a definitive diagnosis of NASH. In this study serum LOX-1 was able to discriminate NASH from healthy controls. The increased levels might indicate the patients who will need histologic evaluation.

Terminology

NAFLD (non-alcoholic fatty liver) is a spectrum of chronic liver diseases where lipid accumulation exceeds 5% in the hepatocytes. Simple steatosis usually has a benign course but NASH may progress to liver fibrosis, cirrhosis and hepatocellular carcinoma due to ongoing necroinflammation. The degree of severity is determined by histopathological scoring systems.

Peer-review

The inclusion and exclusion criteria are well defined in the study. Small sample size is a limitation of this study. It might have a role in the non-invasive diagnosis of NAFLD.

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ORIGINAL ARTICLE

Retrospective Study

Patients with irritable bowel syndrome-diarrhea have lower disease-specific quality of life than irritable bowel syndrome-constipation

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Author contributions: Singh P, Staller K and Kuo B designed the research; Barshop K, Dai E, Castel S and Newman J collected the data and entered the data; Singh P and Staller K analyzed the data; Singh P and Staller K wrote the manuscript; Barshop K, Dai E, Castel S and Newman J also revised the manuscript; Yoon S and Kuo B gave critical inputs to the manuscript.

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Data sharing statement: No additional data are available.

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Abstract

AIM: To determine effect of irritable bowel syndrome (IBS) subtype on IBS-specific quality of life (QOL) questionnaire and its subscales.

METHODS: We studied IBS patients visiting our functional gastroenterology disorder clinic at a tertiary care center of Unites States. IBS and IBS subtype were diagnosed using Rome-Ⅲ questionnaire. QOL was assessed using IBS-QOL questionnaire. IBS-QOL assesses quality of life along eight subscales: dysphoria, interference with activities, body image, health worry, food avoidance, social reactions, sexual health, and effect on relationships. IBS-QOL and its subscales were both scored on a range of 0-100 with higher scores suggestive of better QOL. Results of overall IBS-QOL scores and subscale scores are expressed as means with 95%CI. We compared mean IBS-QOL score and its subscales among various IBS-subtypes. Analysis of variance (ANOVA) was used to compare the mean difference between more than two groups after controlling for age and gender. A post-hoc analysis using Bonferroni correction was used only when *P* value for ANOVA was less than 0.05.

RESULTS: Of 542 patients screened, 243 had IBS as per Rome-Ⅲ criteria. IBS-mixed (IBS-M) was the most common IBS subtype (121 patients, 49.8%) followed by IBS- diarrhea (IBS-D) (56 patients, 23.1%), IBS-constipation (IBS-C) (54 patients, 22.2%) and IBS-unspecified (IBS-U) (12 patients, 4.9%). Overall IBS-QOL scores were significantly different among various IBS-subtypes (P = 0.01). IBS-QOL of patients with IBS-D (61.6, 95%CI: 54.0-69.1) and IBS-M (63.0, 95%CI: 58.1-68.0) was significantly lower than patients with IBS-C (74.5, 95%CI: 66.9-82.1) (P = 0.03 and 0.02 respectively). IBS-D patients scored significantly lower than IBS-C on food avoidance (45.0, 95%CI: 34.8-55.2 *vs* 61.1, 95%CI: 50.8-71.3, P = 0.04) and interference with activity (59.6, 95%CI: 51.4-67.7 *vs* 82.3, 95%CI: 74.1-90.6, P < 0.001). IBS-M patients had more interference in their activities (61.6, 95%CI: 56.3-66.9 *vs* 82.3, 95%CI: 74.1-90.6, P = 0.001) and greater impact on their relationships (73.3, 95%CI: 68.4-78.2 *vs* 84.7, 95%CI: 77.2-92.2, P = 0.02) than IBS-C patients. Patients with IBS-M also scored significantly lower than IBS-C on food avoidance (47.2, 95%CI: 40.7-53.7 *vs* 61.1, 95%CI: 50.8-71.3, P = 0.04) and social reaction (66.1, 95%CI: 61.1-71.1 *vs* 80.0, 95%CI: 72.1-87.7, P = 0.005).

CONCLUSION: IBS-D and IBS-M patients have lower IBS-QOL than IBS-C patients. Clinicians should recognize food avoidance, effects on daily activities and relationship problems in these patients.

Key words: Irritable bowel syndrome; Irritable bowel syndrome subtype; Quality of life; Irritable bowel syndrome-quality of life; Constipation; Diarrhea

Core tip: There is paucity of data on effect of irritable bowel syndrome (IBS)-subtype on disease specific quality of life as most of the earlier studies have utilized general questionnaires like SF-36 *etc*. We studied the effect of IBS subtype on IBS-specific quality of life (QOL), the most validated disease specific questionnaire for IBS. We found that IBS-diarrhea (IBS-D) and IBS-mixed (IBS-M) have lower disease specific QOL than IBS-constipation patients. Our study also points out that clinicians should pay special attention to certain domains of QOL such as food avoidance, relationship problems, effect on daily activities and social reaction in patients with IBS-D and IBS-M as these domains significantly affect QOL in these patients.

Singh P, Staller K, Barshop K, Dai E, Newman J, Yoon S, Castel S, Kuo B. Patients with irritable bowel syndrome-diarrhea have lower disease-specific quality of life than irritable bowel syndrome-constipation. *World J Gastroenterol* 2015; 21(26): 8103-8109 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i26/8103.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i26.8103

INTRODUCTION

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by abdominal pain or discomfort and alteration of bowel habits in the absence of an organic disorder[1-4]. It is the most common gastrointestinal disorder, affecting between 4%-22% of general population[1-4]. IBS is a generally non life-threatening disorder that is not associated with decreased life expectancy, which may potentially lead clinicians to underestimate its impact on patients' lives and the healthcare system as a whole[5]. Nevertheless, studies have shown that patients with IBS make two to three times the number of health care visits per year compared to age-matched controls[6-8]. Additionally, many studies have also shown that patients with IBS have poorer quality of life (QOL) when compared to the general population as well as patients with other chronic diseases such as diabetes and end stage renal disease[8,9]. QOL has also been shown to correlate with disability, healthcare resource utilization, and clinical response to treatment. Thus, it is important to understand the variables affecting the QOL in patients with IBS[10].

While QOL in IBS has been shown to be affected by extraintestinal symptoms, psychiatric symptoms, disease severity, and gender, the effect of IBS-subtype is unclear[10,11]. Previous studies studying the effect of IBS subtype on QOL have failed to show any difference[11-15]. Most of these studies used general health-related QOL questionnaires like the SF-36 and WHO-QOL, which do not capture the specific issues pertinent to IBS[11-14]; thus, these general questionnaires could have minimized the impact of gastrointestinal symptoms on overall QOL. Disease-specific instruments have been developed and validated for IBS, such as the QOL measure specific to IBS (IBS-QOL), the IBS QOL questionnaire, and the functional digestive disorder QOL questionnaire[15-18]. These questionnaires aim to address the specific QOL domains that are most heavily affected by IBS, yet there is a paucity of data on effect of IBS subtype on IBS-QOL.

In order to better understand the interactions between IBS subtype and IBS-QOL, we studied the impact of IBS subtype on disease-specific QOL using the IBS-QOL questionnaire in our cohort of IBS patients.

MATERIALS AND METHODS

Study population

The data was collected between June 2011 and August 2014 at the gastrointestinal motility clinic of Massachusetts General Hospital, Boston. Only adult patients greater than 18 years of age were enrolled in the study. All patients coming to the clinic completed Rome-Ⅲ questionnaires[19]. Patients who were diagnosed with IBS *via* the Rome Ⅲ questionnaires were further classified into four subtypes: IBS-C,

Table 1 Baseline characteristics of three irritable bowel syndrome-subtypes *n* (%)

Baseline characteristics	IBS-C (*n* = 54)	IBS-D (*n* = 56)	IBS-M (*n* = 121)	*P* value
Age (yr) (mean ± SD)	45.4 (16.0)	42.8 (15.3)	41.3 (14.9)	0.250
Gender				
Male	7 (13.0)	5 (9.8)	36 (29.8)	0.002[1]
Female	47 (87.0)	51 (90.2)	85 (70.2)	

[1]There was significant difference between gender distribution of irritable bowel syndrome (IBS)-mixed (IBS-M) *vs* IBS-constipation (IBS-C) ($P = 0.02$) and IBS-M *vs* IBS- diarrhea (IBS-D) ($P = 0.002$).

IBS-D, IBS-mixed type (IBS-M) and IBS-unclassified (IBS-U)[19]. Patients diagnosed with IBS were further assessed for upper gastrointestinal symptom severity and IBS-QOL on their first visit.

Assessment of gastrointestinal symptom severity

Severity of upper gastrointestinal symptoms was assessed using the patient assessment of upper gastrointestinal symptom severity index (PAGI-SYM)[20]. It assesses the severity of upper gastrointestinal symptoms along six subscales: heartburn/regurgitation, fullness/early satiety, nausea/vomiting, bloating, upper abdominal pain, and lower abdominal pain[20]. For each patient, we calculated a PAGI-SYM subscale score by taking the mean of the items in each subscale; the subscale scores varied from 0 (absent) to 5 (very severe)[20]. The total scores were calculated by taking the mean of subscales. If any subscale score was missing, the PAGI-SYM score was also considered to be missing[20]. Although PAGI-SYM is not validated in IBS-patients, it was used in our study as it is routinely administered to every patient coming to our functional gastrointestinal disorder clinic and has many overlapping questions with other IBS-severity scores.

Disease-related QOL

Disease-specific QOL was assessed using the IBS-QOL questionnaire[18]. It is a 34-item questionnaire assessing QOL along eight domains: dysphoria, interference with activities, body image, health worry, food avoidance, social reactions, sexual health, and effect on relationships[18]. All items are negatively-framed with the greatest response scale indicating poorer QOL. As per the IBS-QOL scoring manual, all items were reversed when scored so that as the IBS scores increases, QOL increases as well. All the final raw scores were transformed into a 0 to 100 scale. Using this scale, lowest possible score (worst QOL) and highest possible score (best QOL) were transformed to 0 and 100, respectively. Scores between these values indicate the percentage of the total possible score achieved[18]. Similarly, for each subscale, the raw scores were transformed into a scale of 0 to 100 and results were presented as a percentage of the total possible score achieved[18].

Ethical clearance

Ethics approval for the study was obtained prior to the initiation of the study by Massachusetts General Hospital Institutional Review Board (Protocol number-2012P002255/MGH).

Statistical analysis

Statistical analysis was performed using Stata 11.0 (StataCorp.2009, College station, Texas). Proportion of males and females among various IBS subgroups were compared using χ^2 test (Table 1). Mean age among various subgroups was compared using analysis of variance (ANOVA) (Table 1). Results of overall IBS-QOL scores and subscale scores were expressed as means with 95%CI. These were calculated using model based estimates from ANOVA. Mean differences in overall IBS-QOL scores and their subscales were compared among various IBS subgroups using ANOVA controlling for age and gender. A post-hoc analysis using Bonferroni correction was used only when *P* value for ANOVA was less than 0.05. For both ANOVA and Bonferroni tests, *P* values less than 0.05 were considered statistically significant.

The statistical methods of this study were reviewed by Douglas Hayden of Biostatistics center, Massachusetts General Hospital. This was done with support from Harvard Catalyst, the Harvard Clinical and Translational Science Center (National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health Award UL1 TR001102).

RESULTS

Two hundred fifty one of 572 patients screened during the study period were diagnosed with IBS. The mean age of the study population was 44.3 years. More than three-fourths of the patients (79.7%) were females (200 females, 51 males). Eight patients who were diagnosed with IBS did not have sufficient information to classify them into subtypes. Of the remaining 243 patients, IBS-mixed type (IBS-M) was the most common subtype (121 patients, 49.8%), followed by IBS-diarrhea (IBS-D) (56 patients, 23.1%), IBS-constipation (IBS-C) (54 patients, 22.2%), and IBS-unspecified (IBS-U) (12 patients, 4.9%). For the purpose of statistical analysis, patients with IBS-U were excluded given their small sample size in addition to the uncertainty of what IBS-U category means clinically.

Overall IBS-QOL

Patients with IBS-D had the lowest IBS-specific QOL (61.6; 95%CI: 54.0-69.1) followed by IBS-M (63.0; 95%CI: 58.1-68.0) and IBS-C (74.5; 95%CI: 66.9-82.1) ($P = 0.01$ overall by ANOVA) after controlling for age and gender (Table 2). In post hoc analysis, overall IBS-QOL was significantly-lower in patients with

Table 2 Irritable bowel syndrome specific quality of life subscale scores among three irritable bowel syndrome subtypes

IBS-QOL subscale	IBS-C (*n* = 54)	IBS-D (*n* = 56)	IBS-M (*n* = 121)	ANOVA *F*-test *P* value
Interference with activity	82.3 (74.1-90.6)	59.6 (51.4-67.7)	61.6 (56.3-66.9)	< 0.001[1]
Social reaction	80.0 (72.1-87.7)	70.7 (63.0-78.4)	66.1 (61.1-71.1)	0.008[2]
Food Avoidance	61.1 (50.8-71.3)	45.0 (34.8-55.2)	47.2 (40.7-53.7)	0.020[3]
Relationships	84.7 (77.2-92.2)	75.4 (67.9-83.0)	73.3 (68.4-78.2)	0.030[4]
Dysphoria	69.2 (60.0-78.4)	57.1 (47.9-66.4)	58.0 (51.9-64.0)	0.060
Health worry	64.3 (56.3-72.3)	60.9 (53.0-68.9)	57.3 (52.1-62.4)	0.280
Sexual	73.9 (63.7-84.1)	74.6 (64.6-84.7)	68.8 (62.3-75.3)	0.500
Body Image	69.2 (61.2-77.2)	66.0 (58.1-73.9)	64.9 (59.7-70.1)	0.631
Total	74.5 (66.9-82.1)	61.6 (54.0-69.1)	63.0 (58.1-68.0)	0.010[5]

All the results of mean irritable bowel syndrome (IBS) - quality of life scores and its subscales are compared after controlling for age and gender. [1]IBS-constipation (IBS-C) *vs* diarrhea (IBS-D) ($P < 0.001$) and IBS-C *vs* IBS-mixed (IBS-M) ($P < 0.001$) by Bonferroni test; [2]IBS-C *vs* IBS-M ($P = 0.005$) by Bonferroni test; IBS-C *vs* IBS-D ($P = 0.04$) and IBS-C *vs* IBS-M ($P = 0.04$) by Bonferroni test; IBS-C *vs* IBS-M ($P = 0.02$) by Bonferroni test; [5]IBS-C *vs* IBS-D ($P = 0.03$) and IBS-C *vs* IBS-M ($P = 0.02$) by Bonferroni test. IBS-QOL: Irritable bowel syndrome specific quality of life.

IBS-D than in patients with IBS-C ($P = 0.03$). Also, IBS-M had significantly lower IBS-QOL scores when compared with IBS-C patients ($P = 0.02$). There were no significant differences among IBS-QOL scores of IBS-D and IBS-M.

Individual subscales

Food avoidance: Food avoidance score was significantly different amongst IBS-subtypes ($P = 0.02$). Patients with IBS-D (45.0; 95%CI: 34.8-55.2) and IBS-M (47.2; 95%CI: 40.7-53.7) had a significantly lower food avoidance score than IBS-C (61.1; 95%CI: 50.8-71.3, $P = 0.04$ for both). IBS-D and IBS-M had comparable food avoidance subscale scores.

Interference with activity: There was significant difference in interference with activity subscale score amongst IBS-subtypes ($P < 0.001$). IBS-D patients (59.6; 95%CI: 51.4-67.7) and IBS-M patients (82.3; 95%CI: 74.1-90.6) both had significantly more interference with their activities than patients with IBS-C (81.0; 95%CI: 74.6-87.4, $P < 0.001$ for both).

Relationship: Relationship subscale was also significantly different amongst IBS-subtypes ($P = 0.03$). Relationships were significantly more affected in patients with IBS-M (73.3; 95%CI: 68.4-78.2) when compared with patients with IBS-C (84.7; 95%CI: 77.2-92.2, $P = 0.02$).

Social reaction: There was significant difference in social reaction subscale score amongst IBS-subtypes ($P = 0.008$). IBS-M patients had significantly lower social reaction score (66.1; 95%CI: 61.1-71.1) than IBS-C patients (80.0; 95%CI: 72.1-87.7, $P = 0.005$).

Other subscales: There was no statistical difference among the dysphoria, sexual health, health worries and body image subscale score of the various IBS subtypes (Table 2).

PAGI-SYM scores

PAGI-SYM scores were comparable among patients with IBS-C (2.6; 95%CI: 2.2-2.9), IBS-D (2.7; 95%CI: 2.4-3.1) and IBS-M (2.9; 95%CI: 2.7-3.1). There was no significant difference among IBS-subtypes for mean upper abdominal pain scores ($P = 0.30$), mean lower abdominal pain score ($P = 0.6$), mean bloating scores ($P = 0.52$) and early satiety scores ($P = 0.49$) using ANOVA.

DISCUSSION

In this analysis of IBS patients presenting for evaluation at a tertiary care clinic, we found that IBS-D and IBS-M patients have a significantly-worse disease-specific QOL than IBS-C subtype. IBS-D patients also have more interference with their daily activities and avoided food more commonly when compared to patients with IBS-C. Similarly, patients with IBS-M also had more interference in their activities, greater impact on their relationships and lower social reaction score than IBS-C patients. To our knowledge, this data is unique in demonstrating subtype-specific QOL differences in this population.

Most of the previous studies studying the effect of IBS subtype on QOL failed to show any difference in QOL between the subtypes[12-15,18,21-23]. Like our study, most of these studies are from tertiary healthcare settings and have comparable sample sizes. However, most of these studies used general health-related QOL questionnaires like the SF-36 and WHO-QOL[11,12,14,22,24]. The use of such general QOL questionnaires could have minimized the impact of gastrointestinal symptoms on overall QOL. Our study used IBS-QOL, which captures the specific concerns of patients with IBS.

Notably, other studies using IBS-QOL have failed to show similar differences when IBS-QOL is compared by IBS subtype[15,21,25]. All three of these studies analyzed patients from outside the United States and therefore may be examining a slightly-different IBS population than our own. In line with our results, Patrick *et al*[18] and Schmulson *et al*[23] also found lower IBS-QOL scores in United States patients with IBS-D and IBS-M compared to those with IBS-C, but these differences failed to reach statistical significance. Both these studies enrolled fewer IBS patients than ours.

By overall IBS-QOL and all eight individual subscale scores, IBS-D and IBS-M were very similar to each other and distinct from IBS-C patients. Mearin *et al*[26] previously reported that many patients with IBS-D

or IBS-C may qualify for the diagnosis of IBS-M over time. It is possible that these mixed patients were more similar to IBS-D patients than those with IBS-C in our population. Others have reported similarities between IBS-M patients and IBS-D in terms of defecation urgency, which at least partly explains low subscale scoring in the food avoidance and interference with activity domains in patients with IBS-M[14].

We found that patients with IBS-D and IBS-M had significantly more food avoidance when compared with IBS-C patients. Many patients with IBS attribute their gastrointestinal symptoms to diet and - as a result - a majority of patients restrict the consumption of perceived culprit foods[27]. Self-reported food avoidance has also been associated with high symptom burden and a reduced QOL[28]. Studies have shown that fermentable carbohydrates could increase colonic gas production, small intestinal water volume, and small intestinal motility and thus exacerbate IBS symptoms like pain, bloating, and diarrhea. In fact, fasting and diets restricted in fermentable carbohydrates were demonstrated to improve gastrointestinal symptoms including pain, bloating, and diarrhea in patients with IBS[29-31]. Thus, significantly higher food avoidance in IBS-D patients could be result of self- or physician-advised restriction to minimize these gastrointestinal symptoms.

IBS is known to interfere with the physical aspects of health-related QOL including daily activities and work productivity[11,32,33], yet limited data is available on the effect of IBS subtype on daily activities. In our study, patients with IBS-D and IBS-M had significantly more interference with their activities because of their disease than other IBS subtypes. Schmulson *et al*[23] also reported similar findings in female patients with IBS. Increased bowel frequency could understandably limit the ability of individual to go out and thus engage in daily activities like work, travel, and other social/leisure activities.

Thus, bowel frequency appears to be an important determinant of physical activity domain of QOL. This is further confirmed by the observation that the 5HT3-receptor antagonist alosetron, which improves stool consistency and frequency in these patients, also improved physical activity and work productivity[34]. This data argues that clinicians should focus on adequately controlling diarrheal symptoms in IBS-D patients in addition to addressing the pain and abdominal discomfort that are the hallmarks of IBS.

We found that IBS had a significantly greater impact on relationships in IBS-M patients when compared to IBS-C patients. Silk[33] reported that about one-fifth of married or cohabitating IBS patients reported difficulties in personal relationships due to their illness. More than half also reported embarrassment at the workplace. Interpersonal problems are reported to be more pronounced IBS-D patients[35]. Psychodynamic interpersonal therapy and improvement in interpersonal difficulties have not only been shown to improve psychological distress but also overall health status in patients with IBS[36]. Thus, patients with IBS-M might be benefit from counseling and interpersonal therapy.

Our study has some limitations. We did not study other factors known to affect the QOL in IBS such as somatic comorbidities, psychiatric comorbidities, and disease severity - which could have confounded our results. As a surrogate of gastrointestinal symptom severity, PAGI-SYM was used in our study as it is routinely administered to every patient coming to our functional gastrointestinal disorder clinic. Although PAGI-SYM has not been validated as a measure of symptoms severity in IBS patients, the components of the PAGI-SYM score, including bloating, lower abdominal pain, upper abdominal pain, and early satiety are all domains used to assess IBS symptom severity in IBS severity scales[37].

We found that overall PAGI-SYM score as well as individual subcomponent scores did not differ significantly among IBS subtype, suggesting similar disease severity amongst patients in our study population. The sample size of our study was also small, and similar studies should be repeated on larger number of IBS patients. Because these findings are derived from patients attending a tertiary care center, our study may not be easily generalized to IBS patients seen in community gastrointestinal practices or in the primary care setting. However, clinicians in these settings should also pay attention to these QOL domains.

We demonstrated that IBS-D and IBS-M patients have an overall lower IBS-QOL than IBS-C patients. Clinicians should pay special attention to patient-reported food avoidance, interference with daily activities, social reactions and problems with relationships, which are more prominent in IBS-D and IBS-M than other subtypes patients and drive a lower disease-specific QOL.

COMMENTS

Background

Patients with irritable bowel syndrome (IBS) have been shown to have poorer specific quality of life (QOL) than normal population and those with chronic diseases. QOL has been shown to correlate with health-care utilization, economic burden, disability and response to treatment.

Research frontiers

As general QOL questionnaires could minimize the impact of gastrointestinal symptoms on QOL in patients with IBS, researchers have developed several disease specific QOL. IBS-QOL is the most validated disease specific QOL measuring instrument. The current research hotspot is to understand the determinants of IBS-QOL.

Innovations and breakthroughs

The effect of IBS-subtypes on IBS-QOL is not very clear as most of the previous studies have used generalized QOL questionnaires like SF-36. To overcome this problem, the authors studied the effect of IBS-subtype on IBS-QOL and its eight subscales in patients with IBS at a tertiary healthcare center in the United States. The authors showed that IBS- diarrhea (IBS-D) and IBS-mixed (IBS-M)

patients have an overall lower IBS-QOL than IBS-constipation patients.

Applications

The study results suggest that clinicians should pay special attention to patient-reported food avoidance, interference with daily activities, and problems with relationships, which are more prominent in IBS-D and IBS-M than other subtypes patients and drive a lower disease-specific QOL in these patients.

Terminology

IBS is the most common functional gastrointestinal disorder characterized by abdominal pain or discomfort along with alteration of bowel habits (frequency and/or or consistency) in absence of an organic cause. Based on predominant bowel habit, it is further divided into IBS-diarrhea, IBS-constipation, IBS-mixed and IBS-unspecified. IBS-QOL is a disease specific QOL questionnaire that assesses QOL in IBS patients along eight domains: dysphoria, interference with activities, body image, health worry, food avoidance, social reactions, sexual health, and effect on relationships.

Peer-review

This is an interesting study where authors have shown that IBS-subtype could affect the IBS-QOL. This suggests that clinicians (gastroenterologists and primary care doctors) should pay attention to patients' specific complaints like food avoidance, interference with activity, social reactions and relationship problems which lower the QOL in IBS-D and IBS-M patients.

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ORIGINAL ARTICLE

Retrospective Study

Effect of technical parameters on transjugular intrahepatic portosystemic shunts utilizing stent grafts

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Author contributions: Andring B collected the data, analyzed the data, and drafted the manuscript; Pillai AK and Kalva SP provided analytical oversight, supervised the study, and made revisions to the manuscript; Burrell M helped with the study design and data collection tools; Xi Y provided statistically analysis; Pillai AK, Sutphin P, Anene A and Srinivasa R helped with data collection; all authors have read and approved the final version to be published.

Institutional review board statement: The UT Southwestern Institutional Review Board (IRB) reviewed the above-referenced research study via an expedited review procedure on November 27, 2013 in accordance with 45 CFR 46.110(a)-(b)(1). Having met all applicable requirements, the research study is approved. The approval period for this research study begins on November 27, 2013 and lasts until November 26, 2014.

Informed consent statement: Need for informed consent was waived by University of Texas-Southwestern/Parkland hospitals.

Conflict-of-interest statement: The authors have no conflicts of interest to report.

Data sharing statement: The signed statement has been uploaded to the site.

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Abstract

AIM: To assess the effect of technical parameters on outcomes of transjugular intrahepatic portosystemic shunt (TIPS) created using a stent graft.

METHODS: The medical records of 68 patients who underwent TIPS placement with a stent graft from 2008 to 2014 were reviewed by two radiologists blinded to the patient outcomes. Digital Subtraction Angiographic images with a measuring catheter in two orthogonal planes was used to determine the TIPS stent-to-inferior vena cava distance (SIVCD), hepatic vein to parenchymal tract angle (HVTA), portal vein to parenchymal tract angle (PVTA), and the accessed portal vein. The length and diameter of the TIPS stent and the use of concurrent variceal embolization were recorded by review of the patient's procedure note. Data on re-intervention within 30 d of TIPS placement, recurrence of symptoms, and survival were collected through the patient's chart. Cox proportional regression analysis was performed to assess the effect of these technical parameters on primary patency of TIPS, time to recurrence of symptoms, and all-cause mortality.

RESULTS: There was no significant association

between the SIVCD and primary patency (P = 0.23), time to recurrence of symptoms (P = 0.83), or all-cause mortality (P = 0.18). The 3, 6, and 12-mo primary patency rates for a SIVCD $\geqslant$ 1.5 cm were 82.4%, 64.7%, and 50.3% compared to 89.3%, 83.8%, and 60.6% for a SIVCD of < 1.5 cm (P = 0.29). The median time to stenosis for a SIVCD of $\geqslant$ 1.5 cm was 19.1 mo *vs* 15.1 mo for a SIVCD of < 1.5 cm (P = 0.48). There was no significant association between the following factors and primary patency: HVTA (P = 0.99), PVTA (P = 0.65), accessed portal vein (P = 0.35), TIPS stent diameter (P = 0.93), TIPS stent length (P = 0.48), concurrent variceal embolization (P = 0.13) and reinterventions within 30 d (P = 0.24). Furthermore, there was no correlation between these technical parameters and time to recurrence of symptoms or all-cause mortality. Recurrence of symptoms was associated with stent graft stenosis (P = 0.03).

CONCLUSION: TIPS stent-to-caval distance and other parameters have no significant effect on primary patency, time to recurrence of symptoms, or all-cause mortality following TIPS with a stent-graft.

Key words: Transjugular intrahepatic portosystemic shunt; Stents; Mortality; Portal Hypertension; Technique; Outcomes

Core tip: Current knowledge on the technical factors influencing the patency of transjugular intrahepatic portosystemic shunts (TIPS) is limited to the published data on TIPS created using bare-metal stents. However, stent grafts have replaced bare metal stents in TIPS creation. In this paper, we rigorously analyzed the effects of various technical factors on patency of TIPS created with stent grafts and also demonstrated how these factors influenced time to recurrence of symptoms and all-cause mortality. Our results challenge the accepted assumption that placement of the hepatic venous end of the stent beyond 1.5 cm of the hepatocaval confluence decreases primary shunt patency rates.

Andring B, Kalva SP, Sutphin P, Srinivasa R, Anene A, Burrell M, Xi Y, Pillai AK. Effect of technical parameters on transjugular intrahepatic portosystemic shunts utilizing stent grafts. *World J Gastroenterol* 2015; 21(26): 8110-8117 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i26/8110.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i26.8110

INTRODUCTION

The transjugular intrahepatic portosystemic shunt (TIPS) is widely used in the management of portal hypertension and supplanted the use of surgically created shunts due to its equivalent efficacy, decreased cost, and improved outcomes with future orthotropic liver transplantation[1-4]. Though originally used in the context of variceal bleeding[5-7], the indications for a TIPS quickly expanded to include refractory ascites[8-11], Budd-Chiari syndrome[12,13], and hepatic hydrothorax[14-17]. TIPS were initially created using bare-metal stents, which had poor primary patency rates requiring stent revision up to a rate of 50% within 1 year of creation[18-20]. There has been many theorized causes for the poor primary patency rates: (1) early acute thrombosis, often caused by technical failure (*e.g.,* stent shortening or migration) or a biliary-stent fistula; (2) parenchymal stenosis resulting from a fibrotic healing response to the trauma of shunt creation; and (3) late "pseudointimal" hyperplasia of the hepatic vein occurring between 3 mo and 1 year after TIPS placement[21-25].

Stent grafts including the Viatorr® stent graft (Gore® Flagstaff, Arizona) have shown superior efficacy in terms of primary patency and clinical outcomes compared to bare metallic stents and have, therefore, replaced bare metal stents for TIPS[26-29]. The effect of various technical parameters influencing primary patency of TIPS performed with bare metal stents has been previously studied to varying degrees. A distance greater than 2 cm between the hepatic venous end of the stent and the hepatic vein-inferior vena cava confluence - stent to inferior vena cava distance (SIVCD) (Figure 1), has been identified as one of the main technical factors affecting the patency of TIPS created with a bare-metal stent[30]. Late stenosis (after 3 mo) of a bare metal stent is secondary to pseudo-intimal hyperplasia[21-25]. Prior studies on stent grafts have suggested that they are less predisposed to pseudo-intimal hyperplasia as compared to the bare metal stents[31,32]. The effect of SIVCD, the hepatic vein-parenchymal tract angle (HVTA, Figure 2) and portal vein-parenchymal tract angle (PVTA, Figure 2), stent graft diameter, stent graft length, and the accessed portal vein on the outcomes of TIPS have not been well studied after the introduction of stent grafts for TIPS. The purpose of this study is to evaluate the effect of these selected technical factors on the primary shunt patency, time to recurrence of symptoms and survival following TIPS created with a Viatorr® stent graft (Gore® Flagstaff, Arizona).

MATERIALS AND METHODS

Study population

The institutional review boards of two institutions from which the data were gathered approved this retrospective study and waived the requirement for an informed consent. A database of patients who had TIPS preformed from 2008 to 2014 was generated. Patients lost to follow up were censored from the analysis. Sixty-eight patients with appropriate imaging

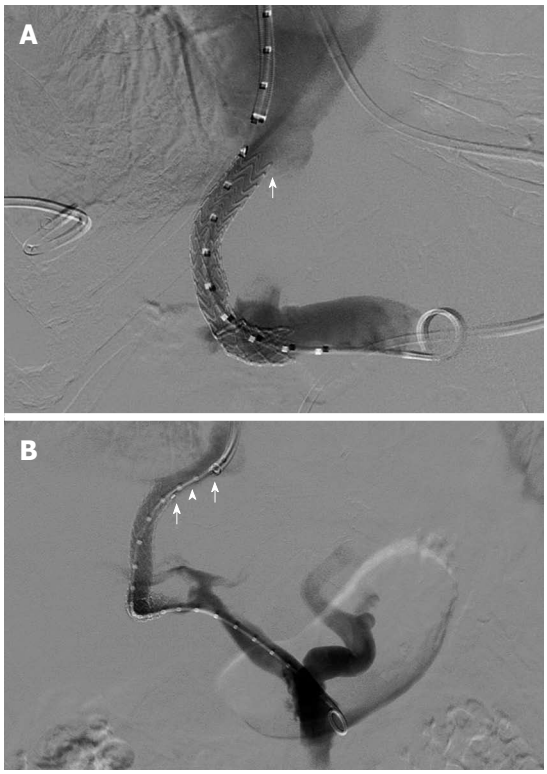

Figure 1 Digital subtraction angiography showing the placement of the hepatic venous end of the transjugular intrahepatic portosystemic shunt stent. A: Hepatic venous end of the stent at the hepatocaval junction (short arrow); B: Placement of the hepatic venous end of the stent away from the hepatocaval junction (arrow denotes end of stent, arrowhead denotes hepatocaval junction, and triangle denotes unstented hepatic vein).

studies and follow up were included in the study. The demographic information of the patients including the indication for TIPS is presented in Table 1. The etiology of cirrhosis included alcoholic (55%), hepatitis C (32%), non-alcoholic steatohepatitis (8%) and other (5%, hepatitis B, autoimmune, primary biliary cirrhosis, and cryptogenic). TIPS was placed according to the latest 2009 American Association for the Study of Liver Diseases 2009 Guidelines[33]. The indications for the procedure included: refractory variceal bleeding, refractory ascites, hepatic hydrothorax, hepatorenal syndrome, or a combination of these factors.

Data review

Two radiologists blinded to the outcomes of this study independently reviewed the angiographic images obtained during TIPS placement. Digital subtraction angiographic images were utilized for the measurement of the SIVCD, HVTA, and PVTA. A measuring catheter positioned within the stent graft was used to calculate the SIVCD. All patients included in this study had a TIPS completion venogram that depicted the confluence of the IVC and the hepatic vein. The HVTA and PVTA were measured in right anterior oblique view. The two observers had excellent correlation with the interclass correlation coefficients for the measured variables of SIVCD, HVTA, and PVTA

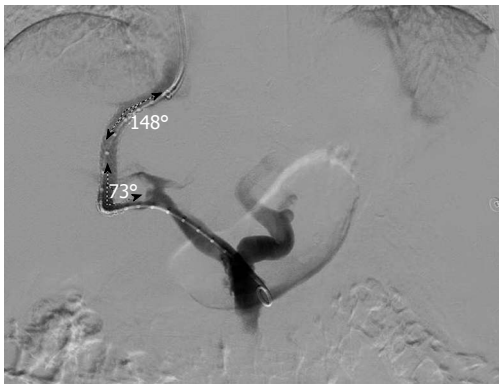

Figure 2 Digital subtraction illustrating the definition of hepatic vein to parenchymal tract angle (148° above) and portal vein to parenchymal tract angle (73° above).

Table 1 Demographic information of 68 patients included in this study including gender, age, Child Pugh class, and indication for transjugular intrahepatic portosystemic shunt

Demographic factor	*n* (%)
Male	35 (51.5)
Female	33 (48.5)
Average age, yr	55.5
Child Pugh Class	
A	10 (14.7)
B	44 (64.7)
C	14 (20.6)
Indication[1]	
Variceal bleeding	38 (55.9)
Ascites	38 (55.9)
Hepatic hydrothorax	8 (11.8)
Hepatorenal syndrome	1 (1.5)

[1]The indications do not sum to 100% as some patients had more than one indication.

of 0.98, 0.91, and 0.93, respectively. No measurement had more than a 10% discrepancy and, therefore, no measurements were discarded for analysis. The accessed portal vein was also determined by review of angiographic images. The TIPS stent graft diameter, TIPS stent graft length, and final TIPS balloon angioplasty diameter s were determined by review of the procedure note. Electronic chart review was performed to review interventions within first 30 d after TIPS creation, the time to recurrence of symptoms, United States Doppler studies, repeat venography and/or TIPS interventions, and mortality.

TIPS procedure

The median pre-TIPS and post-TIPS portosystemic gradient for this cohort was 18 (Range 7-42) mmHg and 6 (Range 1-10) mmHg, respectively. In 64% of patients, a large physiological portosystemic shunt was observed, which could explain the low median pre-TIPS portosystemic gradient. All patients included in this study achieved a portosystemic gradient of < 12 following TIPS. All stents placed were Viatorr® stent

grafts (Gore® Flagstaff, Arizona).

The SIVCD distance ranged from 0.0 to 3.8 cm. The HVTA and PVTA ranged from 117°-180° and 71°-176°, respectively. The accessed portal vein varied and included the main portal vein (*n* = 4, 6.0%), right portal vein (*n* = 31, 46.3%), first order right portal vein (*n* = 25, 37.3%), left portal vein (*n* = 5, 7.5%), and first order left portal vein (*n* = 2, 3.0%). The diameter of the stent graft used included 8 mm (*n* = 45, 66.2%) and 10 mm (*n* = 23, 33.8%). The stent graft length varied based on the patient's anatomy and included 6 cm (*n* = 10, 15.2%), 7 cm (*n* = 28, 42.5%), 8 cm (*n* = 16, 24.2%), 9 cm (*n* = 6, 9.1%), and 10 cm (*n* = 6, 9.1%). An 8 mm balloon was used initially for 10 mm stent grafts with further dilation with a 10 mm balloon if needed. The maximum balloon dilation diameter included either 8 mm (*n* = 45, 66.2%) or 10 mm (*n* = 23, 33.8%). Variceal embolization was performed following TIPS if any significant gastroesophageal varices were still visualized following TIPS.

Follow-up

The median follow up time was 11.2 mo. Patients were regularly followed with clinical and imaging follow-up performed at interval time points using TIPS ultrasound or when the patient presented with recurrence of symptoms. The determination of TIPS stenosis using ultrasound was based on established velocity thresholds of approximately 90-190 cm/s[34,35]. However, given these reference ranges can vary significantly with respiration and Doppler angle, all identified stenosis were confirmed with follow up venography. A stenosis on venography was defined as a portosystemic gradient greater than 12 mmHg and/or 50% narrowing on angiographic images (confirmed on two orthogonal views). A stenosis identified on venography was treated with angioplasty +/- additional stenting. In a few select patients (n-3), venography was performed without preceding ultrasound due to high pre-test probability of stenosis given recurrence of clinical symptoms. Patients lost to follow up were censored at the time of the last known imaging of the shunt (either duplex ultrasound or shunt venography). Patients who underwent liver transplantation were also censored at the time of transplantation (*n* = 3).

Statistical analysis

Cox proportional hazard regression analysis was performed using SIVCD as a continuous variable for its effect on primary patency, time to recurrence of symptoms, and all-cause mortality. Using 1.5 cm as the cutoff, Kaplan-Meier survival analyses were used to test any difference in primary patency, time to recurrence of symptoms, and all-cause mortality between SIVCD $\leqslant$ 1.5 cm or > 1.5 cm. The cutoff of 1.5 cm was chosen as it was similar to the 2.0 cm cutoff used in a prior study[30]. Then, cox proportional hazard multivariate regression analysis was performed on the simultaneous effect of all eight technical parameters (SIVCD, HVTA, PVTA, accessed portal vein, TIPS stent diameter, TIPS stent length, final balloon diameter, and reintervention within 30 d) on primary patency, time to recurrence of symptoms, and all-cause mortality. The association between recurrence of symptoms and stent graft stenosis was also evaluated *via* a χ^2 test. Effects with *P* value less than 0.05 were considered as statistically significant. All analyses were conducted in statistical software package SAS® 9.3. The statistical methods of this study were reviewed by Yin Xi from the University of Texas Southwestern Medical Center.

RESULTS

Cox proportional regression analysis did not show any association between the TIPS stent to IVC distance (SIVCD) and primary patency (*P* = 0.23), time to recurrence of symptoms (*P* = 0.83), and all-cause mortality (*P* = 0.18). The data was also split into two groups for Kaplan-Meier analysis using 1.5 cm as the cutoff (N greater than 1.5 cm- 19, N less than or equal to 1.5 cm- 49). There was no statistically significant difference between the two groups in terms of age, sex, indication for procedure, etiology of cirrhosis, or Child-Pugh class. The 3, 6, and 12-mo primary patency rates for a SIVCD $\geqslant$ 1.5 cm were 82.4% (SE = 9.3%), 64.7% (SE = 11.6%), and 50.3% (SE = 12.7%) compared to 89.3% (SE = 4.5%), 83.8% (SE = 5.7%), and 60.6% (SE = 8.6%) for a SIVCD of < 1.5 cm as shown in Figure 3. The median time to stenosis for a SIVCD of $\geqslant$ 1.5 cm was 19.1 mo (SE = 4.0 mo) compared to 15.1 mo (with SE = 2.2 mo) for an SIVCD < 1.5 cm (Kaplan-Meier analysis, *P* = 0.29). After one year, 57% (SE = 6.6%) of the patients with a SIVCD less than 1.5 cm were without symptoms *vs* 53% (with SE = 10.5%) of the patients with a SIVCD greater than 1.5 cm. (Kaplan-Meier analysis, *P* = 0.64) In the cohort with a SIVCD of greater than 1.5 cm, the patients who had recurrence of symptoms all recurred with the same symptom that served as the primary indication for the procedure. In the cohort with a SIVCD of less than 1.5 cm, there were three patients who had recurrence of symptoms that differed from their primary indication as two patients had a TIPS placed for recurrent ascites who recurred with variceal bleeding and one patient had a TIPS placed for variceal bleeding who recurred with recurrent ascites. After one year, the survival rate of the cohort of patients with a SIVCD less than 1.5 cm were 74% (with SE = 6.0%) *vs* 81% (with SE = 7.8%) for the cohort of patients with a stent graft distance greater than 1.5 cm. Figure 3 shows the primary patency, time to recurrence of symptoms, and all-cause mortality as a function of time. As expected, recurrence of symptoms was associated with stent graft stenosis with *P* value 0.0275 (χ^2 test).

Furthermore, cox proportional hazard regression

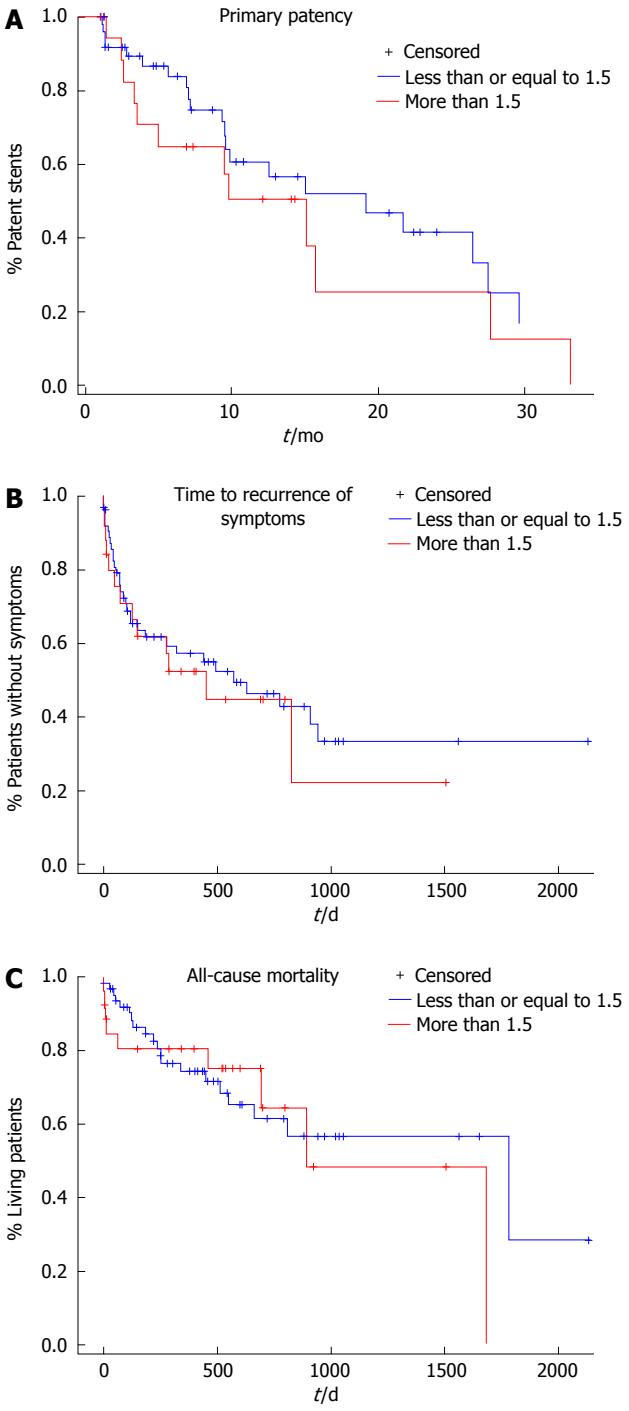

Figure 3 Kaplan-Meier curves following placement of a transjugular intrahepatic portosystemic shunt using a stent-graft when the stent-to-inferior vena cava distance was less than or equal to 1.5 cm and more than 1.5 cm. A: Primary patency as a function of time; B: Time to recurrence of symptoms as a function of time; C: All-cause mortality as a function of time.

analysis did not show any association between the HVTA and primary patency (P = 0.99), recurrence of symptoms (P = 0.62), and all-cause mortality (P = 0.16). Similarly, cox proportional hazard regression analysis did not show any association between the PVTA and primary patency (P = 0.65), recurrence of symptoms (P = 0.25), and all-cause mortality (P = 0.17).

Table 2 Results of cox regression analysis for transjugular intrahepatic portosystemic shunt placed with a stent-graft

	Primary patency *P* value	Time to recurrence of symptoms *P* value	All-cause mortality *P* value
SIVCD	0.23	0.83	0.18
HVTA	0.99	0.62	0.16
PVTA	0.65	0.25	0.17
Accessed portal vein	0.35	0.93	0.67
TIPS stent diameter	0.93	0.69	0.37
TIPS stent length	0.48	0.58	0.69
Final balloon diameter	0.99	0.43	0.22
Variceal Embolization	0.13	0.43	0.30
< 30 d of intervention	0.24	0.33	0.54

There was no statistically significant effect (*i.e.*, $P < 0.05$) of any of the above variables on primary patency, time to recurrence of symptoms, or all-cause mortality. TIPS: Transjugular intrahepatic portosystemic shunt; SIVCD: Stent-to-inferior vena cava distance; HVTA: Hepatic vein to parenchymal tract angle; PVTA: Portal vein to parenchymal tract angle.

Finally, the accessed portal vein, TIPS stent graft diameter, TIPS stent graft length, final TIPS balloon angioplasty diameter, presence of concurrent variceal embolization or reintervention within first 30 d had no statistically significant effect on primary stent patency, time to recurrence of symptoms, or all-cause mortality (Table 2).

DISCUSSION

The standard for positioning the hepatic venous end of the stent graft during TIPS creation is based on the study by Clark *et al*[30] that reported results from TIPS created using bare-metal stents. In this study, authors reported superior primary patency of TIPS when the hepatic venous end of the bare metal stent used for TIPS creation was positioned within 2 cm of the confluence between the hepatic vein and the IVC. Though early stenosis can be attributed to procedural complications (*i.e.,* biliary-stent fistulas), late stenosis of a bare metal stent (especially after 3 mo) is mainly secondary to pseudo-intimal hyperplasia[21-25]. Prior research on stent grafts has suggested that they are less predisposed to pseudo-intimal hyperplasia as compared to the bare metal stents[31,32]. For instance, Huang *et al*[31] found a statistically significant decrease in the incidence of intimal hyperplasia with stent grafts compared to that with bare metal stents (33% *vs* 3%, respectively, $P < 0.01$) in a study involving 60 patients who underwent TIPS.

Our results support the hypothesis that stent grafts are less predisposed to pseudo-intimal hyperplasia as there was no significant effect of SIVCD on primary patency of TIPS. This was further substantiated by the finding that there was no difference in clinical outcomes such as time to recurrence of symptoms or all-cause mortality. Our study shows that positioning the hepatic venous end of the stent graft within 1.5 cm of hepatocaval junction during TIPS creation may

not be necessary, unlike what was reported with bare metallic stents.

Stent graft geometry including the HVTA and PVTA was not well studied with bare metal stents or stent grafts. Despite early thoughts that shear stress from stent angulation may contribute to stenosis, two series presented only in abstract form (Perry *et al*[36] and Weeks *et al*[37]) with 19 and 61 patients, respectively, had shown that stent geometry (*i.e.,* HVTA and PVTA) had no effect on primary patency. Our data further supports there is no significant correlation between HVTA/PVTA and primary patency. Furthermore, our data further showed that stent graft angulation did not affect time to recurrence of symptoms or all-cause mortality.

Our results showed that there was no statistically significant effect of stent graft length, stent graft diameter, and final balloon angioplasty diameter on primary patency, time to recurrence of symptoms, or all-cause mortality. Therefore, during TIPS stent creation, focus should be on the combined effect of these technical factors on the pressure gradient and anatomic considerations as opposed to focusing on any one of the individual above parameters alone for the purposes of increasing primary stent patency.

The accessed portal vein also had no statistically effect on primary patency, time to recurrence of symptoms, or all-cause mortality. The right hepatic vein is usually chosen to access the right portal vein as the anterior approach reduces the likelihood of extra capsular puncture. Our data shows there is no indication to change this choice of hepatic vein to portal vein based on any gain in the primary patency of the stent.

Our data does not show any statistical correlation between reintervention within the first 30 d and more long term primary patency, time to recurrence of symptoms, or all-cause mortality. Therefore, short term patency may not be a good predictor of long term patency. This is consistent with prior reports which showed that the factors resulting in early thrombosis (*i.e.,* stent migration, stent shortening, biliary-stent fistulas) differ from those affecting long term patency (*i.e.,* pseudo-intimal hyperplasia).

This study has limitations. First, it was a retrospective study from only two institutions and included a moderate number of patients. A prospective, large cohort, multi-institutional study is desirable; however, we believe that between the two institutions included in this study, there was a wide range of patient populations. This study is also limited as it only had a moderate follow up time interval. However, as above, prior research has shown that pseudo-intimal hyperplasia typically occurs within 3 mo to one year following placement of the TIPS, which is within the follow up interval. Long term follow up is desirable. Given the similar patient characteristics including gender, age, MELD score, and indication for procedure across the two groups of SIVCD, confounding factors were minimized.

In conclusion, the positioning of hepatic venous end of TIPS stent graft in relation to hepatic vein - inferior vena cava confluence has little effect on the primary patency rate, time to recurrence of symptoms, and all-cause mortality following TIPS. Similarly, other technical factors such as hepatic vein to parenchymal tract angle, portal vein to parenchymal tract angle, TIPS stent graft diameter, TIPS stent length, concurrent variceal embolization and early re-intervention have no effect on primary patency, recurrence of symptoms, or all-cause mortality.

COMMENTS

Background

Patients with cirrhosis have a liver replaced with fibrous tissue that impedes portal venous blood flow to the liver, resulting in ascites and/or gastrointestinal bleeding. A transjugular intrahepatic portosystemic shunt (TIPS) diverts this blood flow through the liver and has been proven to alleviate symptoms. TIPS were originally placed using a bare-metal stents but now stent grafts are used due to their improved patency rates. The current literature on the technical factors to consider when creating a TIPS is based on the data from TIPS created with bare-metal stents.

Research frontiers

This paper reevaluates the effect of various technical parameters on primary shunt patency, time to recurrence of symptoms and all-cause mortality following TIPS created with stent grafts.

Innovations and breakthroughs

Previous studies based on TIPS created with bare metallic stents have suggested that the positioning of the hepatic venous end of the stent within 2 cm of the hepatic vein - inferior vena cava junction improves primary patency of TIPS. The study shows that this may no longer be necessary when TIPS is created with a stent-graft given that it has no effect on primary TIPS patency, time to recurrence of symptoms and all-cause mortality. Other technical factors, previously studied to varying degrees, including the angle the stent makes with the hepatic and portal vein, the length and diameter of TIPS stent, concurrent variceal embolization, and reinterventions within 30 d have little effect on TIPS patency, time to recurrence of symptoms, or all-cause mortality.

Applications

The results of this study can be applied during TIPS creation using a stent graft. Given that the technical parameters have little effect on final outcomes of TIPS, one should focus on physiological end points to improve clinical outcomes.

Terminology

A bare metal stent is a hollow tube lined with a lattice of metal (either stainless steel or Nitinol alloy). A stent-graft is a stent with an external (sometimes internal and external) covering on the metal lattice, typically made of a polymer of expanded polytetrafluoroethylene.

Peer-review

This manuscript retrospectively re-analyzed the gathered clinical data of of the transjugular intrahepatic portosystemic shunt used for patients with portal hypertension.

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ORIGINAL ARTICLE

Retrospective Study

Efficacy of endoscopic ultrasonography-guided fine needle aspiration for pancreatic neuroendocrine tumor grading

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Author contributions: Sugimoto M designed and performed the research and wrote the paper; Takagi T designed the research and supervised the report; Hikichi T designed the research and contributed to the analysis; Suzuki R, Watanabe K, Nakamura J, Kikuchi H, Konno N, Waragai Y, Asama H and Takasumi M provided clinical advice; Watanabe H, Obara K and Ohira H supervised the report.

Institutional review board statement: This study was reviewed and approved by the Ethics Committee of the Fukushima Medical University Hospital.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent. For full disclosure, the details of the study are published on the home page of Fukushima Medical University.

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Data sharing statement: No additional data are available.

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Abstract

AIM: To evaluate the efficacy of endoscopic ultrasonography-guided fine needle aspiration (EUS-FNA) for grading pancreatic neuroendocrine tumors (PNETs).

METHODS: A total of 22 patients were diagnosed with PNET by EUS-FNA between October 2001 and December 2013 at Fukushima Medical University Hospital. Among these cases, we targeted 10 PNET patients who were evaluated according to the World Health Organization (WHO) 2010 classification. Surgery was performed in eight patients, and chemotherapy was performed in two patients due to multiple liver metastases. Specimens obtained by EUS-FNA were first stained with hematoxylin and eosin and then stained with chromogranin, synaptophysin, CD56, and Ki-67. The specimens were graded by the Ki-67 index according to the WHO 2010 classification. Specimens obtained by surgery were graded by the Ki-67 index

and mitotic count (WHO 2010 classification). For the eight specimens obtained by EUS-FNA, the Ki-67 index results were compared with those obtained by surgery. In the two cases treated with chemotherapy, the effects and prognoses were evaluated.

RESULTS: The sampling rate for histological diagnosis by EUS-FNA was 100%. No adverse effects were observed. The concordance rate between specimens obtained by EUS-FNA and surgery was 87.5% (7/8). For the two cases treated with chemotherapy, case 1 received somatostatin analog therapy and transcatheter arterial infusion (TAI) targeting multiple liver metastases. Subsequent treatment consisted of everolimus. During chemotherapy, the primary tumor remained unconfirmed, although the multiple liver metastases diminished dramatically. Case 2 was classified as neuroendocrine carcinoma (NEC) according to the Ki-67 index of a specimen obtained by EUS-FNA; therefore, cisplatin and irinotecan therapy was started. However, severe adverse effects, including renal failure and diarrhea, were observed, and the therapy regimen was changed to cisplatin and etoposide. TAI targeting multiple liver metastases was performed. Although the liver metastases diminished, the primary tumor remained unconfirmed. These chemotherapy regimens had immediate effects for both unresectable neuroendocrine tumor (NET) and NEC cases. These two subjects are still alive.

CONCLUSION: EUS-FNA was effective for PNET diagnosis and Ki-67 index grading for WHO 2010 classification, enabling informed decisions on unresectable PNET treatment by identifying NET or NEC.

Key words: Pancreatic neuroendocrine tumor; Endoscopic ultrasonography-guided fine needle aspiration; Ki-67 index; World Health Organization classification 2010; Chemotherapy

Core tip: This is a retrospective study to evaluate the efficacy of endoscopic ultrasonography-guided fine needle aspiration (EUS-FNA) for grading pancreatic neuroendocrine tumors (PNETs). The concordance rate for grading between specimens obtained by EUS-FNA and surgery using the World Health Organization 2010 classification (Ki-67 indexing) was 87.5% in eight evaluated patients. In the two unresectable cases, chemotherapy was performed after grading was established based on the analysis of specimens obtained by EUS-FNA. Both treatments were adequately effective. EUS-FNA was useful for diagnosing PNET and enabled informed decisions on appropriate treatment plans by identifying neuroendocrine tumor or neuroendocrine carcinoma.

Sugimoto M, Takagi T, Hikichi T, Suzuki R, Watanabe K, Nakamura J, Kikuchi H, Konno N, Waragai Y, Asama H, Takasumi M, Watanabe H, Obara K, Ohira H. Efficacy of endoscopic ultrasonography-guided fine needle aspiration for pancreatic neuroendocrine tumor grading. *World J Gastroenterol* 2015; 21(26): 8118-8124 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i26/8118.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i26.8118

INTRODUCTION

Neuroendocrine tumors (NETs) develop from neuroendocrine cells distributed throughout the body. Pancreatic NET (PNET) and gastrointestinal NET are classified together into G1 and G2 and neuroendocrine carcinoma (NEC) by the World Health Organization (WHO) 2010 classification, which is based on growth morphology (mitotic count and Ki-67 index)[1]. While PNET is rare, accounting for only 2%-5% of pancreatic tumors[2], more cases have been reported in recent years, correlating with the use of more sophisticated diagnostic imaging methods.

To develop PNET treatment plans, guidelines have been issued from the National Comprehensive Cancer Network (NCCN)[3], North American Neuroendocrine Tumor Society (NANETS)[4], and European Neuroendocrine Tumor Society (ENETS)[5]. Regardless of grade, the first treatment choice for resectable tumors is surgery. PNET spreads to the liver in 10%-50% of cases[6-9], and treatment of liver metastasis includes surgery for the primary lesion, transcatheter arterial embolization, or transcatheter arterial chemoembolization[5]. In case of multiple, unresectable liver metastases or metastases in non-hepatic organs, antihormone or antitumor therapies are applied[3-5].

Antitumor therapy varies between NET and NEC. Everolimus or sunitinib has been reported to be useful for NET G1 or G2[10,11]. Meanwhile, approved chemotherapy regimens for NEC based on large clinical trials do not exist due to scarcity of data. Therefore, chemotherapy for small cell lung carcinoma is applied, as its behavior resembles NEC. It has been reported that the response rate to cisplatin and etoposide therapy for NEC is 42%-67%[12,13]. In addition, cisplatin and irinotecan therapy has also been used in NEC, as this therapy has been reported to be more efficient in small cell lung carcinoma[14].

Thus, it is important to sample sufficiently and accurately establish the grade to enable informed decisions on the treatment of unresectable PNET. Endoscopic ultrasonography-guided fine needle aspiration (EUS-FNA) has been reported to be useful for diagnosing pancreatic masses, including NET[15]. The accuracy of EUS-FNA has been reported as 80% for diagnosing PNET[16,17]. In this study, we evaluated the efficacy of EUS-FNA for diagnosing the Ki-67 index grade.

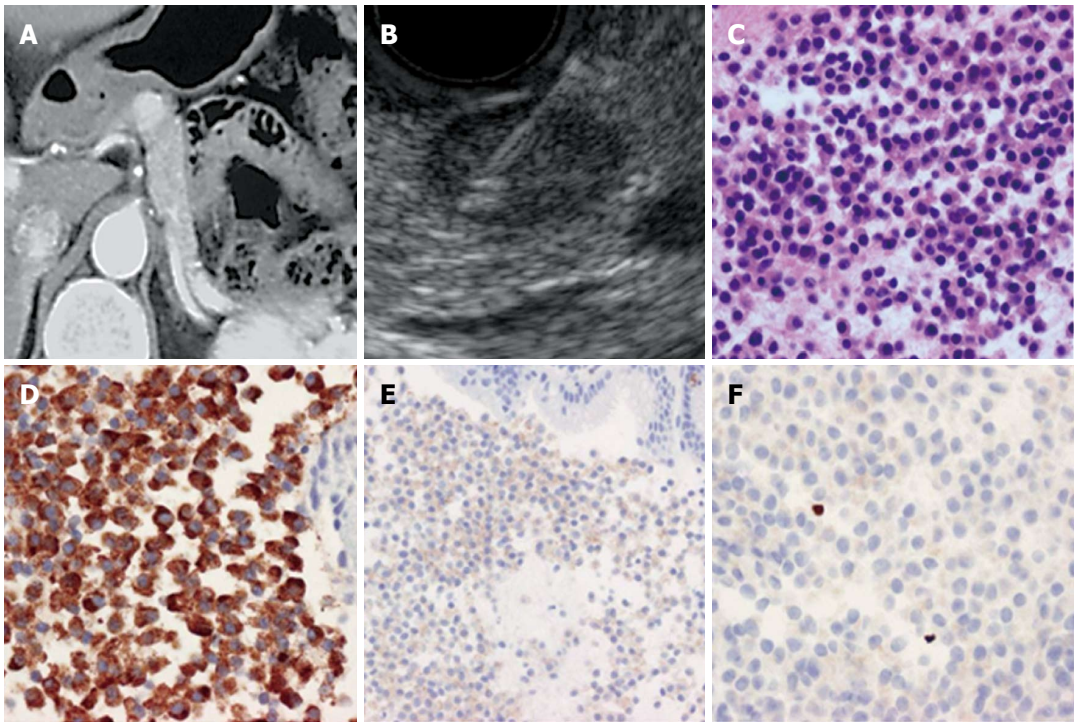

Figure 1 Method of endoscopic ultrasonography-guided fine needle aspiration. A: Abdominal contrast computed tomography. An enhancing tumor was recognized in the body of the pancreas; B: EUS-FNA. The tumor was recognized as a low echoic lesion with distinct boundaries; a 22G needle was inserted into the tumor; C: A specimen obtained by EUS-FNA (HE staining). Tumor cells with oval nuclei and acidophilic granular cytoplasm proliferated diffusely; D: A specimen obtained by EUS-FNA (Chromogranin A staining). Tumor cells were Chromogranin A-positive; E: A specimen taken by EUS-FNA (CD56 staining). Tumor cells were CD56-positive; F: A specimen taken by EUS-FNA (Ki-67 antibody staining). The Ki-67 index was 0.4% with tumor grade G1. EUS-FNA: Endoscopic ultrasonography-guided fine needle aspiration.

MATERIALS AND METHODS

Study design

A total of 22 patients were diagnosed with PNET by EUS-FNA from October 2001 to December 2013 at Fukushima Medical University Hospital. Among these cases, we targeted 10 patients who were evaluated by the 2010 WHO classification. Surgery was performed in eight patients, and chemotherapy was performed in the remaining two patients due to multiple liver metastases. The Ki-67 indices of the eight surgery cases were evaluated using specimens obtained by EUS-FNA and by surgery; for the two cases where chemotherapy was administered, the prognosis was evaluated. The study was approved by the ethics committee of Fukushima Medical University.

EUS-FNA

The endoscopic and ultrasonic equipment used in this study included GF-UCT260 or GF-UC240P, and EU-ME1 or EU-ME2 (Olympus Medical Systems, Tokyo, Japan). The biopsy needles were Expect 22G (Boston Scientific, MA, United States), EZ shot 22G (Olympus Medical Systems), or Echotip 19 or 22 or 25G (Cook Medical Inc., NC, United States).

All patients were sedated with midazolam before endoscopy. After the target lesion was visualized on the monitor, a needle biopsy was performed while confirming no blood flow on the puncture line. The needle was inserted through the gastrointestinal wall into the target lesion under EUS guidance with visualization of the needle in real time. After guiding the needle into the target lesion, the stylet was withdrawn, and the needle was moved back and forth within the lesion 20 times while negative pressure was applied using a 20 mL syringe connected to the end of the needle. Twenty multi-direction strokes were performed toward the target lesion for optimal sampling. An additional puncture was performed toward a different part of the tumor than was previously sampled. Suction was released, and the needle was withdrawn from the target lesion. Microscope slides were prepared from the biopsy sample and stained with Cyto-Quick stain. The slides were assessed by Rapid on-site cytological evaluation (ROSE)[18]. If the sample was adequate, the sampling was complete. If the sample was not sufficient, another needle aspiration was performed. After staining specimens with hematoxylin and eosin, chromogranin (DAKO, Glostrup, Denmark), synaptophysin (DAKO, Glostrup, Denmark), CD56 (ZYMED, Carlsbad, United States), and Ki-67 (DAKO, Glostrup, Denmark), the cases were graded according to the WHO 2010 classification based on the Ki-67 index (Figure 1). The specimens obtained by surgery were graded by the Ki-67 index and mitotic count as

Table 1 Comparison of specimens obtained by endoscopic ultrasonography-guided fine needle aspiration and surgery

	Sex	Age (yr)	Size (mm)	Location of tumor	No. of needle passes	Needle (G)	EUS-FNA specimen		Surgery specimen		
							Ki-67 index	Grade	Ki-67 index	Mitotic count (/10HPF)	Grade
1	M	71	4.4	Tail	5	19	< 2.0%	G1	< 2.0%	0	G1
2	F	79	19	Head	4	22	0.8%	G1	< 2.0%	0	G1
3	M	44	31	Head	2	22	1.8%	G1	0.1%	0	G1
4	F	51	10	Body	3	22	0.4%	G1	1.97%	0	G1
5	M	75	40	Tail	3	22	7.0%	G2	7.13%	2	G2
6	M	49	31	Body	3	22	4.54%	G2	< 20%	11	G2
7	F	46	30	Head	3	22, 25	< 2.0%	G1	7.5%	15	G2
8	M	55	40	Head	2	25	1.8%	G1	< 1.0%	0	G1

Tumor grades were concordant between specimens of EUS-FNA and those obtained by operation, except in case no. 7. The concordance rate between specimens of EUS-FNA and surgery was 87.5% (7/8). M: Male; F: Female; EUS-FNA: Endoscopic ultrasonography-guided fine needle aspiration.

Table 2 Prognosis of patients treated by chemotherapy

	Sex	Age (yr)	Size (mm)	Location of tumor	No. of needle passes	Needle (G)	Metastasis	EUS-FNA		Therapy	Prognosis
								Ki-67 index	Grade		
1	M	64	45	Head	3	22	Liver	10.0%	G2	Everolimus TAI	PR 15 mo survival antemortem
2	M	73	30	Tail	1	22	Liver	48.6%	NEC	IP→PE TAI	PR 12 mo survival antemortem

Two cases were treated by chemotherapy due to multiple liver metastases. Case 1 was diagnosed with NET G1 by EUS-FNA and was started on somatostatin analog therapy. After that, the patient began taking everolimus. Case 2 was diagnosed as NEC by EUS-FNA and therefore started on cisplatin and irinotecan therapy. However, severe adverse effects, including renal failure and diarrhea, were observed; therefore, the regimen was changed to cisplatin and etoposide. In these two cases, transcatheter arterial infusion targeting the multiple liver metastases was performed. During treatment, the primary tumors remained unconfirmed, but the multiple liver metastases diminished dramatically in both cases. M: Male; TAI: Transcatheter arterial infusion; IP: Cisplatin and irinotecan therapy; PE: Cisplatin and etoposide therapy; PR: Partial response; NETs: Neuroendocrine tumors; NEC: Neuroendocrine carcinoma; EUS-FNA: Endoscopic ultrasonography-guided fine needle aspiration.

stipulated by the WHO 2010 classification.

RESULTS

The eight cases for which surgery was performed are summarized in Table 1. The patients were aged 44-79 years and included five males and three females. The major tumor axes ranged from 4.4-40 mm. The pancreatic locations of tumors were the head (n = 4), body (n = 2), and tail (n = 2). The median number of needle passes was 3.5 (2-5). One case was performed using a needle with a diameter of 19G, while the other cases were biopsied with 22G needles. Cases 7 and 8 utilized 22G and 25G needles. The sampling rate for histologic diagnosis was 100%; therefore, sufficient specimens were obtained. No adverse effects were observed. The specimens of EUS-FNA matched those obtained by surgery in eight cases. The concordance rate between EUS-FNA and surgery specimens was 87.5% (7/8) (Table 1). Liver metastases were observed in patient 6; however, tumor metastases or relapses have not been observed in the other surgical cases.

Chemotherapy was administered in two unresectable cases (Table 2). Patient 1 was started on somatostatin analog therapy, and transcatheter arterial infusion (TAI) was performed to target multiple liver metastases. After these initial treatments, the patient began taking everolimus. During chemotherapy, the primary tumor remained unconfirmed, though the countless liver metastases diminished dramatically. Patient 2 was classified as NEC based on the Ki-67 index of a specimen obtained by EUS-FNA and was therefore started on cisplatin and irinotecan therapy. Severe adverse effects, including renal failure and diarrhea, were observed; therefore, the regimen was changed to cisplatin and etoposide. TAI targeting multiple liver metastases was performed, resulting in diminished size of the metastases. However, the primary tumor remained unconfirmed. These two cases are still alive.

DISCUSSION

When Ki-67 indices of specimens obtained by EUS-FNA are evaluated, it is important to take into account whether such indices reflect the entire tumor. Reports on this topic are scarce, as the WHO classification

of NET was revised in 2010, and cases of NET are rare. Here, we evaluated the diagnostic accuracy of PNET grading by EUS-FNA. According to our data, the concordance rate with surgical specimens was 87.5%. Furthermore, the effectiveness was also recognized for patients receiving chemotherapy. Liver metastases after surgery were observed in one case.

Larghi *et al*[19] compared specimens obtained by EUS-FNA with samples obtained by surgery in 12 PNET cases and found a concordance rate of 83.3% (10/12). One case was diagnosed with NET G1 by the EUS-FNA specimen but with NET G2 by the surgery specimen. An additional case was diagnosed with NET G2 by EUS-FNA but NET G1 by surgery. Hasegawa *et al*[20] reported that the concordance rate between EUS-FNA specimens and surgery specimens was 74.0% (20/27) when the EUS-FNA specimens were classified by average Ki-67 index, but the concordance rate was 77.8% (21/27) if the EUS-FNA specimens were classified by the highest Ki-67 index. In that report, one case was diagnosed as NEC by surgery but was diagnosed as NET G2 by the EUS-FNA specimen if classified by the average Ki-67 index. However, if classified by the highest Ki-67 index, the two cases diagnosed as NEC by surgery were diagnosed as NEC by EUS-FNA as well. Unno *et al*[21] compared specimens obtained by EUS-FNA with samples obtained by surgery in 19 PNET cases and found a concordance rate of 89.5% (17/19). One case was diagnosed with NET G2 by the EUS-FNA specimen but with NET G3 by the surgery specimen. Additionally, another case was diagnosed with NET G1 by EUS-FNA but NET G2 by surgery. In our hospital, the only case diagnosed as NET G1 by EUS-FNA was diagnosed as NET G2 by surgery. Moreover, in a report before 2010, Piani *et al*[22] compared specimens obtained by EUS-FNA and those obtained by surgery in 18 cases. They reported that the concordance rate was 89% if 2% was used as the Ki-67 index cut-off value, and the concordance rate was 78% if a Ki-67 index range of 2% to 10% was used as the cut-off value. Although this cut-off differs from the current cut-off value, a high concordance rate between EUS-FNA specimens and surgery specimens was shown. In these three reports and the present report, concordance rates between grades established by EUS-FNA and surgery were consistently between 70%-80%, and the concordance rate in differentiating lesions as NET or NEC was 98.5% (65/66).

In our study, liver metastases after surgery were observed in one case of NET G2. Pape *et al*[23] confirmed an increased survival risk for patients with grade 2 or 3 gastroenteropancreatic NET. Jann *et al*[24] reported that prognoses were statistically worse in patients with grade 2 or 3 NETs in the midgut and hindgut. Therefore, more cautious observation is necessary in patients with NET G2 or G3.

If lesions are unresectable, chemotherapy differs between NET and NEC. Therefore, diagnosing the grade of NET by EUS-FNA must be efficient for determining the chemotherapy choice. The chemotherapy administered in the two cases in this study was sufficiently effective, as evidenced by the fact that the expected progression-free survival of everolimus is 11 mo, and the expected response rate of cisplatin + etoposide therapy ranges from 42% to 67%[12,13]. Although there were only two cases in the present study, both were diagnosed and treated adequately based on conclusions drawn from the EUS-FNA data.

When using EUS-FNA for diagnosing PNETs, there are expected difficulties in obtaining specimens due to bleeding and blood contamination from abundant blood flow. Therefore, methods for obtaining sufficient specimens for immunostaining are required. In two previous reports, Larghi *et al*[19] used 19G needles, and Hasegawa *et al*[20] adopted the fanning method reported by Bang *et al*[25] in 2013. Moreover, Eloubeidi *et al*[26] performed EUS-FNA without negative pressure and diagnosed 13 cases of PNET. In this study, we applied the fanning method, and Ki-67 indices were evaluable for 10/11 cases for which Ki-67 staining was performed; one case was not evaluable. Therefore, a good concordance rate of grading between EUS-FNA and surgery specimens was observed by this approach.

There are certain limitations to the present study. First, the research was carried out at a single institution with a small number of patients, and it was a retrospective study. Further accumulation of study data is needed for a better comparison of Ki-67 indices between EUS-FNA and surgery specimens. Considering that PNET is relatively rare, it is useful to describe decisions on treatment regimens guided only by EUS-FNA specimens for future medical care. Second, the research evaluated only the Ki-67 index of EUS-FNA specimens. It was reported that K-ras mutations were observed in certain NET patients[27,28], and K-ras status might be correlated with malignancy of NET in the future. The Ki-67 index correlates with the prognosis of NET[24] and is a predictive factor for the effect of chemotherapy. Sorbye *et al*[29] reported that gastrointestinal NEC patients with Ki-67 < 55% had a lower response rate to platinum-based chemotherapy than patients with Ki-67 ⩾ 55% (15% *vs* 42%). Scoazec *et al*[30] reported that 20% of gastrointestinal NET and PNET cases diagnosed as NEC by the WHO 2010 classification were well differentiated; therefore, it is thought that well differentiated tumors could be included in NEC cases that do not respond to platinum-based chemotherapy. In this report, the Ki-67 index of the NEC patients was 48.6%; however, the specimens obtained by EUS-FNA showed poor differentiation. Therefore, platinum-based chemotherapy was indicated in this patient, and it was effective.

In conclusion, EUS-FNA is useful for diagnosing the Ki-67 index grade of PNET and for deciding treatment regimens.

COMMENTS

Background

Neuroendocrine tumors (NETs) develop from neuroendocrine cells distributed throughout the body. Pancreatic NETs (PNETs) are classified into G1, G2 and neuroendocrine carcinoma (NEC) by the WHO 2010 classification based on growth morphology (mitotic count and Ki-67 index). The first choice in PNET treatment is surgery, though in cases of multiple, unresectable liver metastases or metastases in non-hepatic organs, antihormone or antitumor therapies are applied. Antitumor therapy differs for NET and NEC. Everolimus or sunitinib is useful for NET G1 or G2, while small lung cell carcinoma chemotherapy is used for NEC, as their behaviors are similar. Thus, it is important to sample sufficiently and to accurately establish the grade in order to choose treatments for unresectable PNET. In this study, we evaluated the efficacy of endoscopic ultrasonography-guided fine needle aspiration (EUS-FNA) for diagnosing the Ki-67 index grade.

Research frontiers

EUS-FNA is important for determining treatment of PNET, a rare tumor. Few prior reports contain analyses of Ki-67 staining, EUS-FNA, and surgery. The results of this study contribute to clarifying the diagnostic potential of EUS-FNA for PNET grading.

Innovations and breakthroughs

In this study, EUS-FNA was a useful tool for diagnosing the Ki-67 index of PNET. The concordance rate between EUS-FNA and surgery was 87.5%. These results are in agreement with previous reports. However, in this report, two patients received chemotherapy based only on the Ki-67 index of EUS-FNA specimens, and the therapeutic values for both PNET and pancreatic NEC were evaluated. This emphasizes the accuracy of EUS-FNA for diagnosing the Ki-67 index in PNET.

Applications

This study suggests that EUS-FNA is useful for diagnosing PNET or PNEC. If a patient is diagnosed with unresectable PNET, chemotherapy can be chosen based on the Ki-67 index of the EUS-FNA specimen.

Terminology

EUS: An endoscopic procedure that enables observation of the chest and abdominal organs in the gastrointestinal tract. EUS-FNA: A technique to obtain specimens of chest and abdominal lesions by EUS guidance by puncturing the gastrointestinal tract.

Peer-review

The author of this paper evaluated the efficacy of EUS-FNA for grading PNET using the Ki-67 index and compared the results with those obtained by surgery from 10 patients. A promising concordance rate (7/8) between the specimens obtained by EUS-FNA and those obtained by surgery was found, and further clinical trials in a large population of PNET patients will be valuable.

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ORIGINAL ARTICLE

Retrospective Study

Risk factors for complications associated with upper gastrointestinal foreign bodies

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Abstract

AIM: To investigate predictive risk factors associated with complications in the endoscopic removal of foreign bodies from the upper gastrointestinal tract.

METHODS: We retrospectively reviewed the medical records of 194 patients with a diagnosis of foreign body impaction in the upper gastrointestinal tract, confirmed by endoscopy, at two university hospital in South Korea. Patient demographic data, including age, gender, intention to ingestion, symptoms at admission, and comorbidities, were collected. Clinical features of the foreign bodies, such as type, size, sharpness of edges, number, and location, were analyzed. Endoscopic data those were analyzed included duration of foreign body impaction, duration of endoscopic performance, endoscopic device, days of hospitalization, complication rate, 30-d mortality rate, and the number of operations

related to foreign body removal.

RESULTS: The types of upper gastrointestinal foreign bodies included fish bones, drugs, shells, meat, metal, and animal bones. The locations of impacted foreign bodies were the upper esophagus (57.2%), mid esophagus (28.4%), stomach (10.8%), and lower esophagus (3.6%). The median size of the foreign bodies was 26.2 ± 16.7 mm. Among 194 patients, endoscopic removal was achieved in 189, and complications developed in 51 patients (26.9%). Significant complications associated with foreign body impaction and removal included deep lacerations with minor bleeding (n = 31, 16%), ulcer (n = 11, 5.7%), perforation (n = 3, 1.5%), and abscess (n = 1, 0.5%). Four patients underwent operations because of incomplete endoscopic foreign body extraction. In multivariate analyses, risk factors for endoscopic complications and failure were sharpness (HR = 2.48, 95%CI: 1.07-5.72; P = 0.034) and a greater than 12-h duration of impaction (HR = 2.42, 95%CI: 1.12-5.25, P = 0.025).

CONCLUSION: In cases of longer than 12 h since foreign body ingestion or sharp-pointed objects, rapid endoscopic intervention should be provided in patients with ingested foreign bodies.

Key words: Emergency department; Foreign body; Upper gastrointestinal tract; Endoscopy; Complication

Core tip: We investigated the status of foreign bodies in the upper gastrointestinal tract and assessed risk factors for complications associated with the endoscopic removal of ingested foreign objects. We concluded that a longer duration of impaction, above 12 h, and sharp-pointed objects were related to the occurrence of endoscopic complications and failure. A strength of this study is that we evaluated risk factors for complications according to particular impaction time, in contrast to published studies that reported simply "long" impaction duration as a risk factor or that impaction time was not associated with the risk of complications.

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INTRODUCTION

Foreign body ingestion can be defined as materials swallowed accidentally or intentionally, or objects swallowed naturally when taking medication or food. It is frequently seen in the emergency department and occurs commonly in the pediatric population[1,2]. In adults, most foreign body ingestion occurs accidentally, but may be a result of contributory factors, such as psychiatric disorders, mental retardation, alcohol consumption, and an edentulous state[3-8]. However, practically, we also encounter not a few seemingly healthy adults with no apparent risk factor with unintentional foreign body ingestion. Although pre-endoscopic series have shown that 80% or more of the ingested objects are likely to pass spontaneously, in approximately 20% of cases, foreign bodies may require endoscopic or surgical intervention[9,10].

Endoscopic removal of foreign bodies generally has a low probability of complications, including impaction, perforation, and obstruction[11-15]. However, it can also sometimes be associated with severe or even life-threatening complications[16,17]. In previous reports, age, long duration of impaction, and impaction site, such as the upper esophagus or upper esophageal sphincter, were considered risk factors for endoscopic intervention[18-20]. However, there has been no consensus regarding risk stratification for the prevention for complications related to endoscopic foreign body removal[18,21]. Because an understanding of the predictive factors for complications with endoscopic removal may reduce morbidity and mortality, we investigated risk factors for complications in endoscopic upper gastrointestinal tract foreign body removal.

MATERIALS AND METHODS

Patients

We retrospectively reviewed the records of patients with a diagnosis of foreign bodies or food bolus impaction in the upper gastrointestinal tract, confirmed by endoscopy, at Kwandong University Myongji Hospital from January 2004 to August 2012, and at Dongguk University Ilsan Hospital from October 2005 to May 2013. After excluding pediatric patients, younger than 10 years, and those with insufficient data for analysis, in total, 194 cases were enrolled in this study. The protocol for the study was approved by the Institutional Review Boards of both hospitals.

Endoscopic procedures

All patients underwent an esophagoduodenoscopy (EGD) under local pharyngeal anesthesia, sedation using midazolam and/or pethidine, or general anesthesia using propofol and desflurane or sevoflurane. All examinations were undertaken by gastroenterologists who had performed at least 5000 EGD examinations and were qualified as Board of Gastrointestinal Endoscopy Specialists (Korean Society of Gastrointestinal Endoscopy) using flexible endoscopes (GIF-H260, Olympus Optical Co., Ltd., Tokyo, Japan). Cardiopulmonary function was

Table 1 Type of foreign bodies *n* (%)

Type of foreign body	Value
Fish bone	63 (32.5)
Drug	39 (20.1)
Shell	19 (9.8)
Meat	15 (7.7)
Metal	14 (7.2)
Animal bone	12 (6.2)
Stone	11 (5.7)
Plastic	9 (4.6)
Dental prosthetic	7 (3.6)
Tooth brush	2 (1.0)
Bean	1 (0.5)
Others	2 (1.0)
Total	194

monitored with pulse oximetry throughout the exam. All patients gave informed consent for the procedure. Endoscopic devices used for the removal of foreign bodies included alligator forceps, biopsy forceps, rat-tooth forceps, and a net. A protective cap, an overtube, or a latex protector hood was used to protect the pharyngeal and esophageal walls in cases of suspected sharp or pointed foreign bodies. In cases of failure to remove the foreign bodies using an endoscope, the patient was referred to the surgical department.

Data collection

Patient demographic data that were analyzed included age, gender, intention to ingestion, symptoms at admission, and comorbidities. Clinical features of foreign bodies were analyzed, including type, size, sharpness of edges, number, and location. Endoscopic data that were analyzed included duration of foreign body impaction, duration of endoscopic performance, endoscopic device, days of hospitalization, complication rate, 30-d mortality rate, and the number of operations related to foreign body removal.

Statistical analysis

For the identification of risk factors for complications after endoscopic intervention, categorical variables were analyzed using the χ^2 test or Fisher's exact test. OR with 95%CIs were calculated using logistic regression analysis for the evaluation of relative risk of complication occurrence and their association with variable parameters. *P* values < 0.05 were considered to indicate statistical significance in each analysis. All statistical analyses were performed using SPSS Statistics (ver. 13.0; SPSS Inc., Chicago, IL, United States).

RESULTS

Patient characteristics

In total, 194 patients with ingested foreign bodies underwent endoscopic management. The mean age at diagnosis was 54.84 ± 18.03 (range: 10-89) years.

Table 2 Anatomic location of foreign bodies *n* (%)

Location	Value
Esophagus	
Upper 1/3 (< 25 cm from incisor)	111 (57.2)
Mid 1/3 (≥ 25 cm, < 40 cm)	55 (28.4)
Lower 1/3 (≥ 40 cm)	7 (3.6)
Stomach	21 (10.8)
Total	194

Most patients were adults (age > 14 years, 191/194, 98.5%), while few were children (age ≤ 14 years, 3/194, 1.5%). There were 105 (54.1%) females and 89 (45.9%) male patients.

Characteristics and location of foreign bodies

The types of foreign body detected in the upper gastrointestinal tract varied markedly. The most common type of foreign object was a fish bone (63 patients; 32.5%), followed by drugs (39 patients; 20.1%), shells (19 patients; 9.8%), meat (15 patients; 7.7%), metal (14 patients; 7.2%), and animal bones (12 patients; 6.2%). Others included stones, plastic, dental prosthetics, teeth, beans, and a toothbrush (Table 1). The median size of the foreign bodies was 26.2 ± 16.7 (range: 3-140) mm. Regarding anatomical location, foreign bodies were located mainly in the upper esophagus (111 patients; 57.2%) and mid esophagus (55 patients; 28.4%), followed by the stomach (21 patients; 10.8%) and lower esophagus (7 patients; 3.6%; Table 2).

Endoscopic management

The median time interval between ingestion and endoscopic removal was about 5 h with a range of approximately 1 to 383 h. The median duration of the endoscopic procedure was 11.76 ± 25.05 (range: 1-320) min. The preferred accessory devices for extraction varied according to the type and location of the foreign bodies. For retrieval, frequently used devices were biopsy forceps (*n* = 96, 49.5%), a net (*n* = 32, 16.5%), and alligator forceps (*n* = 30, 15.4%). A push into the stomach was performed in 16 (8.2%) cases; 189 (97.4%) foreign bodies were removed successfully, while endoscopic removal failed in five patients (2.6%), and all then underwent additional surgery (Tables 3-5). There was no death associated with any foreign body ingestion or endoscopic procedure.

Complications associated with foreign body impaction

Among the 189 patients who underwent endoscopic removal of foreign bodies, minor mucosal injuries, such as abrasions or small erosions were noted in 58 cases (30.7%). Significant complications related to foreign body impaction and removal included deep lacerations with minor bleeding (*n* = 31, 16%), ulcer (*n* = 11, 5.7%), perforation (*n* = 3, 1.5%), and abscess

Table 3 Methods used for removal of foreign bodies *n* (%)

Method of removal	Value
Pull with biopsy forcep	96 (49.5)
Pull with net	32 (16.5)
Pull with alligator	29 (14.9)
Pull with snare	6 (3.1)
Pull with basket	5 (2.6)
Push into stomach	16 (8.2)
Others	4 (2.1)
Surgery	6 (3.1)
Total	194

Table 4 Complications by foreign bodies *n* (%)

Complication	Value
Ulcer	11 (5.7)
Laceration	31 (16.0)
Perforation	3 (1.5)
Abscess	1 (0.5)
Total	46 (23.7)

(*n* = 1, 0.5%) (Table 4). Of these, four patients with perforations or an abscess underwent surgical intervention (Table 5). Because foreign body impaction duration was a continuous variable, we divided it into two categorical variables using receiver-operating characteristic (ROC) curves for different durations. A cut-off value of 12 h had the highest sensitivity (76%) and specificity (43%) for significant complications. Both failure of endoscopic foreign body removal and related significant complications were categorized according to dependent variable stratification. In a multivariate analysis, risk factors were time interval beyond 12 h between ingestion and endoscopic management and the sharpness of the foreign body (Table 6).

DISCUSSION

Foreign body ingestion is a commonly encountered problem in the endoscopic department. According to previous reports, 80%-90% of ingested foreign bodies pass spontaneously and the complication rate is generally low[5,21-23]. However, they are sometimes impacted in a physiological or pathological luminal narrowing or angulation site, which may lead to potentially life-threatening complications. A recent study showed that the rate of endoscopic intervention may be much higher (63%-76%) than expected, and long delays from ingestion to presentation and intervention may account for the relatively high rates of surgery and perforation in patients with intentional ingestion[4]. Generally, identification and radiographic localization are the initial preferred steps in the management of foreign bodies[24]. Biplane radiographs are useful for confirming ingested materials and complications, such as free air and lung aspirates, prior to attempts at endoscopic extraction[8,9,24]. In the current study, routine neck AP X-rays could not detect foreign bodies in two-thirds of patients, and radiopaque material was found in only 38% of patients (75/194). Although no small bony, thin metal, or plastic materials were detected on routine radiologic examinations, failure to locate an object on X-rays does not preclude its presence. Thus, in patients with typical clinical presentations or with highly suspected foreign body ingestion, an endoscopic evaluation should be performed even with a normal finding in radiography[12,25-27].

Recently, the ASGE Standards of Practice Committee published guidelines for foreign body management and divided the timing of endoscopic intervention into three groups - emergency, urgent, and non-urgent - according to the characteristics of the foreign object[9]. Emergency endoscopic intervention is required for patients with high-grade esophageal obstruction and ingestion of disk batteries or sharp-pointed long objects[9]. Urgent endoscopic intervention is needed for esophageal foreign objects that are not sharp-pointed, food impaction without complete obstruction, sharp-pointed objects in the stomach or duodenum, objects longer than 6 cm in length, and magnets within endoscopic reach[9]. They also recommended that because delay decreases the likelihood of successful removal and increases the risk of complications, including risk of perforation, endoscopic removal should not be delayed beyond 24 h for patients with esophageal foreign bodies or food impaction[9]. However, even in that report, a relationship between impaction duration and risk of complications, such as perforation, bleeding, or ulcer formation, was not demonstrated and the differences between "emergency" and "urgent" were debatable.

In practice, the need for and timing of an endoscopic intervention depend on various factors, including the patient's age, clinical condition, foreign body size, shape, content, anatomical location, and duration time since ingestion[9,28]. Additionally, because most patients visited the emergency department, we frequently encounter the problem of making a decision regarding the timing of an endoscopic intervention. A strength of our study is that we assessed the risk factors for complications according to particular impaction time, in contrast to some other published studies that mentioned simply "long" impaction duration as a risk factor or that impaction time showed no association with the risk of complications[19,29]. The question of whether impaction time is a risk factor for complications has been controversial. As in the ASGE guidelines mentioned above, Loh *et al*[30] suggested that if the foreign body had been impacted for more than 1 d, there was a 14-fold greater risk of a major complication *vs* less than 24 h. Wu *et al*[20] reported that patients with delayed endoscopic intervention (> 24 h) may have additional symptoms, such as

Table 5 Cases of surgical intervention

No.	Cause of surgery	Foreign body type	Foreign body size (mm)	Foreign body location
1	Failure of endoscopic removal	Animal bone	25	Upper esophagus
2	Failure of endoscopic removal	Stone	35	Mid esophagus
3	Failure of endoscopic removal	Stone	30	Mid esophagus
4	Failure of endoscopic removal	Fish bone	35	Mid esophagus
5	Failure of endoscopic removal	Pin	35	Stomach
6	Perforation	Metal	25	Stomach
7	Perforation	Fish bone	25	Upper esophagus
8	Perforation	Shell	35	Upper esophagus
9	Abscess	Fish bone	15	Upper esophagus

Table 6 Results of multivariate analysis following univariate analysis of risk factors for foreign body removal related with complications

	Yes (n = 51)	No (n = 143)	P value	OR	P value
Age (yr, mean ± SD)	61.18 ± 17.26	52.58 ± 17.82	0.003		
> 60/≤ 60	27/24	57/86	0.106	1.84 (0.87-3.91)	0.112
Gender					
Male/female	26/25	63/80	0.394	1.42 (0.71-2.86)	0.327
Foreign body					
Location			0.381		
Upper esophagus	27	84		1	0.213
Mid and lower esophagus	20	42		0.55 (0.25-1.24)	0.151
Stomach	4	17		1.61 (0.46-5.64)	0.460
Size (cm, mean ± SD)	2.22 ± 0.83	1.87 ± 0.85	0.012		
> 3 cm /≤ 3 cm	24/27	43/100	0.028	1.70 (0.82-3.54)	0.155
Sharpness (Yes/No)	36/15	74/69	0.02	2.48 (1.07- 5.72)	0.034
Radio-opacity (Yes/No)	20/31	55/88	0.924	0.98 (0.46-2.08)	0.955
Esophageal stricture (Yes/No)	3/48	24/119	0.053	0.27 (0.06-1.12)	0.071
Duration of impaction (min), mean ± SD, median (min-max)	2304 ± 4245, 390 (56-23000)	853 ± 1195, 270 (60-18720)	0.022, 0.094		
> 12 h/≤ 12 h	22/29	35/108	0.012	2.42 (1.12-5.25)	0.025

odynophagia and esophageal ulceration, although they also concluded that severe complications, including esophageal perforation and bleeding, showed no correlation with impaction time. Jung *et al*[19] reported a "long" duration of impaction without mentioning a particular time as a predictive factor for complications after foreign body ingestion. However, Park *et al*[29] reported no correlation of impaction time (> 24 h) with risk of complication, and that sharp-pointed objects, greater length of foreign bodies, and the presence of symptoms were significant risk factors for complications. In contrast, our results showed that impaction duration and sharpness of foreign bodies were the two important risk factors for the development of major complications. In particular, impaction duration over 12 h but less than 24 h had a 2.4-fold increased risk for major complications, whereas age over 60 years, presence of stricture, radio-opacity, foreign body location, and size over 30 mm did not show correlations with the development of complications.

Our study had several limitations. First, although emergency endoscopic removal should be advocated, and we did not experience asphyxia or aspiration, development of these complications due to insufficient fasting time is a possibility. Second, our analysis was limited to a retrospective review of available still EGD images and medical records, so the degree of complications, such as mucosal damage, was not defined objectively, and some potential bias might have been added. Third, due to the relatively small numbers of cases with major complications, such as perforations, in this population, it is difficult to analyze the risk ratio for serious complications. Thus, further prospective studies with larger numbers of patients are needed to confirm these results.

In conclusion, when patients with foreign bodies in the upper gastrointestinal tract present for care, predictive factors for complications should be considered carefully. Because delayed endoscopic removal, beyond 12 h, and sharp objects decrease the likelihood of successful endoscopic removal and increase the risk of complications, early recognition and urgent endoscopic intervention after ingestion are necessary.

COMMENTS

Background

Foreign body ingestion is commonly encountered in the emergency department. Although 80% or more of the ingested objects are likely to pass spontaneously, in approximately 20% of cases, foreign bodies may require endoscopic or surgical intervention. Endoscopic removal of foreign bodies can sometimes be associated with severe or even life-threatening complications. However, there has been no consensus regarding risk stratification for the prevention for complications related to endoscopic foreign body removal.

Research frontiers

Many researchers have discussed the risk factors for complications associated with upper gastrointestinal foreign body impaction. And recently the ASGE Standards of Practice Committee published guidelines for foreign body management. However, there are still many debates regarding risk factors affecting complication occurrence.

Innovations and breakthroughs

Previous published studies reported age, impaction site and long duration of foreign body ingestion were considered the risk factors for complications of endoscopic foreign body removal. However, because consensus has not been reached regarding impaction time, the authors evaluate the risk factors for complications according to particular impaction time.

Applications

If foreign body ingestion is longer than 12 h or sharp-pointed object impaction is suspected, rapid endoscopic intervention may benefit patients with ingested foreign bodies.

Terminology

Foreign body ingestion in the upper gastrointestinal tract can be defined as objects swallowed accidentally or intentionally, or materials swallowed naturally when taking medication or food. Significant complications associated with foreign body impaction include deep lacerations with minor bleeding, ulcer, perforation, and abscess.

Peer-review

This article is to address the risk factors for complications associated with endoscopic removal of ingested foreign bodies in upper gastrointestinal tract, which is a retrospective study conducted on 194 patients at two university hospitals. In the article, sharpness (HR = 2.48, 95%CI: 1.07-5.72, P = 0.034) and longer than 12 h of impaction duration (HR = 2.42, 95%CI: 1.12-5.25, P = 0.025) are major risk factors for complications. These results suggest that early recognition and urgent endoscopic intervention are necessary for the management of foreign body ingestion.

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ORIGINAL ARTICLE

Retrospective Study

Fourteen- *vs* seven-day bismuth-based quadruple therapy for second-line *Helicobacter pylori* eradication

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Abstract

AIM: To compare the efficacy of 14- and 7-d bismuth-based quadruple therapies as second-line eradication treatment for *Helicobacter pylori* (*H. pylori*) infection.

METHODS: Between 2004 and 2014, the medical records of 790 patients who had experienced failure of first-line proton pump inhibitor (PPI)-based eradication therapy and were then treated with bismuth-based quadruple therapy were retrospectively reviewed. Those who received bismuth-based quadruple therapy [PPI, bismuth, metronidazole, and tetracycline (PBMT)] for either 7 d or 14 d were assigned to a PBMT-7 group (n = 543) or a PBMT-14 group (n = 247), respectively. The eradication rates for both groups were determined by intention-to-treat (ITT) and per-protocol (PP) analyses. ITT analysis compared the treatment groups as originally allocated while the PP analysis including only those patients who had completed the treatment as originally allocated. Successful eradication therapy for *H. pylori* infection was defined as a negative ^{13}C-urea breath test 4 wk after the end of eradication treatment.

RESULTS: The overall ITT eradication rate was 69.1% (546/790). Final ITT eradication rates were 67.4% (366/543; 95%CI: 63.1%-71.7%) in the PBMT-7 group and 72.8% (180/247; 95%CI: 67.4%-78.2%) in the PBMT-14 group (P = 0.028). The overall PP eradication rate was 80.0% (546/682), and the final PP eradication rates were 78.2% (366/468; 95%CI: 72.1%-84.0%) in the PBMT-7 group and 84.1% (180/214; 95%CI: 76.8%-90.8%) in the PBMT-14 group (P = 0.009). The *H. pylori* eradication rates in the PBMT-14 group were

significantly higher than in the PBMT-7 group according to both ITT (P = 0.028) and PP analysis (P = 0.009). Compliance was similar in both groups (PBMT-7 group: 97.9%; PBMT-14 group: 96.4%). Adverse event rates were 10.7% (51/478) and 17.1% (38/222) in the PBMT-7 and PBMT-14 groups, respectively (P = 0.487).

CONCLUSION: The 14-d bismuth-based quadruple therapy is a significantly more effective second-line eradication treatment for *H. pylori* infection than the 7-d alternative.

Key words: *Helicobacter pylori*; Treatment failure; Second-line treatment; Bismuth; Eradication rate

Core tip: This study evaluated the efficacy of 14-d bismuth-based quadruple therapy compared with a corresponding 7-d quadruple therapy as second-line eradication treatment of *Helicobacter pylori* (*H. pylori*) infection in South Korea. *H. pylori* eradication rates in the 14-d treatment group were significantly higher than in the 7-d treatment group for both the intent-to-treat and per-protocol analysis. The high eradication rate, excellent compliance, and safety of the 14-d regimen suggest its potential suitability as a second-line eradication treatment. The 14-d bismuth-based quadruple therapy is a significantly more effective second-line eradication treatment than the 7-d alternative for *H. pylori* infection in Korean patients.

Hwang JJ, Lee DH, Lee AR, Yoon H, Shin CM, Park YS, Kim N. Fourteen- *vs* seven-day bismuth-based quadruple therapy for second-line *Helicobacter pylori* eradication. *World J Gastroenterol* 2015; 21(26): 8132-8139 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i26/8132.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i26.8132

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is a primary risk factor for chronic gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma, and gastric cancer[1]. The most effective first-line eradication therapy known to date is a standard triple therapy consisting of a proton pump inhibitor (PPI), clarithromycin, and amoxicillin (or metronidazole)[2,3]. Although many clinical studies have shown standard triple therapy to be highly effective, a number of meta-analyses have found eradication rates for standard triple therapy to vary from 70% to 95%[4,5], and there have been reports of increased antibiotic resistance to clarithromycin and metronidazole[6,7]. Standard triple therapy is recommended in South Korea as first-line eradication therapy for *H. pylori*[8], but eradication rates for standard triple therapy range from 60% to 90%[9], with decreasing eradication rates of first-line eradication therapy due to increased antibiotic resistance rates among *H. pylori* strains in South Korea[10,11].

Various eradication regimens are being studied as possible alternative treatments to overcome decreasing eradication rates. The Maastricht IV/ Florence Consensus Report currently recommends a bismuth-based quadruple therapy consisting of PPI, bismuth, metronidazole, and tetracycline (PBMT) as the preferred second-line therapy following failure of first-line eradication therapy[2]. Metronidazole, one of the key antibiotics used in bismuth-based quadruple therapy, has been reported to have high antibiotic resistance rates of 34.4%-66% in South Korea[6,7,12]. Although metronidazole resistance is known to have little influence on successful eradication[13], eradication rates of 7-d bismuth-based quadruple therapy in per-protocol (PP) analysis have been reported to be 70% in South Korea[14].

There is much controversy about bismuth-based quadruple therapy treatment duration. Recommended treatment duration varies according to individual guidelines; for instance, a treatment duration of more than 1 wk is recommended in Europe, while 1-2 wk and 1 wk are recommended in the United States and South Korea, respectively[8,15,16]. One report indicated that in some metronidazole-resistant areas, extending the bismuth-based quadruple therapy treatment duration to 10-14 d was highly effective[17]; similarly, another study found that administering metronidazole for 14 d could overcome the negative influence of metronidazole resistance[18]. There is also much debate about the value and efficacy of 7- *vs* 14-d of bismuth-based quadruple therapy as a second-line eradication therapy in South Korea[14,19,20]. However, few studies have examined the efficacies of these treatments; most had relatively low samples sizes, making adequate efficacy comparisons between the two treatment regimens difficult. Accordingly, the aim of the present study was to investigate effective treatment duration for bismuth-base quadruple therapy by comparing eradication rate, compliance, and adverse event rate between 7- and 14-d bismuth-based quadruple therapies administered to patients after failure of first-line eradication therapy in South Korea.

MATERIALS AND METHODS

Patient selection

This study was conducted at Seoul National University Bundang Hospital between January 2004 and August 2014. The medical records of 790 patients who had experienced failure of first-line PPI-based eradication therapy for *H. pylori* infection were reviewed in this retrospective study. Eradication failure was defined by at least one of the following three tests: a positive ^{13}C-urea breath test (^{13}C-UBT); histologic evidence

of *H. pylori* by modified Giemsa staining in the lesser and greater curvature of the body and antrum; and/or a positive rapid urease test (CLOtest; Delta West, Bentley, Australia) by gastric mucosal biopsy from the lesser curvature of the body and antrum. None of the patients had previously received *H. pylori* eradication therapy before their first-line treatment. Patients were excluded if they had received PPIs, H_2 receptor antagonists, or antibiotics in the previous 4 wk, or if they had used non-steroidal anti-inflammatory drugs or steroids in the 2 wk prior to the ^{13}C-UBT. Other exclusion criteria were as follows: (1) age below 18 years; (2) previous gastric surgery or endoscopic mucosal dissection for gastric cancer; (3) advanced gastric cancer; (4) severe current disease (hepatic, renal, respiratory, or cardiovascular); (5) pregnancy; and (6) any condition likely to be associated with poor compliance (*e.g.*, alcoholism or drug addiction). The study protocol was approved by the Ethics Committee at Seoul National University Bundang Hospital (IRB number: B-1412/278-106).

Study design

Patients were classified into two groups. Those who received bismuth-based quadruple therapy [orally: 300 mg tripotassium dicitrato bismuthate 4 times/d, 500 mg tetracycline 4 times/d, 500 mg metronidazole 3 times/d, and 20 mg rabeprazole (or 40 mg esomeprazole)] for either 7 d or 14 d were assigned to PBMT-7 and PBMT-14 groups, respectively. Compliance was evaluated by counting remnant pills and asking direct questions during physician evaluation 1 wk after completion of treatment, and was defined as good when drug intake was at least 85%. All patients were asked about adverse events. Successful eradication therapy for *H. pylori* infection was defined as a negative ^{13}C-UBT test 4 wk after the cessation of eradication treatment. Data on demographics (age, gender distribution, smoking status, alcohol use, diabetes, and hypertension) and endoscopic diagnosis were recorded.

^{13}C-urea breath test

Before ^{13}C-UBT testing, patients were instructed to stop taking medications (*i.e.,* bismuth and antibiotics for 4 wk prior; PPIs for 2 wk prior) that could affect the result, and fast for a minimum of 4 h. After the patient's oral cavity was cleaned by gargling, a pre-dose breath sample was obtained. Then, 100 mg of ^{13}C-urea powder (UBiTkit™; Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) was dissolved in 100 mL of water and administered orally. Breath samplings were performed with special breath collection bags while patients were in the sitting position, both before drug administration (baseline) and 20 min after the powder medication. The samples were analyzed using an isotope-selective, non-dispersive infrared spectrometer (UBiT-IR 300®; Otsuka Pharmaceutical Co. Ltd, Tokyo, Japan).

Statistical analysis

The primary and secondary outcomes of the present study were *H. pylori* eradication rates and treatment-related adverse events, respectively. Eradication rates were determined by ITT and PP analyses. Treatment groups were compared by ITT analysis, which included all patients as originally allocated, and PP analysis, which included only those patients who had completed the treatment as originally allocated. Mean ± SD was calculated for all quantitative variables. Student's *t* test was used to evaluate continuous variables, and chi-square and Fisher's exact tests were used to assess non-continuous variables. All statistical analyses were performed using the Predictive Analytics Software (PASW) 20.0 version for Windows (SPSS Inc., IBM, Chicago, IL, United States). A *P*-value of less than 0.05 was defined as carrying clinical significance.

RESULTS

Characteristics of patients

A schematic diagram of the study is provided in Figure 1. A total of 790 patients who had experienced failure of first-line eradication therapy for *H. pylori* were enrolled (mean age, 54 years; range: 25-89 years). Of the 790 patients, 682 (86.3%) were considered to have completed their allocated regimens. The remaining 108 patients (13.7%) were excluded from the study: 75 (9.4%) from the PBMT-7 group and 33 (4.3%) from the PBMT-14 group. In the PBMT-7 group, the 75 patients were excluded from the study for loss to follow-up (65 patients), medication non-compliance (taking < 85% of the assigned tablets; 2 patients), and treatment discontinuation due to adverse events (8 patients). In the PBMT-14 group, 33 patients were excluded from the study for loss to follow-up (25 patients), non-compliance (taking < 85% of the assigned tablets; 2 patients), and treatment discontinuation due to adverse events (6 patients). Finally, 468 PBMT-7 patients and 214 PBMT-14 patients were included in the PP analysis. The enrolled patients' baseline demographic and clinical data are provided in Table 1. There were no statistical differences in age, gender distribution, smoking status, alcohol use, diabetes, hypertension, or endoscopic diagnosis between the two groups ($P > 0.05$).

H. pylori eradication rates

Table 2 shows the eradication rates for *H. pylori* infection according to ITT and PP analyses. Overall ITT eradication rate was 69.1% (546/790). Final ITT eradication rates were 67.4% (366/543; 95%CI: 63.1%-71.7%) in the PBMT-7 group and 72.8% (180/247; 95%CI: 67.4%-78.2%) in the PBMT-14 group ($P = 0.028$, Table 2). The overall PP eradication rate was 80.0% (546/682), and final PP eradication rates were 78.2% (366/468; 95%CI: 72.1%-84.0%) in the PBMT-7 group and 84.1% (180/214; 95%CI:

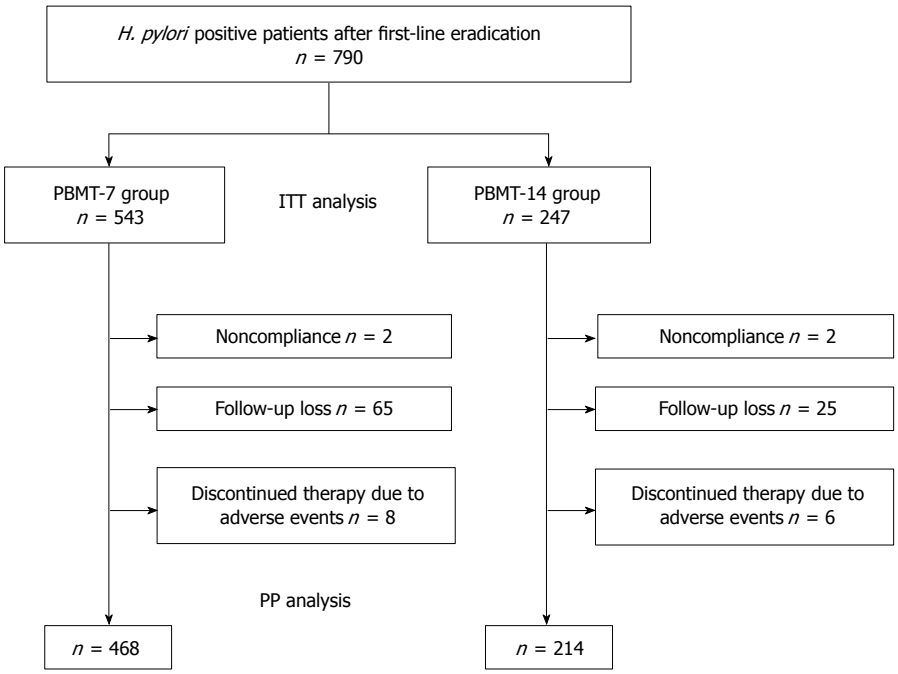

Figure 1 Flow schematic of the study included in intention-to-treat and per-protocol analyses. ITT: Intention-to-treat; PP: Per-protocol; PBMT-7: 7-d bismuth-based quadruple therapy; PBMT-14: 14-d bismuth-based quadruple therapy; ITT: Intention-to-treat; PP: Per-protocol.

Table 1 Demographic and clinical data at baseline (intention-to-treat population) *n* (%)

	PBMT-7	PBMT-14	*P* value
Included in ITT analysis	543	247	ns
Age (mean ± SD, yr)	54.83 ± 11.97	54.69 ± 11.86	0.779
Gender (male)	277 (51.0)	129 (52.2)	0.648
Current smoker	81 (14.9)	47 (19.0)	0.175
Alcohol drinking	118 (21.7)	50 (20.2)	0.641
Diabetes	54 (9.9)	31 (12.6)	0.322
Hypertension	128 (23.6)	53 (21.5)	0.524
Endoscopic diagnosis			0.598
Gastritis	257 (47.4)	146 (59.1)	
Gastric ulcer	93 (17.1)	18 (7.3)	
Duodenal ulcer	170 (31.3)	73 (29.6)	
Gastric and duodenal ulcer	23 (4.2)	10 (4.0)	

PBMT-7: 7-d bismuth-based quadruple therapy; PBMT-14: 14-d bismuth-based quadruple therapy; ITT: Intention-to-treat.

Table 2 *Helicobacter pylori* eradication rates

	PBMT-7	PBMT-14	*P* value
ITT analysis			
Eradication rate	366 (67.4)	180 (72.8)	0.028
95%CI	63.1%-71.7%	67.4%-78.2%	
PP analysis			
Eradication rate	366 (78.2)	18 (84.1)	0.009
95%CI	72.1%-84.0%	76.8%-90.8%	

ITT: Intention-to-treat; PP: Per-protocol; PBMT-7: 7-d bismuth-based quadruple therapy; PBMT-14: 14-d bismuth-based quadruple therapy.

76.8%-90.8%) in the PBMT-14 group (P = 0.009). *H. pylori* eradication rates in the PBMT-14 group were significantly higher than in the PBMT-7 group according to both ITT (P = 0.028) and PP analyses (P = 0.009). Figure 2 shows the eradication rates during 2004-2009 and 2010-2014. The eradication rates during 2004-2009 and 2010-2014 were significantly higher in the PBMT-14 group than those in the PBMT-7 according to both ITT and PP analyses (P < 0.05).

Adverse events and compliance

Table 3 lists the compliance and adverse events for both the PBMT-7 and PBMT-14 groups. Adverse events occurred for 51 of 478 patients (10.7%) in the PBMT-7 group and for 38 of 222 patients (17.1%) in the PBMT-14 group, without statistically significant differences (P = 0.487). The most common adverse events were nausea (16/478, 3.3%) and epigastric discomfort (15/478, 3.1%) in the PBMT-7 group and nausea (13/222, 5.9%) and diarrhea (8/222, 3.7%) in the PBMT-14 group. There were 14 patients with adverse events serious enough to warrant discontinuation of treatment: 8 from the PBMT-7 group and 6 from the PBMT-14 group. Of the 8 patients in the PBMT-7 group who discontinued treatment, 6 did so for nausea and 2 for epigastric discomfort. Of the 6 patients in the PBMT-14 group who discontinued the treatment, 5 did so for nausea, and 1 for diarrhea. Treatment compliance was 97.9% (468/478) in the PBMT-7 group and 96.4% (214/222) in the PBMT-14 group, without statistically significant differences (P = 0.304, Table 3).

DISCUSSION

Single-drug therapeutic effects are considered

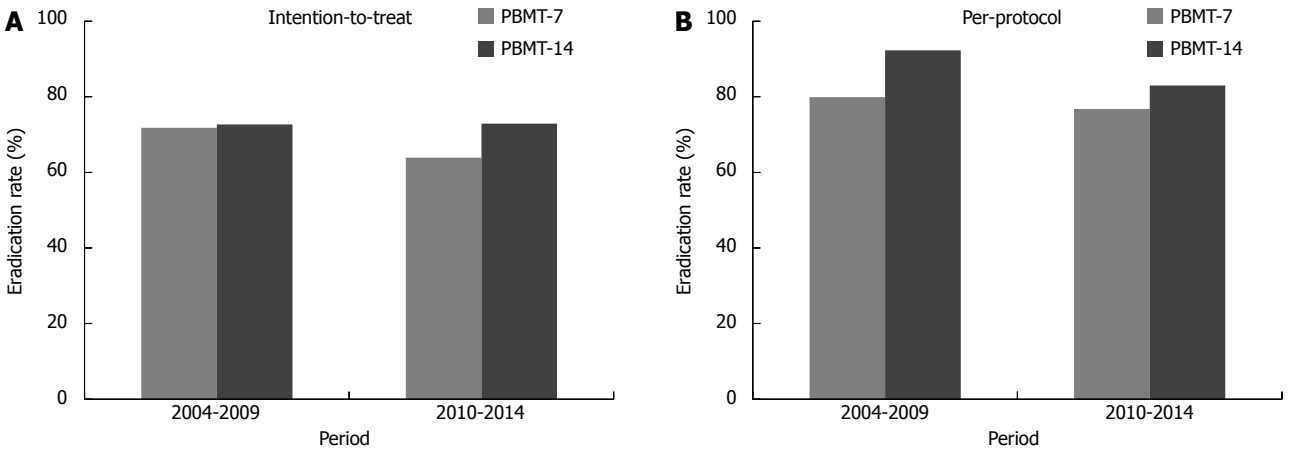

Figure 2 Comparison of the eradication rate between 2004-2009 and 2010-2014 periods in the 7-d bismuth-based quadruple therapy and 14-d bismuth-based quadruple therapy group according to the intention-to-treat (A) and per-protocol analyses (B) ($P < 0.05$). PBMT-7: 7-d bismuth-based quadruple therapy; PBMT-14: 14-d bismuth-based quadruple therapy.

Table 3 Adverse events and compliance *n* (%)

Adverse events	PBMT-7 (*n* = 478)	PBMT-14 (*n* = 222)	*P*-value
Bloating/dyspepsia	3 (0.6)	7 (3.2)	
Dry mouth	4 (0.8)	0 (0.0)	
Taste distortion	2 (0.4)	1 (0.5)	
Epigastric discomfort	15 (3.1)	5 (2.3)	
Nausea	16 (3.3)	13 (5.9)	
Diarrhea	8 (1.7)	7 (3.2)	
Urticaria	3 (0.6)	3 (1.4)	
Headache	0 (0.0)	1 (0.5)	
Chest discomfort	0 (0.0)	1 (0.5)	
Total	51 (10.7)	38 (17.1)	0.487
Compliance	468 (97.9)	214 (96.4)	0.304

PBMT-7: 7-d bismuth-based quadruple therapy; PBMT-14: 14-d bismuth-based quadruple therapy.

weak and likely to lead to increased *H. pylori* drug resistance. Hence, multidrug combination therapies using various antibiotics with bismuth preparations and PPI are recommended. However, because antibiotics used for *H. pylori* eradication are increasingly prescribed for treatment of other diseases, eradication therapy failures due to antibiotic resistance are increasing, resulting in 10%-20% failure rates for first-line eradication therapy even with effective eradication regimens[21,22]. Consequently, a significant number of patients require second-line eradication therapy, which has led to increased interest in related eradication rates. In response to decreasing eradication rates in first-line *H. pylori* eradication therapy, the present study examined effective treatment duration for bismuth-based quadruple therapy consisting of PPI, bismuth, tetracycline, and metronidazole, which is currently recommended as the preferred second-line eradication therapy following failure of first-line eradication therapy. Eradication rates, compliance, and adverse event rates were compared between 7- and 14-d bismuth-based quadruple therapies.

Although bismuth-based quadruple therapy is currently recommended as second-line eradication therapy for *H. pylori* infections, there is some debate over treatment duration. Comparison of eradication rates based on second-line bismuth-based quadruple therapy treatment durations outside South Korea showed eradication rates of 68%-82% and 76%-90% for 7 and 14 d of treatment administration, respectively; however, these differences are not statistically significant[23]. In South Korea, eradication rates for 7- and 14-d administration groups were 70%-81% and 81%-96%, respectively, suggesting a higher eradication rate in the 14-d group, but this is also not statistically significant[14,20]. The present study, using ITT and PP analyses of 790 patients, found eradication rates with 7-d bismuth-based quadruple therapy to be 67.4% and 78.2%, respectively, and with 14-d therapy to be 72.8% and 84.1%, respectively. Eradication rates during 2004-2009 and 2010-2014 were also significantly higher in the group treated for 14 d with bismuth-based quadruple therapy than those in the group treated for 7 d. These results indicate that the group treated with 14-d bismuth-based quadruple therapy demonstrated statistically significantly higher eradication rates in both ITT and PP analyses, consistent with another recent study from South Korea that reported 70%-80% eradication rates[14,19,24].

Many factors can influence the effectiveness of *H. pylori* eradication therapy, including age, smoking status, compliance, and underlying diseases[25]. However, the most important factor in eradication failure is antibiotic resistance[26]. Resistance has a large influence on selection of both first- and second-line eradication therapy regimens. When possible, use of a second-line regimen that differs from that of the first-line eradication therapy is recommended[27]. Resistance to metronidazole, a component of bismuth-based quadruple therapy, occurs because of functional changes in *H. pylori* nitroreductase-encoding genes[13]. Metronidazole antibiotic resistance rate is reportedly

20%-40% in the United States and Europe, and higher rates of 50%-80% have been reported in developing countries[13]. Reported metronidazole antibiotic resistance rates in South Korea vary from 27.1% to 66.2%[7,12,28,29]. A comparative study based on treatment duration and metronidazole resistance reported no differences in eradication rates based on metronidazole resistance[30]; however, another report indicated that 14 d of metronidazole use can overcome the negative influence of metronidazole resistance[18], suggesting that an extended treatment duration may be helpful to overcome antibiotic resistance and improve eradication rates. Tetracycline, another antibiotic component of bismuth-based quadruple therapy, was reported as not playing an important role in *H. pylori* eradication therapy[31]. However, the role of tetracycline in *H. pylori* eradication requires further study. Furthermore, due to its role in protecting and healing the gastric mucosa, bismuth preparations have been used for some time as therapeutic agents for chronic gastritis and peptic ulcer diseases[32,33]. In *H. pylori* eradication therapy, it directly causes bacterial lysis and reduces bacterial density, which in turn increases the therapeutic effects of the antibiotics[34]. Bismuth is also known to reduce bacterial tolerance to eradication therapy by not inducing development of antibiotic resistance[35-37].

While the adverse event rate in the 14-d bismuth-based quadruple therapy group in the present study was higher than that of the 7-d therapy group, the difference was not statistically significant. Adverse events are known to be dosage and duration-dependent[38]; thus, the most serious adverse events were expected to occur during the second week of treatment. Contrary to previous studies[38], most adverse events in the present study, both mild-to-moderate, as well as serious adverse events leading to discontinuation of treatment, appeared during the early treatment stages. These findings may explain the statistically insignificant differences in adverse event rates between the two groups, suggesting that if no serious adverse events appear within the first 7 d of 14-d bismuth-based quadruple therapy, then the probability of any serious adverse events occurring is relatively low. Even when mild-to-moderate adverse events did occur, encouragement and appropriate assistance from medical staff enabled successful completion of 14-d bismuth-based quadruple therapy.

In addition to eradication rate, follow-up loss and medication compliance are also important factors for successful *H. pylori* eradication. High eradication rates and compliance, along with low follow-up loss rates, make successful eradication therapy possible. In the present study, follow-up loss rates for the 7- and 14-d therapy groups were 11.9% (65/543) and 10.1% (25/247), respectively, whereas the compliance for these group were 97.9% (468/478) and 96.4% (214/222), respectively. The results were similar between groups, and the differences were not statistically significant. Although reducing the number of drugs or shortening the treatment duration are important for decreasing follow-up loss rates and increasing compliance, second-line eradication therapy has a high probability of antimicrobial resistance after failure of first-line eradication therapy. Even with a long treatment duration, it is especially important to minimize follow-up loss rates and increase treatment compliance through patient education.

This study has limitations. First, since this retrospective study used medical records, selection errors were possible. Second, since *H. pylori* strain identification and pre- and post-eradication therapy antibiotic susceptibility tests were not performed, the increase in the antibiotic resistance rate in participating patients were not verified. Ideally, it would be possible to utilize a mixture of drugs shown to be effective in antibiotic susceptibility tests in order to overcome reduced eradication rates caused by increasing antibiotic resistance. However, because it is difficult to culture *H. pylori*, the success rates of antibiotic susceptibility tests are low, and there are no established criteria for determining antibiotic resistance. Additional studies on the cost-effectiveness of and appropriate time for conducting antibiotic susceptibility tests are necessary.

The present study examined effective treatment duration for bismuth-based quadruple therapy by comparing eradication rates, compliance, and adverse event rates after administration of 7 and 14 d of bismuth-based quadruple therapies as a second-line eradication therapy following first-line therapy failure in South Korea. The results showed that the eradication rate of the 14-d bismuth-based quadruple therapy group was higher than that of the 7-d bismuth-based quadruple therapy group, while there were no differences in adverse event rates and compliance. The 14-d bismuth-based quadruple therapy is therefore believed to be the more effective second-line eradication therapy in South Korea. Moreover, encouragement and appropriate assistance from medical staff are necessary to address serious adverse events that can occur in the early stages of treatment, and efforts should be made to provide sufficient patient education to increase treatment success rates. In addition, further studies are necessary to develop more effective and safer second-line eradication therapies and evaluate new treatment regimens for second-line eradication therapies based on first-line eradication therapy failures.

COMMENTS

Background

A recent meta-analysis reported a decreased eradication rate using standard triple therapy for *Helicobacter pylori* (*H. pylori*) infection due to increasing antibiotic resistance. For patients whose first-line eradication therapy has failed, second-line eradication therapy for persistent *H. pylori* infection is required.

Research frontiers

There is controversy about the duration of bismuth-based quadruple therapy treatment. There has also been a great deal of debate about bismuth-based quadruple therapy as a second-line eradication therapy in South Korea.

Innovations and breakthroughs

This retrospective study was conducted to evaluate the efficacy of 14-d bismuth-based quadruple therapy as compared with 7-d bismuth-based quadruple therapy as a second-line eradication treatment of *H. pylori* infection. The high eradication rate, excellent compliance, and safety of the 14-d regimen suggest its potential suitability as a second-line eradication treatment of *H. pylori* infection.

Applications

This retrospective study's design and findings could be used to determine adequate sample sizes for a larger, prospective study designed to test the efficacy of 14-d bismuth-based quadruple therapy as a second-line eradication treatment for *H. pylori* eradication.

Terminology

H. pylori, found in the stomach, is associated with the development of gastritis, peptic ulcers, and stomach cancer. To prevent recurrence in patients with these diseases, it is necessary to eradicate *H. pylori* infection.

Peer-review

The study, conducted with 790 patients who failed proton pump inhibitor-based *H. pylori* eradication treatment, reports on the efficacy of 14-d bismuth-based quadruple therapy over that of 7-d therapy as a second-line treatment for *H. pylori* eradication. The results indicate that 14-d quadruple therapy had a significantly higher rate of *H. pylori* eradication, and no adverse effects. This study deals with apparent increased resistance to *H. pylori* eradication by a standard triple therapy among the Korean population, and provides only limited new insights into approaches to *H. pylori* eradication regimens.

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ORIGINAL ARTICLE

Retrospective Study

Liver transplantation for hepatitis B virus: Decreasing indication and changing trends

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Author contributions: Al-hamoudi W, Elsiesy H and Ali S designed the research; Bendahmash A, Al-masri N, Allam N, Al Sofayan M and Al Bahili H worked on data collection and data analysis; Al Sebayel M, Broering D, Saab S and Abaalkhail F contributed to performing the research; Al-hamoudi W wrote the manuscript; all authors approved the final manuscript.

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Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: There is no commercial affiliation or consultancy of an author that could be construed as a conflict of interest with respect to the submitted data.

Data sharing statement: No additional data are available.

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Abstract

AIM: To evaluate the indication and outcome of hepatitis B virus (HBV)-related liver transplantation (LT) in the era of newer antiviral agents.

METHODS: We collected data on all patients who underwent transplantation at King Faisal Specialist Hospital and Research Center. These data included demographic, perioperative and long-term postoperative follow-up data including viral serological markers, HBV DNA, and repeated liver imaging. Between January 1990 and January 2012, 133 patients (106 males and 27 females) underwent LT for HBV-related cirrhosis at our center. All patients were followed up frequently during the first year following transplantation, according to our standard protocol; follow-up visits occurred every 3-6 mo thereafter. Breakthrough infection was defined

as re-emergence of HBV-DNA or hepatitis B surface antigen (HBsAg) while on treatment. Five patients transplanted prior to 1992 did not receive immediate posttransplant anti-HBV prophylaxis; all other patients were treated with HBIG and at least one nucleos(t)ide analog.

RESULTS: One hundred and thirty-three patients underwent LT for HBV and were followed for a median of 82 mo (range: 1-274). The rates of post-LT survival and HBV recurrence during the follow-up period were 89% and 11%, respectively. The following factors were associated with disease recurrence: younger age (44.3 ± 16.2 years *vs* 51.4 ± 9.9 years, $P = 0.02$), positive pretransplant hepatitis B e antigen (HBeAg) (60% *vs* 14%, $P < 0.0001$), detectable pretransplant HBV DNA (60% *vs* 27%, $P = 0.03$), positive posttransplant HBsAg (80% *vs* 4%, $P < 0.0001$) and positive posttransplant HBeAg (27% *vs* 1%, $P < 0.0001$). Forty-four (33%) patients had hepatocellular carcinoma (HCC). In the first (pre-2007) group, HBV was the second leading indication for LT after hepatitis C virus infection. A total of 64 transplants were performed, including 46 (72%) for decompensated HBV cirrhosis, 12 (19%) for decompensated cirrhosis complicated by HCC and 6 (10%) for compensated cirrhosis complicated by HCC. In the second group, nonalcoholic steatohepatitis surpassed HBV as the second leading indication for LT. A total of 69 HBV related transplants were performed, including 43 (62%) for decompensated HBV cirrhosis, 7 (10%) for decompensated cirrhosis complicated by HCC and 19 (27.5%) for compensated cirrhosis complicated by HCC. There was a significant ($P = 0.007$) increase in the number of transplants for compensated cirrhosis complicated by HCC.

CONCLUSION: The use of potent anti-HBV agents has led to a changing trend in the indications for LT. HBV is currently the third leading indication for LT in this hyperendemic area.

Key words: Hepatitis B; Hepatitis C; Non-alcoholic steatohepatitis; Liver transplantation; Hepatocellular carcinoma

Core tip: Hepatitis B virus (HBV) is considered hyperendemic in the Middle East. In the 1980s, the overall prevalence of HBV infection in Saudi Arabia was 8.3%, making it one of the most highly endemic areas in the world. This high prevalence made HBV-related disease a leading indication for liver transplantation (LT). The use of potent anti-HBV agents has led to a changing trend in the indications for LT. HBV is currently the third leading indication for LT in this hyperendemic area. Additionally, there has been a shift in the indication for transplantation from hepatic decompensation to hepatocellular carcinoma.

Al-hamoudi W, Elsiesy H, Bendahmash A, Al-masri N, Ali S, Allam N, Al Sofayan M, Al Bahili H, Al Sebayel M, Broering D, Saab S, Abaalkhail F. Liver transplantation for hepatitis B virus: Decreasing indication and changing trends. *World J Gastroenterol* 2015; 21(26): 8140-8147 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i26/8140.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i26.8140

INTRODUCTION

The World Health Organization (WHO) estimates that approximately 2 billion people worldwide have been infected with hepatitis B virus (HBV), and approximately 350 million people live with chronic infection[1,2]. HBV is considered hyperendemic in Saudi Arabia. In the 1980s, the overall prevalence of HBV infection in Saudi Arabia was 8.3%, making it one of the most highly endemic areas in the world. In 1989, the HBV vaccine was integrated into the expanded program of immunization, through which all newborn children have been vaccinated for HBV in Saudi Arabia ever since. This effort significantly decreased the prevalence of HBV in younger Saudi Arabians. However, the prevalence remains high for people older than 25 years, which is expected to increase the health care system burden for the next several decades[3,4].

Liver transplantation (LT) is the ultimate therapy for patients with hepatic failure and/or hepatocellular carcinoma (HCC) related to HBV. In the past, patient outcome was suboptimal because of severe disease recurrence resulting in graft loss. Therefore, HBV-related liver disease was previously considered a contraindication for LT[5,6]. Advances in HBV antiviral prophylaxis following LT have dramatically improved transplant outcomes, resulting in increased patient and graft survival. The use of hepatitis B immune globulin (HBIG) in combination with lamivudine has dramatically improved the outcome of HBV-related transplantation[7-11]. The availability of newer antivirals, such as adefovir, tenofovir and entecavir, alone or in combination, offers alternative treatment options, especially in patients with lamivudine resistance[12-14]. Additionally, overall patient survival has improved due to the prevention of disease progression to cirrhosis.

Here, we report the rates and indications of HBV-related LT at our center over the last 10 years. We also evaluated predictors of disease recurrence. In addition, we studied the effects of hepatitis delta virus (HDV) coinfection and HCC on transplant outcome.

MATERIALS AND METHODS

Patient population

In this retrospective study we collected data on all patients who underwent transplantation at King Faisal Specialist Hospital and Research Center. These data included demographic, perioperative and long-term

postoperative follow-up data. Between January 1990 and January 2012, 133 patients (106 males and 27 females) underwent LT for HBV-related cirrhosis at our center. All the patients were followed up frequently during the first year following transplantation, according to our standard protocol; follow-up visits occurred every 3-6 mo thereafter. Breakthrough infection was defined as re-emergence of HBV-DNA or hepatitis B surface antigen (HBsAg) while on treatment.

Transplant listing criteria

For all patients in our institution the criteria for medical urgency and patient waiting list ranking are based on the Model for End Stage Liver Disease (MELD). Patients with HCC typically have low calculated MELD scores because of compensated liver disease. Therefore, patients with HCC that are within the Milan criteria receive exceptional MELD points when their lesions exceed 2 cm in diameter. Additionally, HCC management with various ablation techniques is also discussed in the tumor board and treatment is offered to suitable patients. All patients will have their MELD score assessed regularly while they are on the waiting list.

Posttransplant protocol

Five patients transplanted prior to 1992 did not receive immediate posttransplant anti-HBV prophylaxis, all other patients were treated with HBIG and at least one nucleos(t)ide analog. HBIG was administered intravenously at a dose of 10000 units during the anhepatic phase and then at a dose of 5000 units daily during the first week; the goal was a hepatitis B surface antibody (HBsAb) titer of > 500 IU/mL. Subsequently, the antibody titer was monitored every 1-2 wk for the first 3 mo to maintain a titer of > 250 IU/mL. The HBsAb titer was then monitored every 1-2 mo to maintain the level at > 100 IU/mL during the first year following transplantation. Beginning in 2009, the majority of our patients received HBIG for 1 year after LT. Prior to 2009, the duration of HBIG was variable, and it was extended in many patients at the discretion of the treating physician. In addition to HBIG, the majority of our patients were administered lamivudine. Adefovir, entecavir and tenofovir were used in the more recent transplants after these agents became available in Saudi Arabia.

The standard immunosuppression protocol in our institution includes calcineurin inhibitors and mycophenolate mofetil during the first 6-12 mo after transplantation and oral prednisone for the first 3 mo. The doses of immunosuppressive medications were adjusted according to their serum levels and were modified in patients with renal impairment.

A complete blood count, liver function and serum levels of liver enzymes and immunosuppressive drugs were assessed at each visit. The levels of HBV DNA and HBsAg were tested monthly for the first 3 mo and every 6 mo thereafter. HBV viral load was performed using real time PCR technology. The assay provides a detection limit from 15 to 100000000 IU/mL. Results from earlier transplants utilizing older assays measuring viral loads in copies/mL were converted to IU/mL for standardization of results. Breakthrough infection was defined as the reemergence of HBV DNA and HBsAg while a patient was undergoing treatment.

Pretransplant follow-up

While on the transplant waiting list, all patients were closely followed up every 1-3 mo. Standard biochemical liver function assays were performed at each visit. Each patient on the list underwent Doppler ultrasound of the liver every 3 mo. Forty-four patients were diagnosed with HCC (33%) and all patients achieved viral suppression before transplant. After an examination of explants from these patients, five patients were confirmed to have HCC beyond the Milan criteria. Nine patients received surgical or ablative therapy while they were on the waiting list.

Statistical analysis

All variables were checked for normality. Descriptive statistics were summarized as the mean (standard deviation), median (range) or frequency (percentage), as appropriate. A χ^2 test was used to assess group differences for categorical variables, and a *t*-test was used to assess differences between continuous variables. ORs were calculated to determine associations between the variables of interest and recurrence. All the tests were two-sided with a 5% level of significance. All the analyses were performed using Stata version 10 (StataCorp, Texas, United States). The statistical methods of this study were reviewed by Safiyya Ali and Majid Almadi who are trained biostatisticians with extensive biomedical research experience from the Gastroenterology research unit, King Saud University. This study was approved by the research ethics committee of our hospital.

RESULTS

General characteristics of the study population

The mean ± SD age of all the patients was 50.6 ± 11.0 years and 106 (80%) was males. Ninty-seven (73%) patients underwent deceased donor LT and 36 (23%) patients underwent living donor LT. The median post-LT follow-up was 82 mo (range: 1-274) and HBV recurrence occurred in 15 patients (11%). The median time between LT and HBV recurrence was 42 mo. Pretransplant HBsAg and hepatitis B e antigen (HBeAg) were positive in 126 (95%) and 24 patients (19%), respectively. Thirty-six patients (27%) had detectable HBV DNA levels at the time of LT. Pretransplant lamivudine was taken by 81 (61%) patients; 5 (4%) took adefovir; 2 (2%) began with lamivudine and were then switched to adefovir; 20 (15%) took a

Table 1 Characteristics of the study population, both overall and grouped by recurrence *n* (%)

Characteristics				
Age (yr)	50.6 ± 11.0	44.3 ± 16.2	51.4 ± 9.9	0.02[2]
Gender				
Male	106 (80)	14 (13)	92 (87)	0.16
Female	27 (20)	1 (3.7)	26 (96)	
Posttransplantation follow-up (mo)	82 (1-274)[1]	206 (74-274)	53 (1-254)	< 0.0001
Pretransplantation HBsAg				
Negative	6 (5)	0	6 (5)	0.37
Positive	126 (95)	15 (100)	111 (95)	
Pretransplantation HBeAg				
Negative	108 (81)	6 (40)	102 (87)	< 0.0001[2]
Positive	24 (19)	9 (60)	15 (134)	
Pretransplant HBV DNA				
Negative	86 (73)	4 (60)	82 (73)	0.03
Positive	36 (27)	6 (60)	30 (26)	
HBIG duration (mo)[1]	14 (1-140)	14 (1-140)	14 (1-132)	0.83
MELD score	20.8 ± 7.6	19.2 ± 7.7	21.0 ± 7.6	0.39
Posttransplantation HBsAg				
Negative	114 (88)	3 (20)	111 (97)	< 0.0001[2]
Positive	16 (12)	12 (80)	4 (3)	
Posttransplant HBeAg				
Negative	122 (96)	11 (73)	111 (99)	< 0.0001[2]
Positive	5 (4)	4 (5)	1 (1)	
HDV coinfection				
Negative	70 (69)	8 (80)	62 (67)	0.41
Positive	32 (31)	2 (20)	30 (33)	

[1]The results are expressed as the median (range); [2]Significant at $P < 0.05$. HDV: Hepatitis delta virus; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen; HBV: Hepatitis B virus.

combination of lamivudine and adefovir; 4 (3%) took entecavir; 7 (5%) took tenofovir; and 6 (5%) took interferon. HCC was diagnosed in 44 (33%) patients. The median duration of treatment with HBIG in 102 patients was 14 (range 1-140) mo, and 24 (19%) patients were still undergoing active HBIG treatment at the time of data collection. The average MELD score was 20.8 ± 7.6. HDV coinfection was present in 32 (24%) patients.

Recurrence

The 15 patients who developed breakthrough HBV infection were on monotherapy, and switching to or adding a different oral agent helped control their infection. After transplantation, 16 (12.3%) patients were positive for HBsAg. Patients who experienced recurrence had a significantly greater median duration of posttransplant follow-up compared with patients without recurrence (median 206, range 74-274 and median 53, range 1-254, respectively, $P < 0.0001$). The following factors were associated with disease recurrence: younger age (44.3 ± 16.2 years *vs* 51.4 ± 9.9 years, $P = 0.02$), positive pretransplant HBeAg (60% *vs* 14%, $P < 0.0001$), detectable pretransplant HBV DNA (60% *vs* 27%, $P = 0.03$), positive posttransplant HBsAg (80% *vs* 4%, $P < 0.0001$) and positive posttransplant HBeAg (27% *vs* 1%, $P < 0.0001$). Patients who were positive for HBeAg before transplantation were more likely to experience recurrence compared with those who were negative (OR = 9.6, 95%CI: 2.99-30.85; $P < 0.0001$). Patients who had positive HBV DNA levels before transplantation were almost four times more likely to have recurrence compared with those who did not (OR = 4.1, 95%CI: 1.08-15.5; $P = 0.04$). Similarly, patients who were positive for HBsAg or HBeAg after transplantation were more likely to experience recurrence than those who did not (OR = 111, 95%CI: 22.2-555.9; $P < 0.0001$ and OR = 40.4, 95%CI: 4.1-393.5; $P = 0.001$, respectively). There were no significant associations between the patient's gender or MELD score and recurrence (Table 1).

Hepatitis delta virus coinfection

Patients with HBV and hepatitis delta virus (HDV) coinfection were similar to the HBV-monoinfected patients in terms of their age at the time of transplantation (47.7 years *vs* 51.2 years, $P = 0.15$), pretransplant MELD score (22.1 *vs* 20.2, $P = 0.28$) and post-LT disease recurrence (6% *vs* 11%, $P = 0.41$). HDV infection was not a significant predictor of death: none of the patients who had HDV died.

HCC status

HCC was present prior to LT in 44 (33%) patients. A single lesion was present in 37 patients with an average size of 3.9 cm. Seven patients had multifocal HCC that was within the Milan criteria. Despite strict adhesion to Milan criteria when listing patients for LT, five patients were confirmed to have HCC beyond the Milan criteria after examination of explants. Prior to transplantation all patients underwent a chest CT and a bone scan to rule out distant metastasis. The patients with HCC were significantly younger than those without HCC (48.4 ± 11.3 years *vs* 55.2 ± 8.6 years, $P = 0.0007$). HBV recurrence was not significantly associated with the presence of HCC, the duration of survival or HBV markers. The patients who had HCC had significantly lower MELD scores than those who did not (18 ± 8.2 *vs* 22.2 ± 6.9, respectively, $P = 0.0024$) (Table 2). Our data revealed that an increasing number of the more recent transplants (2007-2012) were for well-compensated cirrhosis complicated by HCC compared with the pre-2007 transplants ($P = 0.007$) (Table 3).

Indications for LT

Between January 2001 and January 2012, 500 patients received transplants and were followed up at our center. All the transplant indications recorded in our database were reviewed. During the first study period, January 2001-December 2006, 50% of the patients received transplants for hepatitis C virus (HCV)-induced cirrhosis, 17% for HBV-induced cirrhosis and 12% for nonalcoholic steatohepatitis (NASH)-related cirrhosis.

Table 2 Characteristics of the study population grouped by the presence of hepatocellular carcinoma *n* (%)

Variable	No HCC *n* = 89 (67)	HCC *n* = 44 (33)	*P* value
Age (yr)	48.4 ± 11.3	55.2 ± 8.6	0.0007[1]
Gender			
Male	69 (78)	37 (84)	0.38
Female	20 (22)	7 (16)	
Pretransplant HBsAg			
Negative	4 (5)	2 (5)	1.00
Positive	84 (95)	42 (95)	
Pretransplant HBeAg			
Negative	69 (82)	33 (79)	0.63
Positive	15 (18)	15 (21)	
Pretransplant HBV DNA			
Negative	57 (72)	29 (67)	0.59
Positive	22 (28)	14 (33)	
MELD score	22.2 ± 6.9	18.0 ± 8.2	0.002[1]
Posttransplant HBsAg			
Negative	75 (86)	39 (91)	0.46
Positive	12 (14)	4 (9)	
Posttransplant HBeAg			
Negative	81 (95)	41 (98)	0.53
Positive	4 (5)	1 (2)	
HDV coinfection			
Negative	50 (68)	20 (71)	0.71
Positive	24 (32)	8 (29)	

[1]Significant at $P < 0.05$. HDV: Hepatitis delta virus; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen; HBV: Hepatitis B virus.

During the second study period (January 2007-January 2012), 35%, 20%, and 16% of the patients received transplants for HCV-, NASH-, and HBV-induced cirrhosis, respectively. During the second study period, NASH-related cirrhosis surpassed HBV as an indication for LT, whereas the rates of transplantation for all other indications remained the same throughout the study.

DISCUSSION

The development of potent HBV drugs with high genetic barriers to resistance has resulted in significant suppression of viral replication. As a result, overall patient survival has improved due to the prevention of disease progression to cirrhosis. Recent data showed the regression of cirrhosis in 71 out of 96 (74%) cirrhotic patients treated with tenofovir, which was confirmed by paired biopsies at baseline and after 5 years. However, the preventive effect of these agents against HCC development is less clear. Furthermore, the outcome of LT for HBV-related liver disease has dramatically changed since the introduction of oral antiviral agents and HBIG[15-18].

Here, we reported the rates and indications of HBV-related LT in a Saudi population. We also reported the long term outcomes of HBV-related LT in our country. According to the WHO classification, HBV is considered hyperendemic in Saudi Arabia, where the overall prevalence is 8.3%[3,4]. Saudi Arabia is among the most highly endemic areas for HBV infection in the world.

Table 3 Comparison of indications for transplantation before and after January 2007

	Before January 2007 (*n* = 64)	After January 2007 (*n* = 69)	*P* value
Transplantations for well-compensated HCC	6	19	0.007
Transplantations for liver decompensation without HCC	46	43	Not significant

HCC: Hepatocellular carcinoma.

However, a comparison of the data from the earlier and later periods of this study has clearly demonstrated that HBV-related transplantation has decreased from being the second leading indication for LT, following HCV-related liver disease, to being the third indication, with a corresponding increase in the proportion of LTs due to NASH-related liver disease. The decline in HBV-related liver disease as an indication for LT is likely related both to the various mass screening programs in the Kingdom, which have enabled the detection of chronically infected HBV patients at various disease stages, and to the introduction of effective antiviral treatment[19-24]. Interestingly, similar observations have been noted in various studies from Europe, the United States, Australia, and New Zealand, where massive reductions in HBV-related disease as an indication for LT have been reported[25,26]. For example, Burra *et al*[27] showed that HBV-related cirrhosis had dropped from 24% to 16% in patients transplanted for solely virus-related liver disease in Europe.

NASH-related liver disease is a rising global concern due to the obesity epidemic. Major risk factors for NASH-related liver disease, including diabetes mellitus, obesity, and hyperlipidemia, are extremely common in Saudi Arabia. Recent data have suggested overall prevalence rates of these risk factors of 23.7%, 35.5% and 54%, respectively[28,29].

Our results demonstrated a change in the indications for HBV-related LT from hepatic decompensation to HCC. European and American studies have also reported this trend, noting that the occurrence of HCC in patients with viral-related liver disease doubled from 2006-2010 compared with 1988-1994[23]. This finding could be related to improvements in the control of viral replication with potent medications (entecavir and tenofovir), which has prevented the progression of liver disease to more advanced stages of fibrosis and has prevented decompensation in already cirrhotic patients. HBV-related carcinogenesis develops independently of the onset of cirrhosis; therefore, antiviral treatments such as nucleo(t)side analogs, which may result in the regression of fibrosis or prevent clinical decompensation, often fail to prevent HCC, especially in Asian and African countries[30]. A randomized study conducted by Liaw *et al*[31] demon-

strated that lamivudine therapy for a median of 32 mo resulted in a decrease in the incidence of HCC in patients with advanced fibrosis, but not in patients with decompensated liver disease. Other studies have similarly failed to show any protective effect of lamivudine against the development of HCC in patients with decompensated liver disease[32,33]. The limitations of these studies include their relatively short follow-up periods and small sample sizes.

In our study population, 15 patients (11%) developed HBV reinfection during the follow-up period, which is in agreement with the rates described in other studies[34-36]. Most of the patients in our study who developed resistance underwent transplantation early during the study period, which explains their significantly longer posttransplant follow-up. The most important predictor of disease recurrence is viral replication at the time of transplantation. Patients who were positive for HBeAg or had detectable HBV DNA pretransplantation were more likely to develop recurrence than patients with negative pretransplantation results. This observation has also been reported in several other studies. Yasunaka *et al*[37] evaluated disease recurrence following LT for HBV-related liver disease and concluded that serum HBV DNA before LT correlated with the HBV reinfection rate even with the successful administration of low-dose HBIG prophylaxis.

Thirty-two (24%) of our patients experienced HBV/HDV coinfection. Of the 15 patients with posttransplantation recurrence, only two had HBV/HDV coinfection. This rate was lower than for the HBV-infected patients (6% *vs* 11%); however, this difference was not statistically significant. Furthermore, HDV infection was not a significant predictor of graft loss or posttransplant mortality; none of the patients with HDV died. This observation is similar to other reports on the outcome of transplantation for HBV/HDV coinfection. Caccamo *et al*[38] reported no recurrence of HBV infection following LT in HBV/HDV-infected patients, whereas others have reported lower rates of HBV recurrence in patients with HBV/HDV coinfection compared with HBV monoinfection[38-40]. Burra *et al*[27] reported that HDV coinfection was associated with better survival outcomes compared to HBV monoinfection which is thought to be related to the inhibitory effect of HDV on the HBV replication cycle. Despite the aggressive course of HBV and HDV coinfection in immunocompetent patients, the LT outcomes in HDV-coinfected patients are similar to those in HBV-monoinfected patients.

Forty-four patients in our study were diagnosed with HCC prior to LT. With the exception of 5 patients, all the patients were within the Milan criteria (a single tumor $\leqslant$ 5 cm or a maximum of 3 tumors < 3 cm each). Patients within the Milan criteria who receive transplants due to HCC have a survival rate similar to that of HCC-negative patients[41,42]. The risk factors for tumor recurrence include a larger pretransplantation tumor size, lymphovascular invasion, and post-LT systemic chemotherapy. Saab *et al*[36] evaluated the effects of both HCC recurrence and HBV reinfection on the long-term survival of patients after LT. They concluded that pre-OLT HCC and the recurrence of HCC after transplantation were associated with HBV reinfection and decreased patient survival. However, in their cohort, 50 out of 88 transplant patients with HCC were beyond the Milan criteria based on pathological examinations. This finding likely explains the inferior outcome of patients transplanted for HBV-HCC compared with the HCC-negative patients in their study. In contrast, Wong *et al*[43] compared the clinical outcomes of LT candidates with chronic hepatitis B who did or did not have HCC. In their study, the patients with HCC had a higher rate of LT and a shorter interval from wait list assignment to transplant compared with the HCC-negative patients; however, the two groups had similar post-OLT survival and HBV recurrence rates. This finding may be explained by the observation that 72% of the HCC patients were within the Milan criteria, and 23 of the 25 patients who exceeded the Milan criteria underwent some form of HCC treatment to downstage their disease.

Although the present study has many strengths, it also has several limitations, including its retrospective design. HBV immunoprophylaxis during the posttransplant follow-up period was not consistent for all the patients. Data on pretransplantation HBV DNA status, pretransplantation HBV serological markers, and HDV coinfection were not available for some of the study individuals. However, posttransplantation HBV DNA and HBV serological data were available and complete. Furthermore, this is the largest study originating from our region, an area that is considered endemic for HBV infection.

In conclusion, the use of highly potent anti-HBV agents has led to adequate control of viral replication, significant biochemical remission, histological improvement, and the prevention of hepatic decompensation. These effects are expected to subsequently result in a reduction in the rate of HBV-related LT and a shift in the indication for transplantation from HBV-related hepatic decompensation to HBV-related HCC. The availability of a newer generation of nucleos(t)ide analogs has also resulted in a significant improvement in the outcome of LT for HBV-related liver disease. Controlling viral replication and adhering to the Milan criteria when performing transplants in patients with HBV-related HCC will improve transplantation outcomes.

COMMENTS

Background

The indications for liver transplantation (LT) for hepatitis B (HBV) infected patients are hepatic de-compensation, fulminant liver failure or development of hepatocellular carcinoma. Currently, adequate control of viral replication can be achieved with potent antiviral therapy. The authors believe that with better control of viral replication using potent medication will result in a drop in HBV related transplantation associated with a change in the indication for LT from

hepatic-decompensation to hepatocellular carcinoma.

Research frontiers

Nonalcoholic steatohepatitis (NASH) related cirrhosis surpassed HBV as an indication for LT while the rates of transplantation for all other indications remained the same. With the increasing prevalence of DM and obesity and the progress in the treatment of hepatitis C virus (HCV) and HBV infection NASH related cirrhosis may replace HCV and HBV as the leading indication for transplantation in the future.

Innovations and breakthroughs

LT for HBV decreased dramatically in our region. And the outcome of LT for HBV-related liver disease has dramatically changed since the introduction of oral antiviral agents resulting in better graft and patient survival. Similar observations have been noted in various studies from Europe, the United States, Australia, and New Zealand, where massive reductions in HBV-related disease as an indication for LT have been reported.

Applications

The advances in treating viral hepatitis resulted in a better outcome and resulted in a decrease in viral related transplantation. NASH related cirrhosis is becoming the leading indication for LT. Therefore, there is a dire need for governmental efforts to control the growing obesity epidemic that is impacting the increasing need for liver transplant due to NASH-related cirrhosis.

Peer-review

In this study, the authors clearly demonstrate a decreasing indication for HBV related transplantation associated with a changing trend in the indication from hepatic de-compensation to hepatocellular carcinoma. Additionally, NASH related cirrhosis surpassed HBV as an indication for LT.

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ORIGINAL ARTICLE

Retrospective Study

Relationship between severity of venous calcifications and symptoms of phlebosclerotic colitis

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Abstract

AIM: To examine the correlation between the severity of venous calcifications and the clinical symptoms of phlebosclerotic colitis.

METHODS: This was a retrospective study. The data, including the numbers of episodes of active disease, were collected from the medical records at Taipei Veterans General Hospital and Wei Gong Memorial Hospital in Taiwan between January 2005 and December 2014. All computed tomography images with or without contrast enhancement were obtained using a multiple detector computed tomography scanner. The scanning range reached from the dome of the diaphragm to the pelvis. The severity of calcification at the tributaries of the portal vein was measured using a four-grade scoring system of the calcification of phlebosclerotic colitis. The episodes of active disease were defined as symptoms of fever, abdominal pain, severe constipation, bowel obstruction, vomiting or diarrhea based on a review of the medical records. Spearman's correlation analysis was used to examine the correlation between the numbers of episodes of active disease and the severity of the calcification of the mesenteric veins.

RESULTS: More than 3000 cases were reviewed from 2005 to 2014, and a total of 12 patients from Taipei Veterans General Hospital and Wei Gong Memorial Hospital were enrolled according to our inclusion criteria. Among these 12 patients, the mean age of the six males and the six females was 61.8 ± 11.5 years. All patients exhibited typical imaging characteristics,

consisting of threadlike calcifications and colonic wall thickening in the standard radiographs and calcifications along the colonic and mesenteric vessels or associated with colonic wall thickening and adjacent fat stranding in the computed tomography images. The median score of the severity of the venous calcifications was 18 ± 13, and the median number of active disease episodes was 1 ± 1.75. Spearman's correlation analysis revealed that the number of episodes of active phlebosclerotic colitis disease significantly positively correlated with the severity of the calcification of the mesenteric veins (r = 0.619, P < 0.05).

CONCLUSION: The extent of mesenteric venous calcification is strongly associated with the number of episodes of active disease among patients with phlebosclerotic colitis.

Key words: Phlebosclerotic colitis; Calcification of the mesenteric veins; Symptoms of phlebosclerotic colitis; Computed tomography; Ischemic bowel disease

Core tip: Phlebosclerotic colitis, which is almost exclusively observed in the Asian population, is a rare condition of ischemic colitis associated with the sclerosis and calcification of the mesenteric venous wall, resulting in the preferential involvement of the right hemicolon and complications during a relatively chronic clinical course. We reviewed the medical records and examined the correlation between the severity of venous calcifications and the clinical symptoms of phlebosclerotic colitis observed on computed tomography images. According to our findings, the extent of mesenteric venous calcifications strongly positively correlates with the number of episodes of active disease.

Yen TS, Liu CA, Chiu NC, Chiou YY, Chou YH, Chang CY. Relationship between severity of venous calcifications and symptoms of phlebosclerotic colitis. *World J Gastroenterol* 2015; 21(26): 8148-8155 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i26/8148.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i26.8148

INTRODUCTION

Ischemic bowel disease is a heterogeneous group of disorders that display a common characteristic of bowel hypoxia caused by alterations in blood flow and is typically evoked by arterial thromboembolic disease[1]. However, disturbed venous return may also result in colitis[2]. Phlebosclerotic colitis, which is almost exclusively observed in the Asian population[3-7], is a rare condition of ischemic colitis associated with the sclerosis and calcification of the mesenteric venous wall. Phlebosclerotic colitis preferentially involves the right hemicolon[8], and its clinical course is relatively chronic[9,10]. The common clinical symptoms caused by phlebosclerotic colitis are nonspecific and include abdominal pain, diarrhea or bloody stool[8]. The severity of venous calcification, which is an imaging finding that is specific to phlebosclerotic colitis[11], based on radiography increases gradually and progresses in the caudal direction[12,13]. Pathological examination may reveal various degrees of occlusion of the affected venous lumen[14]. We therefore hypothesized that the severity of venous calcification positively correlates with clinical symptoms. Thus, the objective of this study was to examine the correlation between the severity of venous calcifications and the number of active episodes involving the clinical symptoms of phlebosclerotic colitis.

MATERIALS AND METHODS

This is a retrospective study, and the data were collected from the medical records at Taipei Veterans General Hospital and Wei Gong Memorial Hospital between January 2005 and December 2014. Written informed consent was waived.

Patient selection

We retrospectively reviewed the medical records for patients who fulfilled the following inclusion criteria: (1) at least one complete abdominal computed tomography (CT) examination with or without intravenous contrast medium injection; and (2) calcification at tributaries of the superior mesenteric veins.

CT acquisition

All CT images with or without contrast enhancement were obtained using a multiple detector CT scanner. The scanning range reached from the dome of the diaphragm to the pelvis. For dual-phase contrast enhanced CT images, nonionic contrast material was administered intravascularly using an automated injector at a rate of 1.5-2 mL/s during a single breath-hold. The axial and coronal images were reconstructed in 5-mm-thick intervals.

Assessment of calcification severity

The CT imaging findings were assessed by two radiologists (with 4 and 10 years of clinical experience, respectively). The threshold value for calcification was set at 130 Hounsfield units (HU) according to the typically used value of 130 HU for the Agatston score[15]. For patients who underwent multiple CT scans, the most recent CT scan was evaluated. The severity of calcification at the tributaries of the portal vein was measured using a 4-grade scoring system of the calcification of phlebosclerotic colitis[16] (Table 1; a schematic of the calcification score calculation is depicted in Figure 1).

Table 1 Scoring system of calcification of phlebosclerotic colitis

Calcifications limited in straight vein of the colon (× 1)
Calcifications extended to the paracolic marginal vein (× 2)
Calcifications extended to the main branch of mesenteric vein (× 3)
The proximal end of the main branch is involved (× 4)

The score of mesenteric venous calcification in every branch was summed in each patient to represent the severity of their mesenteric calcification.

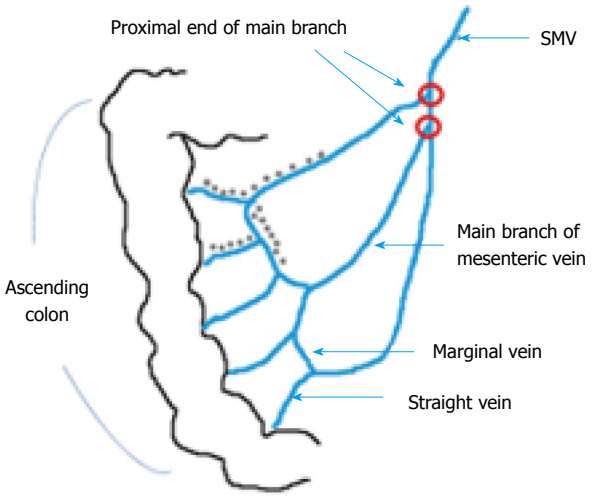

Figure 1 Schematic figure for calculating calcification score. Calcifications (grey dots) at two straight veins, one marginal vein, and one main branch of mesenteric vein are noted. The proximal ends of main branches of mesenteric veins are not involved. Therefore, the calcification score in this case is calculated as 2 × 1 + 1 × 2 + 1 × 3 + 0 × 4 = 7.

Episodes of active disease

The episodes of active disease were defined as symptoms of fever, abdominal pain, severe constipation, bowel obstruction, vomiting, and diarrhea based on a review of the medical records.

Statistical analysis

All data were analyzed using SPSS (Statistical Package for the Social Sciences) version 22.0 software (IBM, Armonk, NY). Descriptive statistics of the distributions of the demographic and clinical characteristics of the patients exhibiting calcification at the tributaries of the portal vein from the continuous data are presented as median values and interquartile ranges, and the statistics from the categorical data are presented as the numbers and frequencies of observations. Spearman's correlation analysis was used to examine the correlation between the number of active disease episodes and the severity of mesenteric vein calcification. A two-tailed *P*-value less than 0.05 was considered to indicate a significant correlation between the variables.

RESULTS

More than 3000 cases were reviewed, and a total of 12 patients were enrolled according to the inclusion criteria. Among these 12 patients, the 6 males and the 6 females had a mean age of 61.8 years, ranging from 49 to 85 years. No patients underwent surgical intervention. Seventy-five percent of the patients exhibited symptoms of phlebosclerotic colitis, and the number of episodes of active disease ranged from 0 to 27 [1 ± 1.75, median ± interquartile range (IQR)] (Table 2). All patients exhibited linear calcification on CT, and the scores for the severity of venous calcification ranged from 9 to 33 (18 ± 13, median ± IQR). Table 3 shows the imaging findings for these patients. Based on the imaging findings, 10 patients displayed threadlike calcifications (Figure 2) on conventional radiographs, eight patients exhibited colonic wall thickening in CT studies (Figure 3), and one patient displayed a typical characteristic, which was thumb-printing appearance, based on barium follow-through study (Figure 4). One patient received a colonoscopic examination, and the findings revealed a typical ischemic change in the ascending colon (Figure 5). Three patients exhibited no apparent symptoms; however, the CT imaging findings of these three

Table 2 Distribution of demographic characteristics and clinical history of 12 cases *n* (%)

Characteristics	Case (*n* = 12)
Age (yr), mean ± SD	61.8 ± 11.5
Sex	
Male	6 (50.0)
Female	6 (50.0)
Underlying disease	
No	2 (16.7)
HCC	2 (16.7)
Bladder cancer	1 (8.3)
Ampulla Vater cancer	1 (8.3)
ESRD	6 (50.0)
Known episode of active disease	
None	3 (25.0)
One	5 (41.7)
Two	2 (16.7)
More than two	2 (16.7)
The score of the severity of venous calcifications (median ± interquartile)	18.0 ± 13.0

HCC: Hepatocellular carcinoma; ESRD: End stage renal disease.

Table 3 Imaging features of 12 cases *n* (%)

Imaging features	Case (*n* = 12)
Plain-film radiographs of the abdomen (kidney ureter bladder)	
Threadlike calcification,	10 (83.3)
Was not performed	2 (16.7)
Abdominal computed tomography	
Finding of thickening of colonic wall	
Negative	4 (33.3)
Positive	8 (66.7)
Finding of threadlike calcification	
Negative	0 (0.0)
Positive	12 (100.0)

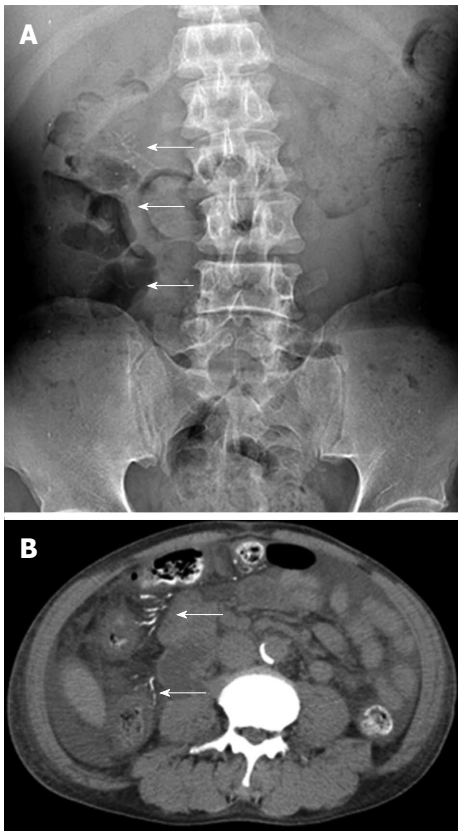

Figure 2 Kidney-ureter-bladder radiography and non-contrast enhanced computed tomography. A 56-year-old male suffered from right upper quadrant abdominal pain and fever. A: The kidney-ureter-bladder radiography showed threadlike calcifications (arrows) at right abdomen; B: The non-contrast enhanced computed tomography study revealed calcifications (arrows) at tributaries of mesenteric vein and wall thickening of the ascending colon. The diagnosis is phlebosclerotic colitis with active episode.

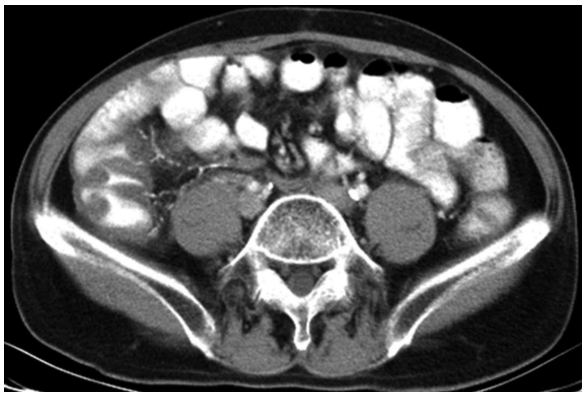

Figure 3 Intravenous contrast enhanced computed tomography with oral contrast ingestion. A 59-year-old male came to emergency room with fever and tenderness at right lower abdomen. The contrast enhanced computed tomography shows wall thickening at the ascending colon and calcifications at straight veins, marginal veins and main branch of mesenteric vein, suggestive of phlebosclerotic colitis.

asymptomatic patients were bowel wall thickening and fat stranding at the paracolic gutters of the involved colon (Figure 6), which were typical imaging characteristics of this rare disease.

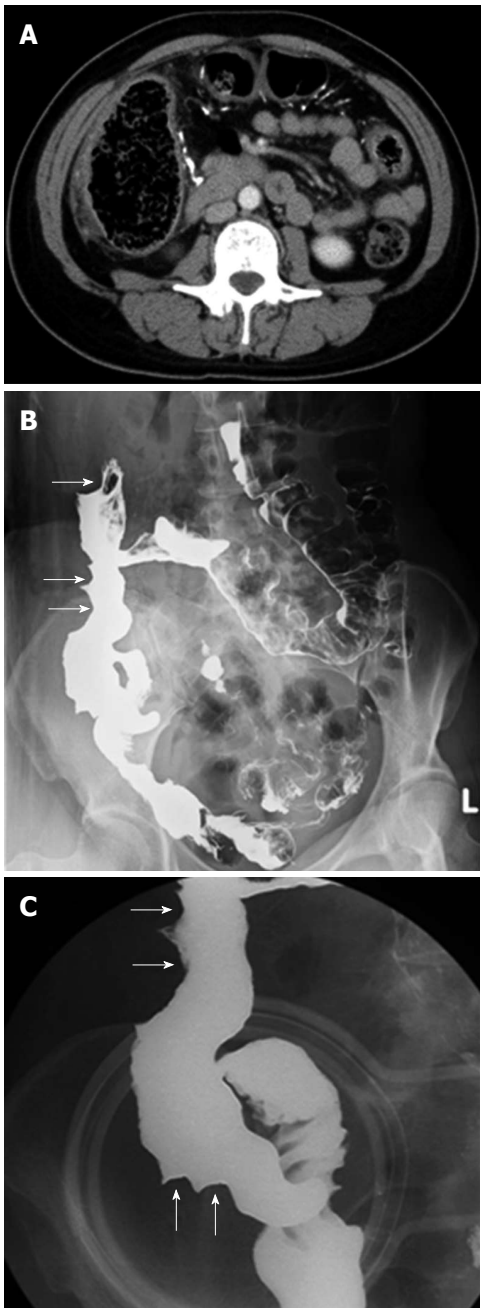

Figure 4 Barium follow-through study and intravenous contrast enhanced computed tomography. A-54-year old female suffered from abdominal pain and constipation. A: The contrast enhanced abdominal computed tomography shows calcifications at tributaries of mesenteric vein and dilatation of the ascending colon; B: The barium follow-through study done after discharge shows thumb-printing appearance (arrows) at the ascending colon; C: The close view of cone compression.

The dispersion diagram (Figure 7) displays the relationship between the number of active disease episodes and the severity of mesenteric venous calcification. Spearman correlation analysis showed that the number of episodes of active disease significantly correlated with the severity of mesenteric venous calcification ($r = 0.619$, $P < 0.05$).

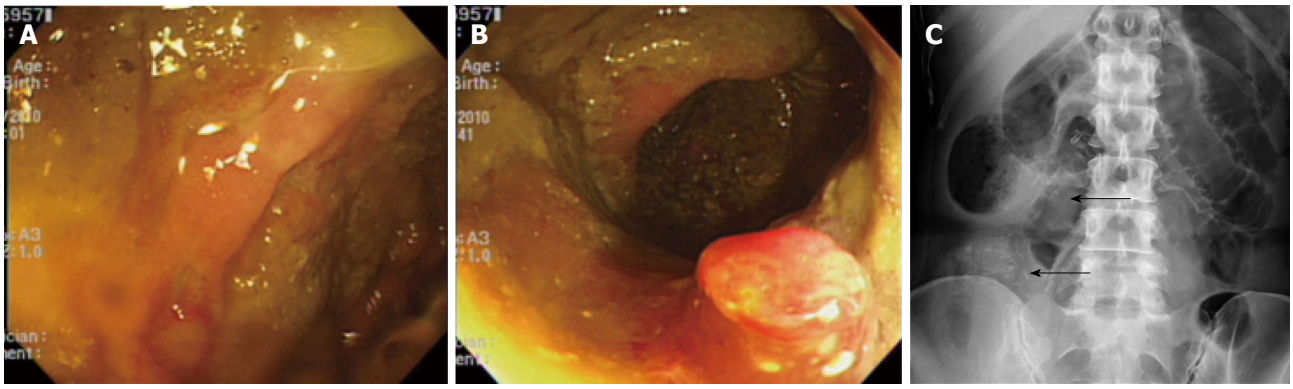

Figure 5 Colonoscopy and plain-film radiographs of the abdomen of one symptomatic patient. A: Cobble stone appearance in colonoscopy was noted; B: A polyp in the ascending colon was noted and biopsy was done. The pathology report revealed granulation tissue with heavy chronic inflammatory cell infiltration; C: The kidney-ureter-bladder radiography shows threadlike calcifications (arrows) at right abdomen with bowel obstruction.

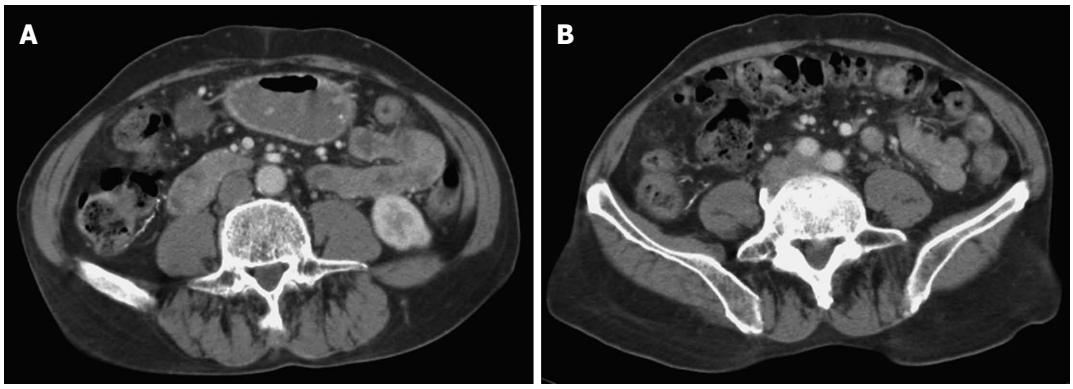

Figure 6 Intravenous contrast enhanced computed tomography of an asymptomatic patient. A 72-year-old male has not experienced any significant abdominal symptoms. A: Computed tomography study showed calcifications at mesenteric veins; B: There was thickening of the wall of the cecum and ascending colon with pericolic stranding, which may indicate active disease.

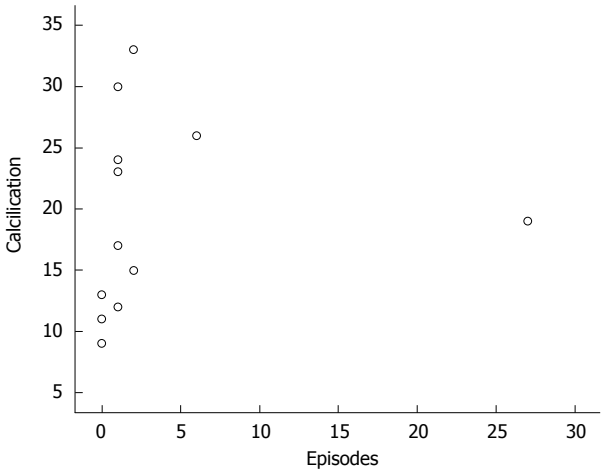

Figure 7 The dispersion diagram between the number of active disease episodes and the severity of mesenteric venous calcifications.

DISCUSSION

Ischemic colitis is caused by a disturbance in the blood supply and is evoked by arterial thromboembolic disease; however, phlebosclerotic colitis affects regions of the colon displaying aberrant venous drainage into the superior mesenteric vein. The first description of phlebosclerotic colitis was in a Japanese study in 1991[5]. The clinical course of phlebosclerotic colitis is chronic, and its clinical symptoms, such as fever, abdominal pain, severe constipation, bowel obstruction, vomiting, and diarrhea, are attributable to the ischemic change in the colon secondary to the sclerosis of the draining veins. Several additional severe symptoms, such as bowel obstruction, bowel perforation, bloody diarrhea, and abscess formation[17], occur over time. However, the etiology and risk factors for sclerosis in the tributaries of the superior mesenteric vein remain unclear. Six patients with phlebosclerotic colitis in our studies suffered from end-stage renal disease (ESRD), all of whom ingested Chinese herbs for different periods. The incidence of ESRD in Taiwan is the highest in the world, and there is evidence that Chinese herb nephropathy is a significant cause of ESRD in Taiwan[18,19]. Chang[20] indicated that these absorbed substances enter the venous return, potentially damaging veins. Moreover, several studies[20-22] reported that certain substances or toxins from ingested Chinese herbs may contribute to phlebosclerotic colitis. Therefore, we propose that ESRD correlates with phlebosclerotic colitis. The

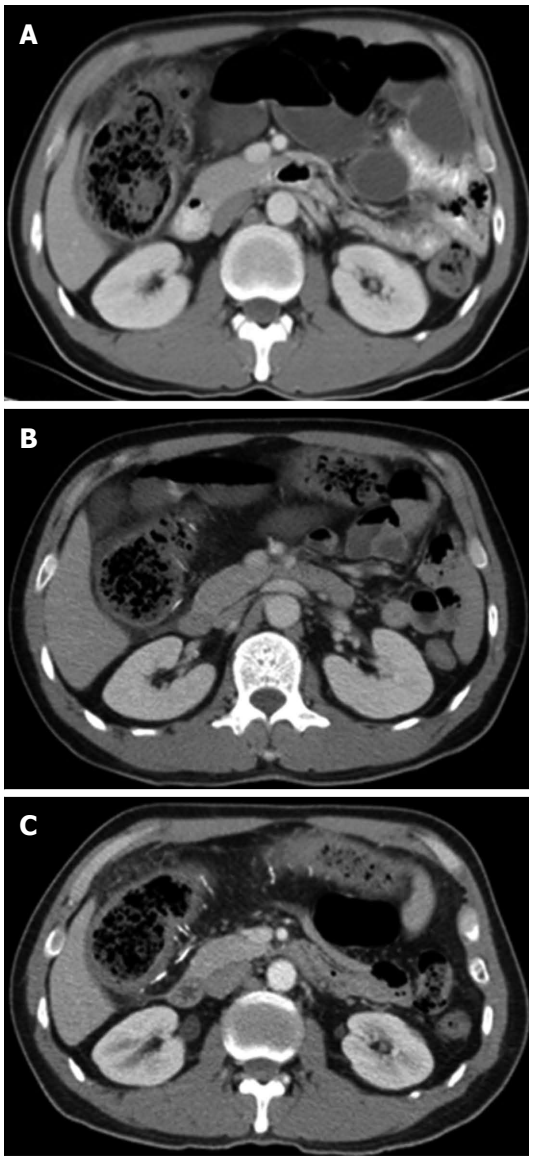

Figure 8 Serial computed tomography studies from 2005 to 2012. A 46-year-old male suffered from recurrent abdominal pain and severe constipation for about 10 years. A: Computed tomography (CT) study done on November 18, 2005 revealed dilated ascending colon and minimal colonic wall thickening without evidence of mesenteric venous calcification; B: CT study of the same case done on November 17, 2009 showed some linear calcifications at colonic wall. Dilated ascending colon with wall thickening was also noted; C: CT study of the same case done on January 3, 2012 showed more linear calcifications at colonic wall and mesenteric veins. The extent of colonic wall thickening was also more prominent in this study.

relationship between ESRD and phlebosclerotic colitis requires further investigation. Although Chang[20] reported 5 phlebosclerotic colitis cases with a history of herbal ingestion, the hypothesis that herb ingestion contributes to phlebosclerotic colitis requires further investigation. Regarding the medical history, the laboratory findings, and the family history of the 12 examined patients, no notable etiologies or risk factors were detected.

The clinical diagnosis of phlebosclerotic colitis is typically based on a combination of symptoms and clinical, radiological, and endoscopic findings. The typical findings from a barium enema are the disappearance of the semilunar folds, luminal narrowing and a thumbprint-like appearance[8,11]. On conventional radiographs, threadlike calcification in the right hemicolon is a pathognomonic imaging finding. CT scans are more reliable for detecting mesenteric venous calcification[23] and colonic wall thickening. Several angiographic findings, such as narrowing and irregularity of the marginal arteries with tortuous vasa recta[23] and delayed venous return[14,25] with or without developed collateral veins[25], have been reported.

In the present study, although three patients exhibited no apparent clinical episodes of active disease, their CT imaging findings were typical of this disease (Figure 6). In contrast, patients at the early stage of this disease may exhibit no substantial calcification based on imaging or microscopic examination[26].

One patient in our study experienced the recurrent symptoms of abdominal pain, constipation, nausea and vomiting many times since 2003. The CT examination conducted in 2005 only revealed a dilated ascending colon without calcification at the mesenteric veins. The barium enema performed in 2006 revealed a loss of haustration in the ascending colon, the transverse colon, and the proximal descending colon and a thumbprint-like appearance of the ascending colon. Colitis with unknown etiology was observed. The severity of colonic wall thickening and calcification in the portal vein tributaries increased in serial CT studies performed in 2005, 2009, 2010 and 2012 (Figure 8). However, the diagnosis of phlebosclerotic colitis was initially determined in 2010 according to typical imaging findings and clinical symptoms. The CT examinations of patients with phlebosclerotic colitis may display nonspecific findings, and thus, accurate diagnosis during the early stage of this disease is difficult.

In this study, Spearman's correlation analysis was used to examine the relationship between the clinical symptoms of phlebosclerotic colitis and the severity of venous calcifications based on the imaging data. The results indicated that more severe mesenteric venous calcification was associated with more numerous clinical symptoms.

The major limitation of this study is the small case number. In addition, because these patients may have experienced mild clinical symptoms[26], they may not have visited the hospital or their condition may have been misdiagnosed as diarrhea or constipation by a clinician. Even if patients visit the hospital and receive an imaging examination, calcification in the colonic wall or along the tributaries of the superior mesenteric vein may not have occurred during the early stage of this disease. Some asymptomatic patients exhibiting typical imaging characteristics may experience symptoms during an extended follow-up duration.

Previously, the majority of phlebosclerotic colitis patients underwent surgery; however, at present,

conservative treatment with close follow-up is preferred if there are no signs of bowel compromise[27-29]. Surgery has been suggested for patients with severe complications, such as intestinal obstruction, perforation, peritonitis and sepsis. The patients in our study received only conservative treatment because they lacked any complications that would require surgical treatment.

In conclusion, radiologic findings play a crucial role in diagnosing phlebosclerotic colitis, and a CT study is the most useful diagnostic tool for evaluating the severity of mesenteric venous calcification. The extent of mesenteric venous calcification is strongly positively associated with the number of episodes of active disease.

COMMENTS

Background

Phlebosclerotic colitis is a rare disease, and its etiology and risk factors remain uncertain. The presence of calcifications in the mesenteric veins is a typical radiographic characteristic of this disease.

Research frontiers

The radiographic and pathological characteristics of this disease were previously described in several reports. However, the correlation between the severity of venous calcification and these clinical symptoms remains unclear.

Innovations and breakthroughs

The author's finding improves the understanding of phlebosclerotic colitis.

Applications

A positive relationship between venous calcifications and the clinical symptoms of phlebosclerotic colitis was observed. The scoring system of venous calcification may help predict the prognosis and guide the treatment of this disease.

Terminology

Phlebosclerotic colitis is a rare disease causing chronic ischemic colitis and sclerosis of the mesenteric veins.

Peer-review

The authors detail the radiographic characteristics of phlebosclerotic colitis and the relationship between imaging findings and clinical presentation. The results show that the extent of mesenteric venous calcifications is positively associated with the number of episodes of active disease.

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ORIGINAL ARTICLE

Retrospective Study

Stereotactic body radiotherapy using CyberKnife for locally advanced unresectable and metastatic pancreatic cancer

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Author contributions: Su TS and Liang P performed the majority of experiments and designed the study; Su TS wrote the manuscript; Lu HZ and Liang JN collected all the clinical materials; Liu JM, Zhou Y, Gao YC and Tang MY provided vital reagents and analytical tools and were also involved in revising the manuscript.

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Abstract

AIM: To evaluate the efficacy and toxicity of stereotactic body radiotherapy using CyberKnife for locally advanced unresectable and metastatic pancreatic cancer.

METHODS: From June 2010 to May 2014, 25 patients with locally advanced unresectable and metastatic pancreatic cancer underwent stereotactic body radiotherapy. Nine patients presented with unresectable locally advanced disease and 16 had metastatic disease. Primary end-points of this study were overall survival, relief of abdominal pain, and toxicity.

RESULTS: Fourteen patients were treated with a total dose of 30-36 Gy in three fractions and the remainder with 40-48 Gy in four fractions. Median follow-up was 11 mo (range: 2-25 mo). The median survival duration calculated from the time of stereotactic body radiotherapy for the entire group, the locally advanced group, and the metastatic group was 9.0 mo, 13.5 mo, and 8.5 mo, respectively. Overall survival was 37% and 18% at one and two years, respectively. Abdominal pain relief was achieved within 2 wk of completing radiotherapy in the patients who received successful palliation (13 of 20 patients had significant pain). Five patients (20%) had grade 1 nausea, and one (4%) had grade 2 nausea. No acute grade 3+ toxicity was seen.

CONCLUSION: Stereotactic body radiotherapy using the CyberKnife system is a promising, noninvasive, palliative treatment with acceptable toxicity for locally advanced unresectable and metastatic pancreatic cancer.

Key words: CyberKnife; Pancreatic cancer; Stereotactic body radiotherapy

Core tip: Locally advanced unresectable and metastatic pancreatic cancer is the most common presentation of pancreatic cancer. The available therapeutic option is chemotherapy or chemoradiotherapy. The low-dose radiation of conventional radiotherapy has unsatisfactory results for survival and local control, at a cost of increased hematologic toxicity. Doses > 54 Gy may be considered if clinically appropriate. Stereotactic body radiation therapy has become an important research topic to provide a higher biologically effective dose. We evaluated the efficacy and toxicity of stereotactic body radiation therapy using the CyberKnife system for patients with locally advanced unresectable and metastatic pancreatic cancer.

Su TS, Liang P, Lu HZ, Liang JN, Liu JM, Zhou Y, Gao YC, Tang MY. Stereotactic body radiotherapy using CyberKnife for locally advanced unresectable and metastatic pancreatic cancer. *World J Gastroenterol* 2015; 21(26): 8156-8162 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i26/8156.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i26.8156

INTRODUCTION

Pancreatic cancer is both an aggressive and prevalent malignancy. It is the fourth leading cause of cancer mortality in men and women in the United States[1]. In the Asia-Pacific region, the age-standardized incidence reached a plateau after 1985, while the incidence continued to rise due to the aging population in the region[2]. Only approximately 20% of patients are amenable to surgery at diagnosis. It is a highly aggressive entity with approximately 40% presenting with locally advanced but unresectable disease and an additional 40% presenting with metastatic disease[3]. Surgical resection remains the only curative therapeutic modality for early-stage pancreatic cancer. Despite improvements in surgical technique and patient selection, as well as adjuvant chemotherapy, the five-year survive rate remains low, ranging from 10% to 20%, following curative surgery[4-6]. In patients with locally unresectable pancreatic cancer, the only therapeutic option is chemoradiotherapy. The local control rate after chemoradiotherapy is still relatively low, ranging from 40% to 55%, with a median survival ranging from 5 mo to 14 mo[7-9]. The conventional radiation dose is usually between 45 and 54 Gy in 1.8-2.5-Gy fractions. These limited doses have a poor curative effect. Doses > 54 Gy may be considered if clinically appropriate[10-12]. Recently, stereotactic body radiotherapy (SBRT) has become an important research topic to provide a higher biologically effective dose. The conformity and rapid dose fall-off associated with SBRT offer the potential for dose escalation[13]. In this study, we analyzed the patients with locally advanced unresectable and metastatic pancreatic cancer who underwent SBRT.

MATERIALS AND METHODS

Patient population

Between June 2010 and May 2014, 25 patients with unresectable or metastatic pancreatic adenocarcinoma were included in this retrospective analysis. Ethical approval was given by the Medical Ethics Committee of Rui Kang Hospital, Guangxi, China. All patients gave written informed consent. Reasons for unresectability included the presence of metastatic disease and radiographic evidence of major vessel involvement, as determined by the surgeon and/or radiologist. Patients with metastatic disease who were treated with SBRT had distant disease that: (1) responded well to initial chemotherapy if the prognosis was that local disease potentially could lead to death or significant morbidity; or (2) the local tumor was causing symptoms of pain or obstruction. All patients' hospital charts and irradiation documents were carefully reviewed.

SBRT

Patients were immobilized in the supine position with arms over the head using a thermoplastic body mask and a styrofoam block provided abdominal compression to minimize internal organ motion (spontaneous or breath-induced). CT was performed with a slice thickness of 3 mm. The gross tumor volume was defined as the tumor visible on the CT scan, and in those with N1 disease, the nodes were not included in the target. The gross tumor volume was expanded by 1 or 2 mm to form the planning target volume (PTV). The dose-volume constraints for organs at risk were: duodenum, V 1 mL < 25 Gy; stomach and small bowel, V 1 mL < 25 Gy, and was strict with regard to keeping any 1 mL < 25 Gy; kidneys, 1/3 V_{tot} < 15 Gy; liver, total spared volume (V_{tot} - V 15 Gy) > 700 mL and V 15 Gy < 1/3 total volume; spinal cord, V 1 mL < 15 Gy, and strict with regard to keeping any 1 mL < 15 Gy. The radiosurgical plan was to deliver a dose of 30-36 Gy in three fractions or 40-48 Gy in four fractions. Plans were devised such that the prescription dose was the isodose line encompassing > 97% of the PTV. No more than 3% of the PTV was to receive < 93% of the prescription dose. For stereotactic localization, patients underwent a 4D-CT treatment

Table 1 Detailed information of pancreatic cancer patients treated with stereotactic body radiotherapy

Patient	Sex	T	N	M	CA19-9	Dose/No. of fractions	Live/dead	Survival time (mo)	Toxicity
1	Female	4	0	0	Positive	36 Gy/3	Live	4	G1
2	Male	3	0	0	Negative	45 Gy/4	Dead	9	
3	Male	3	1	1	Negative	48 Gy/4	Live	2	G1
4	Male	3	0	0	Positive	46 Gy/4	Dead	2	
5	Male	3	1	1	Positive	46 Gy/4	Dead	8	
6	Male	3	1	1	Positive	30 Gy/3	Live	8	G1
7	Female	3	0	0	Positive	36 Gy/3	Dead	4	
8	Female	3	1	0	Negative	31.5 Gy/3	Live	17	
9	Female	3	1	1	Negative	33 Gy/3	Dead	5	
10	Male	3	0	1	Positive	36 Gy/3	Dead	9	G1
11	Male	3	0	1	Positive	35 Gy/3	Dead	14	
12	Female	4	1	1	Positive	33 Gy/3	Dead	9	
13	Female	4	0	1	Positive	36 Gy/3	Dead	3	G1
14	Male	3	0	0	Positive	36 Gy/3	Live	6	
15	Male	3	0	1	Negative	40 Gy/4	Dead	3	
16	Female	3	0	1	Positive	45 Gy/4	Live	15	G1
17	Male	3	1	1	Negative	33 Gy/3	Dead	17	
18	Male	3	0	1	Positive	36 Gy/3	Dead	2	G2
19	Male	3	1	1	Positive	36 Gy/3	Dead	2	
20	Male	3	1	0	Negative	42 Gy/4	Dead	3	
21	Male	3	0	0	Negative	33 Gy/3	Live	3	G1
22	Male	4	1	1	Positive	40 Gy/4	Live	1	
23	Male	3	1	0	Positive	46 Gy/4	Live	25	
24	Male	3	1	1	Negative	48 Gy/4	Dead	9	
25	Male	3	0	1	Positive	40 Gy/4	Live	9	G1

CA19-9: Carbohydrate antigen 19-9.

simulation with the CyberKnife Robotic Radiosurgery System with the Xsight Spine Tracking System (Accuray Inc., Sunnyvale, CA, United States).

Response evaluation and follow-up

Patients were re-evaluated 1 mo after SBRT and then every 3 mo thereafter by the treating radiation oncologist. Clinical examination, determination of carbohydrate antigen 19-9 levels, and contrast-enhanced CT were performed at each step of follow-up. Acute and late toxicity was scored according to the NCI Common Terminology Criteria for Adverse Events version 3.0.

Statistical analysis

Overall survival (OS) was calculated from the date of SBRT to the date of progression and to the day of last follow-up or death using the Kaplan-Meier method. Acute toxicity was defined as that occurring within 90 d of SBRT, and late toxicity as that occurring thereafter. SPSS version 17.0 (SPSS Inc., Chicago, IL, United States) was used for statistical analysis. All enrolled patients were included in the statistical evaluation. The statistical methods of this study were reviewed by Zhen-Dong Yang from Rui Kang Hospital, Guangxi Traditional Chinese Medical University.

RESULTS

Patient characteristics

Twenty-five patients were treated in our hospital with SBRT for pancreatic cancer. The median age was 63 years (range: 44-80 years) and 72.0% (18/25) were male. All patients were considered to have unresectable/locally advanced (9/25; 36.0%) and metastatic (16/25; 64.0%) disease as determined by experienced pancreatic surgeons and/or radiologists. Patients were diagnosed with pancreatic cancer at clinical stages T3 (21/25; 84.0%) and T4 (4/25; 16.0%). The majority of patients were N0, but 48.0% (12/25) had N1 disease. Clinical characteristics of selected patients are described in Tables 1 and 2.

SBRT

Fourteen patients treated with SBRT received a dose of 30-36 Gy in three fractions and the remaining 11 received 40-48 Gy in four fractions. The mean target volume was 43.27 mL (range: 8.80-96.39 mL). The CyberKnife platform utilized 150-180 beams. Maximum spinal cord point dose was a mean 730 cGy (range: 390-1430 cGy), which was strictly maintained at 1 mL < 15 Gy. Maximum bowel point dose was a mean 3361 cGy (range: 2792-4018 cGy) for the PTV, which was strictly maintained at 1 mL < 25 Gy.

Adjuvant therapy

Two patients received neoadjuvant gemcitabine-based chemotherapy. Another two patients received adjuvant gemcitabine-based chemotherapy. The choice of chemotherapy was at the discretion of the medical oncologist. During SBRT, combined adjuvant medication was given, consisting of Chinese herbs and

Table 2 Characteristics of pancreatic cancer patients treated with stereotactic body radiotherapy

Characteristic	*n*
Sex	
Male	18
Female	7
Age, yr	Median 63 (range: 44-80)
Stage[1]	
T3	21
T4	4
N0	13
N1	12
M0	9
M1	16
Dose (Gy)/No. of fractions	
30-36 Gy/3	14
40-48 Gy/4	11
Carbohydrate antigen 19-9	
Positive	16
Negative	9
Primary location of tumor	
Head of pancreas	20
Body or tail of pancreas	5

[1]According to 2010 AJCC staging system.

dexamethasone, vitamins, glutathione, and lansoprazole.

Toxicity

Twelve patients experienced grade 1 fatigue at 2 wk after SBRT, which required no treatment. Five patients (20%) had grade 1 nausea, and ondansetron was administered to one (1/25; 4%) patient with grade 2 nausea. None of these patients had persistent nausea after 1 mo. No acute grade 3+ toxicity was seen. Most toxicity was well tolerated.

Pain relief

According to the numerical rating scale scoring system, 20/25 (80%) patients experienced significant pain before SBRT. Abdominal pain relief was achieved within 2 wk of completing radiotherapy in the patients who received successful palliation. Ten patients achieved pain control after treatment, allowing suspension of analgesic administration. In three patients, analgesic dose was reduced by 50%, or the patients needed fewer analgesic drugs.

Survival

Survival data were available at a median follow-up of 11 mo (range: 2-25 mo). The median survival duration calculated from the time of SBRT for the entire group, the locally advanced group, and the metastatic group was 9.0 mo, 13.5 mo, and 8.5 mo, respectively. OS was 37% and 18% at one and two years, respectively (Figure 1).

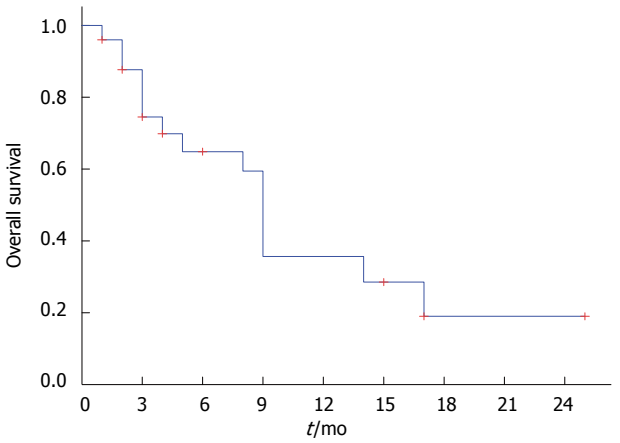

Figure 1 Kaplan-Meier curve for overall survival. Median overall survival was 9 mo, and overall survival was 37% and 18% at one and two years, respectively.

DISCUSSION

Locally advanced unresectable and metastatic pancreatic cancer is the most common presentation of pancreatic cancer. There have been many clinical trials conducted to evaluate novel system regimens for advanced pancreatic cancer. Chemotherapy alone reduces the incidence of distant metastases in patients with localized disease, even though it may hardly improve local disease control. Gemcitabine monotherapy has conventionally been considered the standard regimen for advanced pancreatic cancer on the basis of phase Ⅲ clinical trials. The median overall survival is limited to the range of 4.6 to 9.2 mo with gemcitabine treatment[12,13]. Among non-gemcitabine regimens, the most notable is FOLFRINOX. A phase Ⅲ clinical trial showed that OS was significantly longer in the FOLFRINOX arm (11.1 mo *vs* 6.8 mo). Nevertheless, the FOLFRINOX regimen was at the cost of increased hematologic toxicity[13,14]. The only therapeutic option available is gemcitabine- or capecitabine-based chemoradiotherapy. The median survival ranges from 11.1 mo to 15.2 mo[12,15,16]. The local progression rate reported with conventional fractionation of radiotherapy is still relatively low, at 40%-55%[7-9]. In recent years, the unsatisfactory results of conventional radiotherapy led to several studies that investigated the efficacy and safety of SBRT. Recent encouraging results of SBRT for pancreatic cancer are shown in Table 3. Improvement of local disease control was relevant in these studies, with a success rate of 57%-94%. Median OS was 5.7-20.0 mo. Survival was extended for most of the patients. However, acute and late toxicity are still challenging. The rate of late gastroduodenal toxicity of grade 2 or higher was 4%-47% in several studies.

We investigated the outcomes in a series of patients with locally advanced unresectable and metastatic pancreatic cancer who underwent SBRT. Radiotherapy comprised 30-36 Gy in three fractions or 40-48 Gy in four fractions, and the priority was to evaluate the safety of the surrounding normal tissue. According to the standard equation, 30-36 Gy in three fractions has a biologically effective dose

Table 3 Summary of treatment regimen, local control, progression-free survival, overall survival and late toxicity in previous studies compared with the present study

Ref.	No. of patients	SBRT dose (Gy/No. of fractions)	Gemcitabine-based chemotherapy	LC (%)	PFS (mo)	OS (mo)	Toxicity (≥ G2) (%)
Didolkar *et al*[17]	85	15-30 Gy/3	Sequential	91.7	-	18.6 from diagnosis 8.6 from SBRT	22
Polistina *et al*[18]	23	30 Gy/3	Prior	82.6	7.3	10.6	None
Mahadevan *et al*[19]	39	24-36 Gy/3	Sequential	85	15	20 from diagnosis	9
Schellenberg *et al*[20]	16	25 Gy/1	Sequential	81	9	11.4 from diagnosis	47
Hoyer *et al*[21]	22	45 Gy/3	Sequential	57	4.8	5.7 from diagnosis	18
Koong *et al*[22]	15	15-25 Gy/1	No	77	2	11 from diagnosis	None
Chang *et al*[23]	77	25 Gy/1	Prior	84	-	11.4 from diagnosis	13
Schellenberg *et al*[24]	20	25 Gy/1	Sequential	94	9.2	11.8 from diagnosis	20
Rwigema *et al*[25]	71	18-25 Gy/1	No	64.8	-	10.3	10
Pollom *et al*[26]	167	25-33 Gy/1-5	Sequential or concurrent	-	-	13.6 from diagnosis	12.3
Moningi *et al*[27]	88	20-33 Gy/5	Gemcitabine, cisplatin, FOLFIRINOX or paclitaxel	-	9.8	18.4 from diagnosis	5.7 G2 3.4 G3
Gurka *et al*[28]	38	25-30 Gy/5	Gemcitabine, mFOLFOX or capecitabine	79	9.2	14.3 from diagnosis	-
Present study	25	30-36 Gy/3 or 42-48Gy/4	4 patients, Gemcitabine	-	-	M0 group 13.5 M1 group 8.5 from SBRT	4

LC: Local control; OS: Overall survival; PFS: Progression-free survival; SBRT: Stereotactic body radiotherapy.

of 50-66 Gy, and 40-48 Gy in four fractions has a biologically effective dose of 68-88 Gy (assuming an α/β ratio of 10 for rapidly proliferating tumor cells and 3 for normal tissues). We found that the median OS was 9 mo. OS was 37% and 18% at one and two years, respectively. Palliative treatment with SBRT improved quality of life, especially palliation of pain, with acceptable toxicity. Our results support the use of palliative SBRT. The major advantages of this approach compared with conventional fractionated radiotherapy are: (1) more intensified treatment of the primary tumor; (2) increased patient convenience; and (3) minimal interference with the delivery of maximal systemic chemotherapy. We hypothesize that quality of life and OS benefit from local palliative SBRT for primary tumors, and large prospective clinical trials are warranted.

COMMENTS

Background

Locally advanced unresectable and metastatic pancreatic cancer is the most common presentation of pancreatic cancer. The available therapeutic option is chemotherapy or chemoradiotherapy. However, the low-dose radiation of conventional radiotherapy leads to unsatisfactory results for survival and local control, at a cost of increased hematologic toxicity. Doses > 54 Gy may be considered if clinically appropriate.

Research frontiers

Stereotactic body radiotherapy (SBRT) with conformity and rapid dose fall-off has become an important research topic, to provide a higher biologically effective dose. It has been used to treat many cancers. The current research hotspot is to evaluate the efficacy and toxicity of SBRT for patients with locally advanced unresectable and metastatic pancreatic cancer.

Innovations and breakthroughs

This study presented outcomes in a series of patients with locally advanced unresectable and metastatic pancreatic cancer who underwent SBRT using the CyberKnife system. The radiotherapy plan was 30-36 Gy in three fractions or 40-48 Gy in four fractions. The dose-volume constraints for organs at risk were: duodenum, V 1 mL < 25 Gy; stomach and small bowel, V 1 mL < 25 Gy, and was strict with regard to keeping any 1 mL < 25 Gy; kidneys, 1/3 V_{tot} < 15 Gy; liver, total spared volume (V_{tot} - V 15 Gy) > 700 mL and V 15 Gy < 1/3 total volume; spinal cord, V 1 mL < 15 Gy, and strict with regard to keeping any 1 mL < 15 Gy. According to the standard equation, 30-36 Gy in three fractions has a relative biologic effectiveness of 50-66 Gy, and 40-48 Gy in four fractions has a biologically effective dose of 68-88 Gy (assuming an α/β ratio of 10 for rapidly proliferating tumor cells and 3 for normal tissues). The authors found that median overall survival was 9 mo. Overall survival was 37% and 18% at one and two years, respectively. Palliative treatment with SBRT was effective for pain relief (65%), with acceptable toxicity (grade 1: 20%, grade 2: 4%). These results support the use of palliative treatment with SBRT. The major advantages of this approach compared with conventional fractionated radiotherapy are: (1) more intensified treatment of the primary tumor; (2) increased patient convenience; and (3) minimal interference with the delivery of maximal systemic chemotherapy.

Applications

This study supports the use of palliative treatment with the CyberKnife for locally advanced unresectable and metastatic pancreatic cancer. It is remarkably effective in palliation of pain, with acceptable toxicity.

Terminology

SBRT using the CyberKnife system is a promising noninvasive and palliative treatment with acceptable toxicity for locally advanced unresectable and metastatic pancreatic cancer.

Peer-review

This is an intriguing report on the experience with stereotactic body radiotherapy for locally advanced/metastatic pancreatic cancer, a topic of great interest for oncologists given the very difficult issue of local treatment/palliation in the setting of an aggressive histology with a high propensity to disseminate.

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ORIGINAL ARTICLE

Retrospective Study

Management of hepatocellular carcinoma rupture in the caudate lobe

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Author contributions: Hong DF, Liu YB and Peng SY designed the clinical study and surgical techniques; Pang JZ, Shen GL and Zhang YB participated in the management of the patients including working as the assistant in the operations; Cheng J did clinical data collection and analysis; Hong DF and Wang ZF wrote the paper.

Institutional review board statement: This work has been improved by the ethics committee of Zhejiang Provincial People's Hospital.

Informed consent statement: Informed consent was obtained from all the patients at the admission to the hospital.

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Data sharing statement: No additional unpublished data are available.

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Abstract

AIM: To demonstrate that caudate lobectomy is a valid treatment in cases of hepatocellular carcinoma (HCC) rupture in the caudate lobe based on our experience with the largest case series reported to date.

METHODS: A retrospective study of eight patients presenting with spontaneous rupture and hemorrhage of HCC in the caudate lobe was conducted. Two patients underwent ineffective transarterial embolization preoperatively. Caudate lobectomy was performed in all eight patients. Bilateral approach was taken in seven cases for isolated complete caudate lobectomy. Left-sided approach was employed in one case for isolated partial caudate lobectomy. Transarterial chemoembolization was performed postoperatively in all patients.

RESULTS: Caudate lobectomy was successfully completed in all eight cases. The median time delay from the diagnosis to operation was 5 d (range: 0.25-9). Median operating time was 200 min (range: 120-310) with a median blood loss of 900 mL (range: 300-1500). Five patient remained in long-term follow-

up, with one patient becoming lost to follow-up at 3 years and two patients currently alive at 7 and 19 mo. One patient required reoperation due to recurrence. Gamma knife intervention was performed for brain metastasis in another case. Two patients survived for 10 and 84 mo postoperatively, ultimately succumbing to multiple organ metastases.

CONCLUSION: Caudate lobectomy is the salvage choice for HCC rupture in the caudate lobe. Local anatomy and physiologic features of the disease render caudate lobectomy a technically difficult operation. Postponement of surgical intervention is thus recommended while the rupture remains hemodynamically stable until an experienced surgeon becomes available. Prognosis is confounded by numerous factors, but long-term survival can be expected in the majority of cases.

Key words: Caudate lobectomy; Hepatocellular carcinoma; Emergency; Rupture; Transarterial embolization

Core tip: Management of spontaneous rupture and hemorrhage of hepatocellular carcinoma is seldom reported due to its rareness and severity. This article demonstrates that caudate lobectomy is a valid treatment as well as the salvage choice for management in such cases based on our experience with the largest case series reported to date. Based on limited long-term follow-up data, overall 1-year and 3-year survival rates have been achieved. In addition, delayed surgery is advised over prudent resection until an experienced surgical team becomes available.

Hong DF, Liu YB, Peng SY, Pang JZ, Wang ZF, Cheng J, Shen GL, Zhang YB. Management of hepatocellular carcinoma rupture in the caudate lobe. *World J Gastroenterol* 2015; 21(26): 8163-8169 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i26/8163.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i26.8163

INTRODUCTION

Hepatitis B virus (HBV) infection remains a major public health problem with more than 350 million chronic HBV sufferers worldwide[1]. Chronic HBV infection is the cause of approximately one third of all cases of liver cirrhosis and more than three quarters of all hepatocelluar carcinoma (HCC) cases worldwide[2,3]. Longstanding history of hepatitis B infection and liver cirrhosis prior to HCC confirmation comprise the typical pathological process for HCC patients in China.

Spontaneous tumor rupture is one of the most severe complications of HCC. Incidence of rupture is reported at rates varying from 3% to 15% of all HCC cases, with approximately 10% of HCC patients dying of this complication annually in Asia[4]. Spontaneous rupture and hemorrhage of HCC at the caudate lobe is especially rare and catastrophic[5]. Rupture is mainly a consequence of increased tension from tumor progression, central necrosis, malacosis, or liquefaction. Although transarterial embolization (TAE) is the first choice in the treatment algorithm, the outcome is not always satisfactory and hepatectomy cannot be avoided. Management of tumor rupture in the caudate lobe necessitates caudate lobectomy - a technically challenging operation confounded by caudate lobe anatomy and complicated further by intricate vasculature of the main organ[6]. The multitude of branches from both right and left hepatic arteries makes inflow occlusion to the tumor and hemostasis of the caudate lobe equally difficult to achieve. These factors explain the insufficiency and ineffectiveness of TAE, and warrant special considerations for surgical management of these cases.

Caudate lobectomy is the primary salvage treatment for spontaneous HCC rupture in the caudate lobe. Our team has accumulated significant experience with surgical management of the caudate lobe, detailing relevant anatomy and methodology in publication for better global understanding of caudate lobectomy[5]. The cases are sparse, however, leaving few medical centers with necessary exposure. Comprehensive case reporting remains pivotal to systematic validation of surgical treatment. We present our experience with eight cases of spontaneous rupture and hemorrhage of HCC in the caudate lobe, constituting the largest case series reported to date.

MATERIALS AND METHODS

A retrospective study of eight patients presenting with spontaneous rupture and hemorrhage of HCC in the caudate lobe was conducted. Patients were evaluated at admission to our center between January 2010 and July 2013 and were managed surgically by caudate lobectomy. Patient demographics and disease profiles are summarized in Table 1. All patients were male with a median age of 41.5 years (range: 33 to 47 years). Previous interventions included left hepatectomy for HCC resection in one patient two years prior to our involvement, and two cases of prior TAE. Seven patients presented Child-Pugh A liver function, with one patient classified as Child-Pugh C. Portal vein thrombus was absent in all eight patients and no other medical co-morbidities were identified. Seven patients were haemodynamically stable on admission and underwent planned hepatectomy. Emergency surgery was performed on admission in one haemodynamically unstable patient with confirmed rupture of the lesser sac and intra-abdominal bleeding. Postoperative pathology confirmed liver cirrhosis and HCC in all eight subjects.

Table 1 Patient demographics and disease history

Patient number	Age	Gender	Liver function Child-Pugh classification	Hepatitis	Cirrhosis	Preoperative TAE	Intact lesser sac	Intact liver capsule	Tumor size (cm)	Time delayed for operation (d)
1	46	M	A	B	Y	N	Y	Y	7 × 8 × 6	93
2	33	M	C	B	Y	N	N	N	9 × 10 × 9	0.25
3	47	M	A	B	N	N	Y	Y	5 × 4 × 8	1
4	41	M	A	B	Y	Y	Y	Y	3 × 3 × 3	1
5	42	M	A	B	Y	Y	Y	N	10 × 6 × 6	5
6	45	M	A	B	Y	N	Y	Y	7 × 7 × 6	8
7	39	M	A	B	Y	N	Y	N	4 × 5 × 6	6
8	40	M	A	B	Y	N	Y	Y	5 × 6 × 6	5

Table 2 Surgical findings and outcomes

Patient number	Surgical approach	Operating time (min)	Intraoperative blood loss (mL)	Intraoperative blood transfusion (RBC: U; plasma: mL)	Complications	Survival time (mo)
1	Combined complete	210	1000	3.5; 800	N	10
2	Left partial	190	1500	12; 2540	Wound infection	19
3	Combined complete	310	1200	7; 1100	Wound infection	36[1]
4	Combined complete	270	1000	8; 1600	N	84
5	Combined complete	150	300	None	N	7
6	Combined complete	160	500	2; 200	N	Lost to follow-up
7	Combined complete	290	800	3; 400	N	Lost to follow-up
8	Combined complete	120	350	None	N	Lost to follow-up

[1]Patient was lost to follow-up after 3 years. Survival time taken at 36 mo.

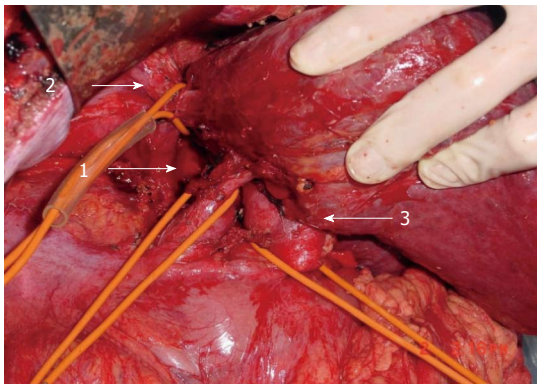

Figure 1 Inflow and outflow control during combined bilateral approach. Control of infrahepatic inferior vena cava (IVC) (arrow 1), suprahepatic IVC (arrow 2), and hepatoduodenal ligament (arrow 3) during bilateral approach for ruptured hepatocellular carcinoma repair.

Surgical procedure

Surgical repair occurred on average 9 d after symptom onset (range, 6 h to 93 d). Tumor size and location dictated the choice of procedure. Isolated caudate lobectomy was performed in seven cases. Patient 2 underwent partial caudate lobectomy. A reversed L-shaped incision was made in seven cases. A roof incision overlaying the existing incision line was made in the patient with a prior history of hepatectomy.

Procedure details are summarized in Table 2. The liver was mobilized by dissection of hepatic ligaments. The hilum was exposed and hepatoduodenal ligament was encircled with a No. 8 urine catheter in preparation for Pringle's maneuver. Infrahepatic inferior vena cava (IVC) was exposed by parting overlying retroperitoneum and encircled with an additional urine catheter in preparation for inflow and outflow control (Figure 1). Both controls were applied immediately if the lesser sac was found damaged and active bleeding was confirmed. Dissection was then performed along the anterior surface of the retrohepatic IVC. To facilitate this process, several short hepatic veins draining the caudate lobe were identified, ligated, and divided. Left hepatic lobe was thus mobilized and rotated to the right. With the caudate process exposed, the hepatoduodenal ligament became available for mobilization to the right, revealing the portal triad of the caudate lobe and opening it to transection. The stump was closed with 4-0 prolene suture. Attention was then shifted to short hepatic veins supplying the IVC from the caudate lobe. Both were ligated and divided, marking separation and cauterization of the connection between right and left portions of the liver. Finally, the caudate lobe containing the tumor became open to resection (Figure 2).

Resection was completed under intermittent inflow occlusion with Pringle's maneuver. Occlusion time limit was 10 min with 2 min reperfusion. The liver parenchyma was transected by means of curettage and aspiration[1] using Peng's Multifunctional Operative Dissector (PMOD). Figure 3 shows the anatomy of caudate lobe fossa and IVC. The abdomen was irrigated with large volumes of hypoosmotic fluids. One gram of 5-fluorouracil (5-FU) was administered intra-

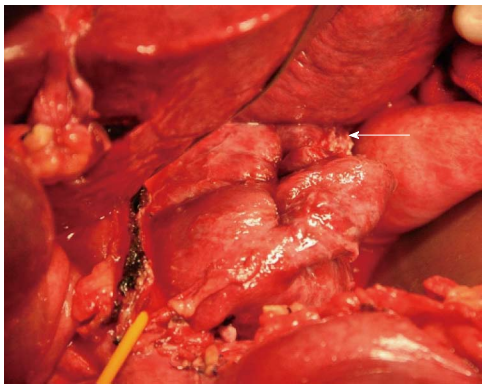

Figure 2 Rupture site access during left-sided approach. Left-sided approach for ruptured hepatocellular carcinoma repair in Spiegel lobe. Rupture site is shown (white arrow).

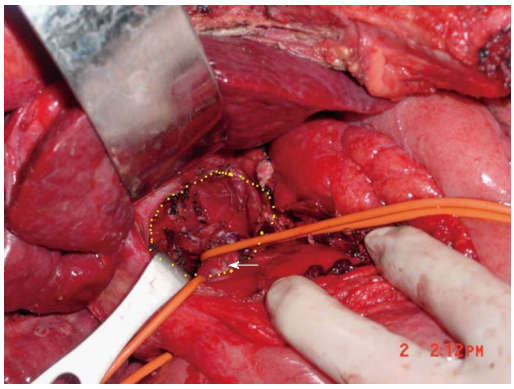

Figure 3 Anatomy of caudate lobe fossa and inferior vena cava. Caudate lobe fossa (circumscribed by yellow dotted zone) and inferior vena cava (arrow) following removal of caudate lobe.

abdominally. Two to three abdominal drainages were placed for each patient and subsequently removed 3 to 5 d following confirmation by abdominal ultrasound.

RESULTS

All eight patients underwent caudate lobectomy between 0.25 and 9 d following diagnosis of HCC rupture. Median operating time was 200 min (range: 120 to 310 min) with a median blood loss of 900 mL (range: 300 to 1500 mL). Two to four RBC units and 400 mL plasma were transfused. Surgical findings are detailed in Table 2. Three cases were characterized by an intact lesser sac in the presence of ruptured hepatic capsule. Resected specimens showed blood clots and caudate lobe tissue resembling that of a compromised lesser sac. Encapsulated clot and liver tissue samples were obtained from the caudate lobe in patients presenting intact hepatic capsules. All patients underwent transarterial chemoembolization (TACE) postoperatively as necessitated by liver function, presence of recurrence on radiologic findings, and serum AFP.

Zero mortality was observed in the early postoperative period (Table 2). Postoperative complications were limited to two incidences of incision infection. Patients 6-8 became unavailable for further postoperative follow-up.

Two patients expired in the course of long-term follow-up. Patient 1 underwent gastric endoscopy 9 mo after caudate lobectomy due to upper gastrointestinal bleeding. Patient 4 was treated conservatively for a month until death due to tumor metastasis. Patient 4 developed multiple organ metastases; brain metastasis was found 4 years after caudate lobectomy and treated with gamma knife therapy, succeeded by a liver recurrence 2 years later and treated with TACE. Patient 3 had a recurrence of HCC on the left liver 8 mo after the operation, receiving RF treatment and ultimately becoming lost to follow-up at 3 years.

Two patients remain in follow-up. Patient 2 was found with a mass in the perigastric region just lateral to the liver at 8 mo postoperatively. The mass was removed by reoperation revealing multiple intraabdominal and abdominal wall metastases. Pathology confirmed metastatic infiltrative HCC. This individual received regular chemotherapy and one TACE treatment. Tumor progression was noted with a growing number of metastatic lymph nodes and portal tumor thrombi. Patient 5 is currently on the 7th mo of postoperative observation, having undergone two TACE treatments during that time. Patient's recent CT and laboratory findings show no recurrence or metastasis.

DISCUSSION

While HCC occurrence in the caudate lobe is not uncommon[7,8], rupture of the tumor is exceptionally rare. The diagnosis is not difficult, typically characterized by clinical symptoms of sudden-onset upper abdominal pain and imaging revealing hematoma lesion in the lesser sac tracing from the caudate lobe[9]. TAE is recommended as initial treatment. However, its efficacy suffers tremendously from the multitude of venous branches draining the caudate lobe and its proximity to major vessels[5,10].

The caudate lobe of the liver is notable for its anatomic location - easily identified as the segment straddling right and left lobes of the liver[11] and fundamentally difficult to approach surgically. It lies between major vascular structures - the IVC posteriorly, the portal triads inferiorly, and the hepatic venous confluence superiorly[12], - all carrying great volume and posing tremendous danger during the operation. These anatomic and physiologic circumstances render caudate lobectomy a formidable technical challenge, and demand substantial experience to guarantee safe surgical management. As a major regional institution, our center treats numerous cases of HCC rupture and accepts transfers from outside hospitals, affording our team critical exposure to this scarce population. A comprehensive text was published introducing our findings and providing a detailed account of surgical

interventions for the hepatic caudate lobe[6]. While the body of experience and the volume of published work detailing tumor rupture management have earned our team expert recognition in this methodology, continued discussion of notable surgical cases will inevitably advance global understanding of caudate lobectomy.

Caudate lobectomy remains the salvage procedure in many cases following TAE failure[5] despite its technical challenges, associated mortality rate of 5.3%-14%[6], and inadvertent lack of surgeons wielding necessary expertise. Since the caudate lobe resides within the lesser sac, post tumor rupture hemorrhage is contained by the lesser sac for as long as it remains intact. The bleeding of ruptured tumors in the caudate lobe thus becomes a secondary problem, not subject to immediate nor aggressive management. This explains the relative haemodynamic stability in seven cases observed at the time of admission to our center. Once the lesser sac does undergo rupture, the emergent bleeding becomes fatal if it is not controlled.

All eight cases in this series were initially haemodynamically stable as confirmed by imaging studies and intraoperative findings due to anatomically benevolent circumstances. One patient presenting for emergent surgical management at transfer to our hospital delivers a comprehensive lesson in management of HCC rupture in the caudate lobe. His clinical course vividly demonstrates the role of proper treatment algorithm, timing, and surgical specialization in the setting of a rare medical catastrophe. This individual initially presented in stable condition to a local hospital with the diagnosis of HCC rupture in the caudate lobe confirmed by CT scan, as attributable to the intact lesser sac. Transfer to another medical center for further management entailed an 8 h transit by train and immediate surgical intervention was necessary at the time of arrival. Unfortunately, the admitting surgeon was not sufficiently experienced in caudate lobectomy and the IVC was accidently injured. Massive hemorrhage resulted in hemorrhagic shock, driving the decision for fast track surgery. A local gauze packing was applied and the patient was temporarily stabilized for transfer to our center. Emergency surgery was performed immediately on admission to our hospital and the patient was successfully repaired. Since the start of the second operation, the patient sustained three instances of cardiac arrest and massive blood loss. Another notable issue in this patient's course is the prompt recovery of liver function despite preoperative classification as Child-Pugh C. This is largely attributable to low serum protein level caused by sustained hemorrhage.

Optimal timing for caudate lobectomy following HCC rupture in the caudate lobe is subject to critical evaluation. While the lesser sac is intact, conservative treatment is preferred until an experienced surgical team becomes available. However, in emergency conditions instigated by lesser sac rupture, ongoing uncontrolled hemorrhage, and unsuccessful TAE, fast track surgery must be considered even if a specialized hepatobiliary surgeon is unavailable. In this series, every patient undergoing caudate lobectomy at our center had previously undergone surgical intervention that should have been avoided. Nevertheless, secondary fast track surgical strategy proved to be an appropriate and necessary salvage choice.

Four types of lobectomy approaches have been described[13-19]: Left-sided, right-sided, anterior transhepatic, and bilateral, combining left and right-sided approaches. In this series, left-sided approach was taken in the case of ruptured HCC in Spiegel lobe (Figure 2). This patient underwent isolated partial caudate lobectomy. Bilateral approach was elected in seven cases for an isolated complete caudate lobectomy. Anterior transhepatic approach was unnecessary as the tumors were not exceedingly large.

As is ubiquitous to all oncology practices, surgical outcomes correlate closely with several patient-specific variables. Physiological profile of HCC and anatomical complexity of the caudate lobe put this patient population at a particularly high risk of postoperative recurrence. An accurate postoperative prognosis is therefore difficult to deliver, and the disease often warrants continued aggressive treatment following removal of the ruptured tumor.

An intact lesser sac may prevent peritoneal dispersion of ruptured HCC. Among cases treated at our center, the patient whose lesser sac did rupture was found to have a recurrence at the original tumor site 8 mo postoperatively. Analogously, the patient with a prior history of HCC and left hepatectomy presenting to us with a tumor recurrence at the caudate lobe appreciated the shortest survival time of 10 mo. The patient with the longest survival (84 mo) had the smallest caudate lobe tumor in the series, measuring 3 cm^3. He underwent six TACE treatments following the first surgery and seven subsequent rounds of radiotherapy for brain metastasis spanning 48 and 80 mo, respectively. Three patients in this series expired in the course of postoperative treatment. Long-term evaluation of the remaining five patients revealed metastasis or recurrence. These patients underwent TACE postoperatively on a minimum of two occasions.

This case series underscores another notable issue: the prevalence of hepatitis B infection and liver cirrhosis in patients prior to HCC confirmation. This is a typical pathological process for HCC patients in China[20-27]. Impaired liver function and possible hypersplenism secondary to cirrhotic liver are evident in these cases. Resultant coagulopathy predisposes to uncontrolled bleeding and multiplies the risks and technical demands of surgery[28]. In this report, all patients presented abnormalities in coagulation function preoperatively. The median blood loss was 900 mL (range: 300-1500 mL) - a relatively large volume. The fact that minimal blood loss closely correlates with better HCC prognosis augments the challenges of surgical involvement in the caudate lobe. However, in the context of varying time from symptom

onset to surgical repair, these data are revealed to be less influential to surgical decision making and prognosis.

The experience summarized in this report constitutes the largest case series presented to date. Published findings regarding caudate lobectomy are sparse[6,29] relative to publications depicting other types of hepatectomy[30]. While our group has accumulated significant experience with caudate lobectomy and detailed relevant anatomy and techniques, surgical management of HCC tumor rupture in the caudate lobe remains an isolated domain. Equally obscure, caudate lobe rupture of non-oncologic etiology presents another extreme for the application of caudate lobectomy techniques, as do giant caudate lobe tumors. Clinical considerations and intraoperative recommendations applicable to HCC rupture in the caudate lobe may be extended to the management of these rare and complex cases. For improved understanding and mastery of caudate lobectomy, the efforts to document these cases must be continued.

Caudate lobectomy is the salvage choice for management of spontaneous rupture and hemorrhage of HCC in the caudate lobe. Anatomic and physiologic circumstances render caudate lobectomy a remarkable technical challenge. Delayed surgery is advised over prudent resection until an experienced surgical team becomes available. Hepatitis infection, cirrhosis, and HCC recurrence can be expected to compound the treatment algorithm and postoperative course. Sustained blood loss should be considered as the underlying cause of low serum protein in the evaluation of liver function.

The majority of reviewed cases presenting with HCC rupture in the caudate lobe demonstrated no hemodynamic instability and were managed conservatively until caudate lobectomy was performed by an experienced hepatobiliary surgeon. Based on limited long-term follow-up data, overall 1-year and 3-year survival rates have been achieved.

COMMENTS

Background

Spontaneous rupture and hemorrhage of the hepatocellular carcinoma (HCC) at the caudate lobe is especially rare and catastrophic.

Research frontiers

Management of spontaneous rupture and hemorrhage of HCC is seldom reported due to its rareness and severity.

Innovations and breakthroughs

The authors present the experience with eight cases of spontaneous rupture and hemorrhage of the HCC in the caudate lobe, constituting the largest case series reported to date. This experience shows that caudate lobectomy is a valid treatment as well as the salvage choice for management in such cases based on our experience with the largest case series reported to date. Based on limited long-term follow-up data, overall 1-year and 3-year survival rates have been achieved. In addition, delayed surgery is advised over prudent resection until an experienced surgical team becomes available.

Applications

This article provides important issues to consider in the situation of such cases. Among the advices, postponement of surgical intervention while the rupture remains hemodynamically stable until an experienced surgeon becomes available, is the key suggested principle.

Peer-review

The authors present a case series on a rare clinical condition, the management of which cannot be examined in higher-level studies because of low incidence. The approach they suggest is well accepted and is presented with all necessary details. The paper is well written and provides a sound basis for further discussion.

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ORIGINAL ARTICLE

Observational Study

Japanese apricot improves symptoms of gastrointestinal dysmotility associated with gastroesophageal reflux disease

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Informed consent statement: Written, informed consent was obtained from all participants.

Conflict-of-interest statement: There is no conflict of interest in this study.

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Abstract

AIM: To investigate the effects of Japanese apricot (JA) consumption on gastroesophageal reflux disease (GERD)-related symptoms.

METHODS: Participants included individuals living in Minabe-cho, a well-known JA-growing region, who received specific medical check-ups by the local community health service in 2010. GERD-related symptoms were examined in 1303 Japanese individuals using a validated questionnaire, the Frequency Scale for Symptoms of GERD (FSSG), which consists of 7 questions associated with acid reflux symptoms and 5 questions asking about gastrointestinal dysmotility symptoms. Each question was answered using a 4-point scale, with higher scores indicating more severe GERD-related symptoms. Subjects were divided into two groups according to their intake of dried and pickled JA: daily intake (≥ 1 JA daily) (392 subjects) and none or

occasional intake (< 1 JA daily) (911 subjects). FSSG scores were compared between subjects who consumed JA daily and those who did not. Next, subjects were stratified by age, gender and *Helicobacter pylori* (*H. pylori*) status for subanalyses.

RESULTS: Those who ate JA daily were significantly older than those who did not (60.6 ± 10.5 years *vs* 56.0 ± 11.0 years, $P < 0.001$). Total FSSG scores were significantly lower in subjects with daily JA intake than in those with none or only occasional intake (2.13 ± 3.14 *vs* 2.70 ± 3.82, $P = 0.005$). In particular, subjects who consumed JA daily showed significantly improved FSSG dysmotility scores compared with subjects who did not (1.05 ± 1.58 *vs* 1.46 ± 2.11, $P < 0.001$). In contrast, the FSSG reflux score did not differ between subjects with and without daily intake of JA (1.08 ± 1.90 *vs* 1.24 ± 2.11, $P = 0.177$). Subanalysis indicated that improvement in dysmotility by JA intake was specifically observed in non-elderly (1.24 ± 1.68 *vs* 1.62 ± 2.22, $P = 0.005$) and *H. pylori*-negative subjects (0.99 ± 1.58 *vs* 1.57 ± 2.06, $P < 0.001$). GERD patients (total FSSG score ⩾ 8) were less frequently observed among subjects with daily intake of JA as compared to those without daily intake of JA (6.1% *vs* 9.7%, $P = 0.040$).

CONCLUSION: Daily JA intake may improve digestive dysmotility symptoms, resulting in relief of GERD symptoms. The effect is more obvious in non-elderly and *H. pylori*-negative subjects.

Key words: Japanese apricot; *Umeboshi*; Gastroesophageal reflux disease; Frequency Scale for Symptoms of gastroesophageal reflux disease; Dysmotility

Core tip: Japanese apricot (JA), which is eaten as a daily food in Japan, is considered to have medicinal benefits. We focused on the effect of JA on gastroesophageal reflux disease (GERD)-related symptoms in this study. First, GERD-related symptoms were examined in 1303 Japanese individuals using the validated questionnaire, the Frequency Scale for Symptoms of GERD (FSSG). JA was consumed daily by 392 subjects. Then, FSSG scores were compared between subjects who took one or more JA daily and those who did not. This study indicates that daily intake of JA improves digestive dysmotility symptoms, resulting in relief of GERD-related symptoms.

Maekita T, Kato J, Enomoto S, Yoshida T, Utsunomiya H, Hayashi H, Hanamitsu T, Inoue I, Maeda Y, Moribata K, Muraki Y, Shingaki N, Deguchi H, Ueda K, Iguchi M, Tamai H, Ichinose M. Japanese apricot improves symptoms of gastrointestinal dysmotility associated with gastroesophageal reflux disease. *World J Gastroenterol* 2015; 21(26): 8170-8177 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i26/8170.htm

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INTRODUCTION

The Japanese apricot (JA) ("*ume*" in Japanese; *Prunus mume*, Siebold et Zucc.) enjoys great popularity in Japan. Since ancient times, the JA has been processed into a dried and pickled form ("*umeboshi*"), and made into liquor and soft drinks. These products have been known to possess various medicinal benefits and have been frequently prescribed as a traditional folk remedy. JA has been reported to possess such beneficial biological activities as improving blood fluidity[1], anti-fatigue effect[2], protection from the human influenza A virus[3], and anti-cancer effect[4]. The biological basis of the efficacy of JA is partly attributable to anti-oxidative and free radical scavenging activities[5], and partly to immune enhancement[6,7].

In Eastern countries, although JA is widely believed to improve various gastrointestinal disorders, including gastrointestinal motility, dyspepsia and gastrointestinal infections, the effect of JA on the gastrointestinal system has scarcely been evaluated in a scientific manner. We previously reported that JA intake exerted a preventive effect on chronic atrophic gastritis by inhibiting *Helicobacter pylori* (*H. pylori*) infection and reducing mucosal inflammation[8]. Meanwhile, animal models have suggested that JA or the alimentary fiber provided by it enhances gastrointestinal motility. JA showed laxative effects in the low-fiber diet-induced constipation rat model[9], and the increased alimentary fiber resulting from JA ingestion produced fecal lipid excretion effects and feces bulking effects in adult mice by inducing changes in the composition of intestinal flora[10].

Gastroesophageal reflux disease (GERD) is a common disease in Western countries. Recently, the number of GERD patients has also been increasing in Japan, owing to the Westernization of diets and the decrease in prevalence of *H. pylori* infection. The main symptoms of GERD can be attributed to reflux of gastric acid and gastrointestinal dysmotility. Subjects with GERD often get heartburn, an unusual sensation in the throat, and bitter liquid coming up into the throat due to reflux of gastric acid, with or without esophageal mucosal injury. Furthermore, they often feel full, sick or heavy during and after meals, and often burp due to gastrointestinal dysmotility[11]. The traditional Japanese folk remedy of ingestion of JA is probably based on its effects on gastric acid secretion and gastrointestinal motility, resulting in improvement of GERD symptoms.

In this study, therefore, we investigated and compared GERD symptoms between Japanese inhabitants who took and did not take JA daily, using a questionnaire that can quantify the severity of GERD

FSSG Questionnaire

Do you have any of the following symptoms? If so, please circle the appropriate response below.

Question		Frequency				
		Never	Occasionally	Sometimes	Often	Always
Q1	Do you get heartburn?	0	1	2	3	4
Q2	Does your stomach get bloated?	0	1	2	3	4
Q3	Does your stomach ever feel heavy after meals?	0	1	2	3	4
Q4	Do you sometimes subconsciously rub your chest with your hand?	0	1	2	3	4
Q5	Do you ever feel sick after meals?	0	1	2	3	4
Q6	Do you get heartburn after meals?	0	1	2	3	4
Q7	Do you have an unusual (*e.g.*, burning) sensation in your throat?	0	1	2	3	4
Q8	Do you feel full while eating meals?	0	1	2	3	4
Q9	Do some things get stuck when you swallow?	0	1	2	3	4
Q10	Do you get bitter liquid (acid) coming up into your throat?	0	1	2	3	4
Q11	Do you burp a lot?	0	1	2	3	4
Q12	Do you get heartburn if you bend over?	0	1	2	3	4

Figure 1 Frequency Scale for Symptoms of gastroesophageal reflux disease questionnaire. Of the 12 FSSG questions, 7 questions (Q1, Q4, Q6, Q7, Q9, Q10, and Q12; shaded lines) deal with acid reflux symptoms, and the other 5 questions (Q2, Q3, Q5, Q8, and Q11, white lines) ask about dysmotility symptoms. FSSG: Frequency Scale for Symptoms of gastroesophageal reflux disease.

symptoms and differentiate between acid-related symptoms and dysmotility-related symptoms.

MATERIALS AND METHODS

Subjects and study design

Participants in this study included adult individuals living in Minabe-cho, Wakayama Prefecture, a well-known JA-growing region, who received specific medical check-ups provided by the local community health service. In Minabe-cho, specific medical check-ups are performed every year for all inhabitants aged 35 years or older. To remind people about the medical check-up program, a guidance postcard is sent from the local community health service to each subject once a year. In Japan, these types of health check-up programs are performed to detect diseases, including cancer, at an early stage. Therefore, subjects who had specific serious symptoms requiring medical care were excluded from the program.

The participation rate in the check-up program in Minabe-cho town in 2010 was about 36%, and a total of 1902 subjects underwent the check-ups. The participants received the following tests and procedures: physical examination, chest X-ray, electrocardiogram, blood laboratory tests, including *H. pylori* IgG antibody titer, urinalysis, upper gastrointestinal barium X-ray examination, and a fecal occult blood test. *H. pylori* IgG antibody titers were measured by ELISA (SRL Inc., Tokyo, Japan). Subjects with *H. pylori* antibody titers $\geqslant$ 10 U/mL were considered positive for *H. pylori* infection and those with titers < 10 U/mL were considered negative.

The subjects also completed a self administered questionnaire and an interview to determine general health status. The questionnaire included an assessment of the daily intake of dried and pickled JA or processed JA, the frequency scale for symptoms of GERD (FSSG)[11], history of therapy for eradication of *H. pylori*, regular medication, and past history of diseases affecting the gastrointestinal system. Subjects were divided into two groups according to the intake of dried and pickled JA: daily intake ($\geqslant$ 1 JA daily) and none or occasional intake (< 1 JA daily).

Subjects who had a previous history of surgical resection of gastrointestinal cancer (16 subjects), or *H. pylori* eradication (167 subjects), and those who had been prescribed a proton pump inhibitor (PPI) or other digestive medicines (167 subjects) that might affect gastrointestinal function were excluded from the study. Those who did not reply to the FSSG questionnaire (12 subjects) were also excluded. In addition, subjects who regularly took processed JA, for example, JA extract, powdered JA or JA juice, other than dried and pickled JA, were excluded (321 subjects) to avoid confounding by additional intake of JA ingredients. Thus, a total of 1303 subjects (589 males and 714 females) were analyzed in this study. The ethics committee of Wakayama Medical University approved the study protocol. Written, informed consent was obtained from all participants.

Assessment of the FSSG questionnaire and the definition of GERD

The FSSG questionnaire for GERD-related symptoms, which was developed by Kusano *et al*[11] in Japan (Figure 1), was evaluated. The questionnaire consists of 12 questions regarding GERD-related symptoms. Of the 12 FSSG questions, 7 questions (Q1, Q4, Q6, Q7, Q9, Q10, and Q12) are related to acid reflux symptoms, while the remaining 5 questions (Q2, Q3, Q5, Q8, and Q11) pertain to gastrointestinal dysmotility symptoms. Patients assigned each question 0 (never), 1 (occasionally), 2 (sometimes), 3 (often), or 4 (always) points. Acid reflux-related, gastrointestinal dysmotility-related, and total scores were calculated by adding the points for the 7, 5 and 12 questions, respectively. GERD was defined as a total score $\geqslant$ 8, which is the

Table 1 Characteristics and Frequency Scale for Symptoms of gastroesophageal reflux disease scores of subjects on the basis of Japanese apricot intake

	Daily intake (≥ 1 JA daily)	None or occasional (< 1 JA daily)	*P* value
n	392	911	
Age (yr)	60.6 ± 10.5	56.0 ± 11.0	< 0.001
Sex (male/female)	201/191	388/523	0.004
Helicobacter pylori infection rate	203 (51.8%)	451 (49.5%)	0.469
FSSG			
Total score	2.13 ± 3.14	2.70 ± 3.82	0.005
Acid reflux score	1.08 ± 1.90	1.24 ± 2.11	0.177
Dysmotility score	1.05 ± 1.58	1.46 ± 2.11	< 0.001
Total FSSG score ≥ 8	24 (6.1%)	88 (9.7%)	0.040

Data are expressed as mean ± SD. JA: Japanese apricot; FSSG: Frequency Scale for Symptoms of gastroesophageal reflux disease.

Table 2 Scores of each Frequency Scale for Symptoms of gastroesophageal reflux disease question on the basis of Japanese apricot intake

	Daily intake (≥ 1 JA daily)	None or occasional (< 1 JA daily)	*P* value
n	392	911	
Q1 score	0.30 ± 0.62	0.33 ± 0.66	0.369
Q2 score	0.31 ± 0.63	0.44 ± 0.75	0.002
Q3 score	0.18 ± 0.50	0.28 ± 0.62	0.001
Q4 score	0.10 ± 0.37	0.13 ± 0.44	0.182
Q5 score	0.08 ± 0.32	0.13 ± 0.41	0.036
Q6 score	0.19 ± 0.49	0.19 ± 0.49	0.865
Q7 score	0.10 ± 0.43	0.11 ± 0.42	0.845
Q8 score	0.11 ± 0.38	0.23 ± 0.61	< 0.001
Q9 score	0.11 ± 0.41	0.14 ± 0.44	0.436
Q10 score	0.18 ± 0.43	0.24 ± 0.52	0.043
Q11 score	0.37 ± 0.70	0.38 ± 0.75	0.792
Q12 score	0.09 ± 0.36	0.11 ± 0.40	0.537

Data are expressed as mean ± SD. JA: Japanese apricot; FSSG: Frequency Scale for Symptoms of gastroesophageal reflux disease.

recommended cut-off FSSG score for GERD[11,12].

Statistical analysis

The data are expressed as mean ± SD. Data were analyzed using the unpaired *t*-test and Fisher's exact text. The level of statistical significance was $P < 0.05$. All analyses were performed using the SPSS 21.0 software package (SPSS Inc., Chicago, IL, United States).

RESULTS

Upper gastrointestinal symptoms according to intake of JA

A total of 1303 subjects (589 men and 714 women, mean age 57.4 (34-79) years old) received the medical check-up and answered the questionnaire. These subjects were analyzed according to intake of JA: daily intake versus none to occasional intake. Of the 1303 subjects, 392 (30%) were categorized into the daily intake group, while 911 (70%) were included in the none or occasional group. The characteristics of subjects in each group are summarized in Table 1. Those who ate one or more JA daily were significantly older than those who did not (60.6 ± 10.5 years *vs* 56.0 ± 11.0 years, $P < 0.001$). In addition, the proportion of male subjects was higher in the daily intake group.

The total FSSG score was significantly lower in subjects with daily intake of JA than in those with none or occasional intake (2.13 ± 3.14 *vs* 2.70 ± 3.82, $P = 0.005$). In particular, those with daily JA intake had significantly better FSSG dysmotility scores than subjects who did not take JA daily (1.05 ± 1.58 *vs* 1.46 ± 2.11, $P < 0.001$). In contrast, the FSSG acid reflux score did not differ between subjects with and without daily intake of JA (1.08 ± 1.90 *vs* 1.24 ± 2.11, $P = 0.177$). GERD patients (total FSSG score ≥ 8) were less frequently observed among subjects with daily intake of JA as compared to those without (6.1% *vs* 9.7%, $P = 0.040$) (Table 1).

Table 2 shows the scores for each question according to JA intake. Significant differences were observed in Q2, Q3, Q5, Q8, and Q10 between subjects with and without daily intake of JA. Four of these 5 questions relate to dysmotility symptoms.

Subgroup analysis

Since there was a significant difference in intake of JA according to age and gender, subanalyses were stratified by these factors (Tables 3 and 4). In both male and female subjects, FSSG dysmotility scores were significantly lower in subjects with daily intake of JA than those without (male: 1.00 ± 1.45 *vs* 1.33 ± 1.99, $P = 0.021$, female: 0.76 ± 1.38 *vs* 0.95 ± 1.65, $P = 0.005$). In contrast, the FSSG acid reflux score did not differ between subjects with and without daily intake of JA (male: 1.01 ± 1.70 *vs* 1.14 ± 1.89, $P = 0.434$, female: 1.14 ± 2.08 *vs* 1.32 ± 2.27, $P = 0.338$).

FSSG dysmotility scores in the non-elderly (34-64 years) subgroup of subjects with daily intake of JA were significantly lower than those of subjects without daily intake of JA (1.24 ± 1.68 *vs* 1.62 ± 2.22, $P = 0.005$), while in the elderly subgroup (65-79 years), the differences were not significant (0.76 ± 1.38 *vs* 0.95 ± 1.65, $P = 0.268$). The FSSG acid reflux scores, on the other hand, did not differ between subjects with and without daily intake of JA regardless of age. These results suggest that JA intake improves upper gastrointestinal dysmotility in both men and women, specifically in non-elderly subjects.

Since *H. pylori* infection may affect digestive symptoms, subanalysis stratified by *H. pylori* infection status was also performed (Table 5). Significantly lower total FSSG scores and FSSG dysmotility scores were seen in subjects with daily intake of JA who were negative for *H. pylori* infection (total FSSG score: 2.03 ± 2.99 *vs* 2.80 ± 3.69, $P = 0.006$, FSSG dysmotility

Table 3 Characteristics and Frequency Scale for Symptoms of gastroesophageal reflux disease scores of subjects on the basis of Japanese apricot intake stratified by sex

	Male		*P* value	Female		*P* value
	Daily intake (≥ 1 JA daily)	None or occasional (< 1 JA daily)		Daily intake (≥ 1 JA daily)	None or occasional (< 1 JA daily)	
n	201	388		191	523	
Age (yr)	60.0 ± 10.8	56.2 ± 11.6	< 0.001	61.2 ± 10.1	55.8 ± 10.5	< 0.001
Helicobacter pylori infection rate	103 (51.2)	192 (49.5)	0.728	100 (52.4)	259 (49.5)	0.554
FSSG						
Total score	2.01 ± 2.86	2.47 ± 3.53	0.091	2.26 ± 3.41	2.88 ± 4.01	0.057
Acid reflux score	1.01 ± 1.70	1.14 ± 1.89	0.434	1.14 ± 2.08	1.32 ± 2.27	0.338
Dysmotility score	1.00 ± 1.45	1.33 ± 1.99	0.021	0.76 ± 1.38	0.95 ± 1.65	0.005
Total FSSG score ≥ 8	9 (4.5)	34 (8.8)	0.066	15 (7.9)	54 (10.3)	0.391

Data are expressed as mean ± SD or *n* (%). JA: Japanese apricot; FSSG: Frequency Scale for Symptoms of gastroesophageal reflux disease.

Table 4 Characteristics and Frequency Scale for Symptoms of gastroesophageal reflux disease scores of subjects on the basis of Japanese apricot intake stratified by age

	Non-elderly (34-64 yr)		*P* value	Elderly (65-79 yr)		*P* value
	Daily intake (≥ 1 JA daily)	None or occasional (< 1 JA daily)		Daily intake (≥ 1 JA daily)	None or occasional (< 1 JA daily)	
n	239	692		153	219	
Age (yr)	54.0 ± 7.7	51.5 ± 8.5	< 0.001	70.9 ± 3.7	70.2 ± 3.7	0.052
Sex (male/female)	127/112	280/412	0.001	74/79	108/111	0.916
Helicobacter pylori infection rate	129 (54.0)	331 (47.8)	0.151	74 (48.4)	120 (54.8)	0.246
FSSG						
Total score	2.50 ± 3.27	2.93 ± 3.96	0.095	1.56 ± 2.82	1.98 ± 3.21	0.191
Acid reflux score	1.26 ± 1.99	1.31 ± 2.17	0.748	0.79 ± 1.70	1.03 ± 1.91	0.211
Dysmotility score	1.24 ± 1.68	1.62 ± 2.22	0.005	0.76 ± 1.38	0.95 ± 1.65	0.268
Total FSSG score ≥ 8	18 (7.5)	77 (11.1)	0.136	6 (3.9)	11 (5.0)	0.802

Data are expressed as mean ± SD or *n* (%). JA: Japanese apricot; FSSG: Frequency Scale for Symptoms of gastroesophageal reflux disease.

Table 5 Characteristics and Frequency Scale for Symptoms of gastroesophageal reflux disease scores of subjects on the basis of Japanese apricot intake stratified by *Helicobacter pylori* infection status

	Helicobacter pylori infection					
	Negative			Positive		
	Daily intake (≥ 1 JA daily)	None or occasional (< 1 JA daily)	*P* value	Daily intake (≥ 1 JA daily)	None or occasional (< 1 JA daily)	*P* value
n	189	460		203	451	
Age (yr)	60.1 ± 11.5	54.0 ± 11.6	< 0.001	61.0 ± 9.3	58.1 ± 10.0	< 0.001
Sex (male/female)	98/91	196/264	0.037	103/100	192/259	0.062
FSSG						
Total score	2.03 ± 2.99	2.80 ± 3.69	0.006	2.22 ± 3.27	2.60 ± 3.94	0.202
Acid score	1.04 ± 1.80	1.23 ± 2.07	0.251	1.11 ± 1.99	1.25 ± 2.16	0.435
Dysmotility score	0.99 ± 1.58	1.57 ± 2.06	< 0.001	1.11 ± 1.58	1.35 ± 2.16	0.116
Total FSSG score ≥ 8	11 (5.8)	42 (9.1)	0.206	13 (6.4)	46 (10.2)	0.140

Data are expressed as mean ± SD or *n* (%). JA: Japanese apricot; FSSG: Frequency Scale for Symptoms of gastroesophageal reflux disease.

score: 0.99 ± 1.58 *vs* 1.57 ± 2.06, $P < 0.001$). In contrast, in *H. pylori*-positive subjects, there was no significant difference in either total FSSG or dysmotility scores between subjects with and without daily intake of JA.

DISCUSSION

In this study, using a questionnaire, we examined GERD-related symptoms with respect to the intake of JA in a Japanese community cohort. Subjects who took JA daily were less likely to experience GERD-related symptoms than those who did not take JA daily. In addition, there were fewer GERD patients among subjects who took JA daily than those who did not. Moreover, the lower incidence of GERD-related symptoms in subjects with daily intake of JA was mainly attributable to reduced dysmotility of the upper

gastrointestinal system. These results suggest that JA facilitates gastric motility and that daily intake of JA can relieve GERD-related symptoms, resulting in improvement in the patients' quality of life.

The JA tree is a fruit-bearing species belonging to the genus *Prunus*. Dried and pickled JA, called "*umeboshi*" in Japanese, is a typical traditional Japanese pickled food, which is made from ripened JA pickled with salt. The taste of *umeboshi* is extremely sour and salty, and it is usually eaten with rice. This traditional Japanese pickle is considered to have antibacterial activity, and to cause facilitation of salivary secretion and digestion in the gastrointestinal system[1,13-15].

The improvement in gastric motility resulting from intake of JA observed in this study can be attributed to several mechanisms. First, the high acidity of JA may be responsible for this beneficial effect. Dried and pickled JA contains strong acids, including citric acid and malic acid. A previous report showing that JA has laxative effects in a low-fiber diet-induced constipation rat model[9] indicated that citric acid and malic acid accelerate spontaneous contraction of the isolated rat colon. In this regard, such strong acid components may affect gastrointestinal motility. Second, the increased alimentary fiber due to ingestion of JA could also reduce dysmotility. Alimentary fiber increases fecal output and improves gastrointestinal motility by promoting gastrointestinal tract emptying[16-18]. In addition, it has been reported that the fiber contained in JA changes the composition of the intestinal flora, and possesses fecal lipid excretion effects and feces bulking effects in adult mice[10]. Gastrointestinal dysmotility is related to the type of intestinal flora[19,20], and the change in the composition of intestinal flora induced by the fiber in JA may improve gastrointestinal motility. Finally, when we ingest or imagine sour foods, secretion of saliva and gastric juice is facilitated. In this regard, the sour taste of JA, largely resulting from the citric acid content, can induce saliva secretion[14,15,21]. Saliva helps chewing, initial swallowing, and absorption of food particles[22]; hence, increased saliva secretion induced by the sour taste of JA can improve dysmotility.

Daily intake of JA did not improve GERD-related acid reflux symptoms in our study. In addition, improvement of gastric dysmotility by JA intake was observed only in *H. pylori*-negative subjects. Previously, we reported that JA intake inhibits *H. pylori* infection and reduces active mucosal inflammation[8]. *H. pylori* has the ability to neutralize gastric acid, and therefore, suppression of the activity of *H. pylori* by intake of JA may improve gastric-acid secretion. Conversely, the anti-*H. pylori* effect of JA may worsen acid reflux symptoms in *H. pylori*-positive subjects, although the effect of *H. pylori* suppression on GERD is not well established[23,24]. Meanwhile, gastric emptying is reportedly significantly slower in *H. pylori*-positive patients than *H. pylori*-negative patients[25]. Absence of improvement in dysmotility by JA intake in *H. pylori*-positive subjects suggests that the effect of JA on gastric dysmotility may not overcome the negative effects of *H. pylori* infection on gastric motility.

Daily intake of JA improved dysmotility symptoms in both men and women. In contrast, improvement of dysmotility by JA intake was seen only in non-elderly subjects. One of the reasons for absence of the effect of JA in elderly subjects may be impaired visceral sensations due to aging[26]. Hence, the effect of JA on improvement of gastrointestinal motility may not surpass the depression of gastrointestinal motility due to aging.

This study has several limitations. First, because JA is considered to be a "healthy" food with curative or beneficial effects, it is possible that those who have a higher intake of JA may also be more likely to have a healthier lifestyle. Such lifestyles, rather than JA intake, may affect GERD-related symptoms. Second, neither smoking habit nor body weight data were collected in this study. Smoking and body weight are factors that are known to affect the GERD condition[27,28]. Third, subjects in this study did not undergo endoscopy to assess for GERD. However, all GERD patients do not show positive endoscopic findings, and therefore, other methods, such as the questionnaire survey used in this study, may also provide sufficient information to make the diagnosis of GERD. Fourth, the presence of diabetes may affect dysmotility-like symptoms, particularly in elderly subjects[29,30]. Although we investigated the presence of diabetes in some of our patients, significant correlations with dysmotility-like symptoms were not observed (data not shown because of the limited number of subjects analyzed). Finally, our cohort included only a few GERD (FSSG $\geqslant$ 8) patients. To further demonstrate the effectiveness of JA on improvement of GERD, the correlation between intake of JA and GERD symptoms should be examined in GERD patients in the future.

Daily intake of JA improves digestive dysmotility symptoms, resulting in relief of GERD symptoms. The effect is more obvious in non-elderly and *H. pylori*-negative subjects. Hence, JA should be recommended as a dietary supplement in young adults in developed countries who have GERD symptoms. Moreover, improvement of hygiene will lower the prevalence of *H. pylori* infection, particularly in Asian countries, where people eat mainly rice, since intake of JA with rice would alleviate GERD symptoms in susceptible populations. Elucidation of the precise mechanism of improvement in gastric motility by JA may lead to the development of new drugs for GERD.

COMMENTS

Background

Japanese apricot (JA), which is eaten as a daily food in Japan, is considered to have medicinal benefits. Gastroesophageal reflux disease (GERD) is a common disease in Western countries. Recently, the number of GERD patients has also been increasing in Japan. The authors focused on the effect of JA on

GERD-related symptoms in this study.

Research frontiers

The authors investigated and compared GERD symptoms between Japanese inhabitants who took and did not take JA daily, using the validated questionnaire, "the Frequency Scale for Symptoms of GERD", to quantify the severity of GERD symptoms and differentiate between acid-related symptoms and dysmotility-related symptoms.

Innovations and breakthroughs

Daily intake of JA improves digestive dysmotility, resulting in relief of GERD symptoms. The effect is more obvious in non-elderly and *Helicobacter pylori*-negative subjects. Hence, JA should be recommended as a dietary supplement in young adults in developed countries who have GERD symptoms.

Applications

Elucidation of the precise mechanism of improvement of gastric motility by JA may lead to development of new drugs for GERD.

Terminology

The FSSG questionnaire for GERD related symptoms, which was developed by Kusano *et al* in Japan, was evaluated. The questionnaire consists of 12 questions regarding GERD-related symptoms, including 7 questions associated with acid reflux symptoms and 5 questions asking about gastrointestinal dysmotility symptoms. Each question was answered on a 4-point scale [0 (never), 1 (occasionally), 2 (sometimes), 3 (often), or 4 (always)], with higher scores indicating more severe GERD-related symptoms. GERD was defined as a total score $\geqslant$ 8, which is the recommended cut-off FSSG score for GERD.

Peer-review

This is an interesting manuscript reporting the effect of JA on upper gastrointestinal symptoms using a validated questionnaire. This is a well-written paper, limited by the absence of objective measurement of GERD, limitations already reported by the Authors. Discussion is complete and the limitations of the study have been fully highlighted. Prospective studies are needed to assess the role of JA in the management of GERD and functional hp negative dyspepsia.

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ORIGINAL ARTICLE

Prospective Study

Procedure for prolapse and hemorrhoids *vs* traditional surgery for outlet obstructive constipation

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Abstract

AIM: To compare the clinical efficacies of two surgical procedures for hemorrhoid rectal prolapse with outlet obstruction-induced constipation.

METHODS: One hundred eight inpatients who underwent surgery for outlet obstructive constipation caused by internal rectal prolapse and circumferential hemorrhoids at the First Affiliated Hospital of Xinjiang Medical University from June 2012 to June 2013 were prospectively included in the study. The patients with rectal prolapse hemorrhoids with outlet obstruction-induced constipation were randomly divided into two groups to undergo either a procedure for prolapse and hemorrhoids (PPH) (n = 54) or conventional surgery (n = 54; control group). Short-term (operative time, postoperative hospital stay, postoperative urinary retention, postoperative perianal edema, and postoperative pain) and long-term (postoperative anal stenosis, postoperative sensory anal incontinence, postoperative recurrence, and postoperative difficulty in defecation) clinical effects were compared between the two groups. The short- and long-term efficacies of the two procedures were determined.

RESULTS: In terms of short-term clinical effects, operative time and postoperative hospital stay were significantly shorter in the PPH group than in the control group (24.36 ± 5.16 min *vs* 44.27 ± 6.57 min, 2.1 ± 1.4 d *vs* 3.6 ± 2.3 d, both $P < 0.01$). The incidence of postoperative urinary retention was higher in the PPH group than in the control group, but the difference was not statistically significant (48.15% *vs* 37.04%). The

incidence of perianal edema was significantly lower in the PPH group (11.11% *vs* 42.60%, $P < 0.05$). The visual analogue scale scores at 24 h after surgery, first defecation, and one week after surgery were significantly lower in the PPH group (2.9 ± 0.9 *vs* 8.3 ± 1.1, 2.0 ± 0.5 *vs* 6.5 ± 0.8, and 1.7 ± 0.5 *vs* 5.0 ± 0.7, respectively, all $P < 0.01$). With regard to long-term clinical effects, the incidence of anal stenosis was lower in the PPH group than in the control group, but the difference was not significant (1.85% *vs* 5.56%). The incidence of sensory anal incontinence was significantly lower in the PPH group (3.70% *vs* 12.96%, $P < 0.05$). The incidences of recurrent internal rectal prolapse and difficulty in defecation were lower in the PPH group than in the control group, but the differences were not significant (11.11% *vs* 16.67% and 12.96% *vs* 24.07%, respectively).

CONCLUSION: PPH is superior to the traditional surgery in the management of outlet obstructive constipation caused by internal rectal prolapse with circumferential hemorrhoids.

Key words: Internal rectal prolapse; Outlet obstructive constipation; Procedure for prolapse and hemorrhoids; Prospective study; Randomized controlled study

Core tip: This study included 54 patients with rectal prolapse hemorrhoids and compared procedure for prolapse and hemorrhoids (PPH) with a traditional operation. The PPH group had a significantly shorter operative time, shorter hospital stay, and lower incidence of postoperative edema perianal, postoperative pain, and sensory incontinence compared to the group receiving traditional surgical treatment. PPH surgery has an obvious effect that can be widely used in clinical treatment.

Lu M, Yang B, Liu Y, Liu Q, Wen H. Procedure for prolapse and hemorrhoids *vs* traditional surgery for outlet obstructive constipation. *World J Gastroenterol* 2015; 21(26): 8178-8183 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i26/8178.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i26.8178

INTRODUCTION

Constipation is the most common chronic digestive symptom of many causes. It is characterized by decreased defecation frequency, decreased amount of feces, dry feces, and difficulty in defecation. The incidence of constipation is associated with many factors including sex, age, dietary habit, and occupation. Statistics show that the incidence of constipation is as high as 20% in the general population. In recent years, due to the continuous improvement of living standards, the incidence of constipation has been increasing, and thus has become one of the important factors that seriously affect human health. Based on the dynamics of defecation, constipation can be divided into three types: slow transit constipation, outlet obstructive constipation, and mixed type constipation. Conservative treatment is the main therapy for slow transit constipation, and surgery is not advocated. Outlet obstructive constipation is more common in middle-aged and elderly females and often requires management by surgery. Outlet obstructive constipation may be caused by circumferential hemorrhoids, internal rectal prolapse, rectocele and puborectalis muscle syndrome, with internal rectal prolapse and circumferential hemorrhoids being the most common causes. Currently, there are multiple surgical procedures available for the treatment of outlet obstructive constipation caused by internal rectal prolapse with circumferential hemorrhoids, with traditional ligation of prolapsed rectal mucosa and hemorrhoids and procedure for prolapse and hemorrhoids (PPH) being the most commonly used. The present study was conducted to assess whether PPH is superior to the traditional surgery in the management of outlet obstructive constipation caused by internal rectal prolapse with circumferential hemorrhoids.

MATERIALS AND METHODS

Patients

One hundred eight inpatients who underwent surgery for outlet obstructive constipation caused by internal rectal prolapse and circumferential hemorrhoids at the First Affiliated Hospital of Xinjiang Medical University from June 2012 to June 2013 were prospectively included in the study. Hemorrhoids were diagnosed by history, digital rectal examination, and anoscopic examination according to the Diagnostic Criteria for Hemorrhoids formulated in 2004 by the Anorectal Surgery Group of Surgery Branch of China Association of Chinese Medicine[1]. Internal rectal prolapse was graded according to the criteria formulated in 1975 at the National Conference of Anorectal Medicine. The patients were divided into two groups using a randomized block design to undergo either PPH (n = 54) or traditional surgery (ligation of prolapsed rectal mucosa and hemorrhoids; n = 54). The PPH group was comprised of 20 men and 34 women, with a mean age of 55.4 ± 8.5 years; the control group was comprised of 23 men and 31 women, with a mean age of 54.1 ± 9.1 years. For the PPH group, the mean disease duration was 11.4 ± 3.7 years; there were 39 cases of grade Ⅲ hemorrhoids, 15 cases of grade Ⅳ hemorrhoids, 24 cases of grade Ⅱ internal rectal prolapse, and 30 cases of grade Ⅲ internal rectal prolapse. For the control group, the mean disease duration was 10.2 ± 4.1 years; there were 37 cases of grade Ⅲ hemorrhoids, 17 cases of grade Ⅳ hemorrhoids, 26 cases of grade

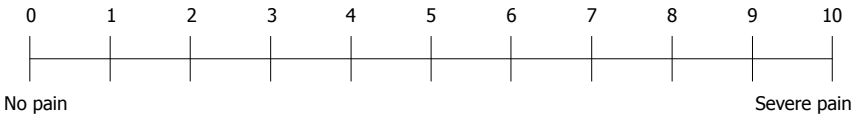

Figure 1 Visual analogue scale. Mild pain: 1-3 points; moderate pain: 4-6 points; severe pain: 7-10 points.

Ⅱ internal rectal prolapse, and 28 cases of grade Ⅲ internal rectal prolapse. Baseline data including age, sex, grade of hemorrhoids, and grade of internal rectal prolapse were not significantly different between the two groups.

Inclusion criteria

Inclusion criteria were: (1) grade Ⅲ or Ⅳ hemorrhoids; (2) age 45-65 years; (3) grade Ⅱ or Ⅲ internal rectal prolapse diagnosed by defecography; and (4) clinical manifestations including difficulty in defecation, sensation of anorectal obstruction, anal tenesmus or discomfort, prolonged defecation, and the frequent need of manual maneuvers to facilitate defecations.

Exclusion criteria

Exclusion criteria were: (1) patients with severe anal stenosis or anal incontinence; (2) patients with a previous history of surgery or injection therapy for hemorrhoids; (3) patients with malignant tumors of the colon, rectum, or anal canal; and (4) patients with severe diseases of the heart, brain, liver, kidney, hematologic or endocrine system, or the disabled.

Operative procedures

For PPH, sacral anesthesia was performed and the patients were placed in the right lateral decubitus position. After the surgical area was disinfected with 0.5% iodophor liquid, the anal canal was expanded to insert a circular anal dilator and obturator. The obturator was then removed to make the prolapsed mucosa fall into the canal dilator. Subsequently, a purse-string anoscope was inserted and used to place a circumferential mucosal/submucosal purse-string suture with 2-0 Prolene 3-4 cm above the dentate line in a clockwise manner. A stapler was opened to its maximum extent, and its anvil was advanced across the purse string. The purse string suture was then cinched closed and tied. The stapler was fired and held closed for approximately 20 s to aid in hemostasis. Finally, the stapler was opened and removed. The anoscope was reinserted into the anal canal to evaluate hemostasis. Any small bleeding areas could be managed by oversewing. Perianal skin tags were finally removed.

For ligation of prolapsed rectal mucosa and hemorrhoids, sacral anesthesia was performed and the patient was placed in the lithotomy or lateral decubitus position. After the perineal area, rectum, and anal canal were disinfected with 0.5% iodophor liquid, and the anal canal was expanded to the extent that three fingers could be placed in. After the anus fully relaxed, a di-wing anoscope was inserted to fully expose the prolapsed rectal mucosa. The ligation of the prolapsed rectal mucosa was then performed, followed by the ligation of hemorrhoids. Sufficient skin and mucosal bridges were retained between adjacent hemorrhoids. After careful detection of possible active bleeding points, Vaseline gauze was placed into the anal canal for compression hemostasis. After aseptic dressing was applied, adhesive tape and T-bandage were used for fixation.

Outcome measures

Both short- and long-term outcome measures were evaluated in this study. Short-term outcome measures were arbitrarily defined as those observed within 3 mo after surgery, whereas long-term outcome measures were those observed 6 mo or longer after surgery. Short-term outcome measures included operative time, postoperative hospital stay, postoperative urinary retention, postoperative perianal edema, and postoperative pain, whereas long-term outcome measures included postoperative anal stenosis, postoperative sensory anal incontinence, postoperative recurrence, and postoperative difficulty in defecation.

Postoperative pain evaluation

Postoperative pain was evaluated using the visual analogue scale (VAS). The VAS is a 10-cm line with the two ends marked "0" (no pain) and "10" (worst pain) (Figure 1). The patient was asked to place a mark that corresponds to his/her current pain intensity. The mean scores of mild, moderate and severe pain were 2.57 ± 1.04, 5.18 ± 1.41, and 8.41 ± 1.35, respectively.

Statistical analysis

All statistical analyses were performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, United States). Numerical data including operative time, postoperative hospital stay, and postoperative pain score are expressed as mean ± SD and were compared using the Student's *t* test. Categorical data including the incidences of postoperative urinary retention, perianal edema, anal stenosis, sensory anal incontinence, recurrent internal rectal prolapse and difficulty in defecation were compared using the χ^2 test. $P < 0.05$ was considered statistically significant.

RESULTS

Short-term efficacy and safety

After surgical treatments, the symptoms improved in

Table 1 Outcome measures for short-term efficacy and safety

Outcome measure	PPH group (n = 54)	Control group (n = 54)	χ^2 or t value	P value
Operative time, min	24.36 ± 5.16	44.27 ± 6.57	17.514	< 0.001
Postoperative hospital stay, d	2.1 ± 1.4	3.6 ± 2.3	4.094	0.001
Urinary retention, n (%)	26 (48.15)	20 (37.04)	1.363	0.243
Perianal edema, n (%)	12 (11.11)	23 (42.60)	5.115	0.024
Postoperative VAS score				
At 24 h	2.9 ± 0.9	8.3 ± 1.1	27.923	< 0.001
At first defecation	2.0 ± 0.5	6.5±0.8	35.055	< 0.001
At one week	1.7 ± 0.5	5.0 ± 0.7	28.205	< 0.001

VAS: Visual analogue scale; PPH: Procedure for prolapse and hemorrhoids.

Table 2 Outcome measures for long-term efficacy and safety n (%)

Outcome measure	PPH group (n = 54)	Control group (n = 54)	χ^2 value	P value
Anal stenosis	1 (1.85)	3 (5.56)	0.26	0.610
Anal tenesmus	15 (27.78)	13 (24.07)	0.193	0.661
Sensory anal incontinence	2 (3.70)	7 (12.96)	3.951	0.041
Recurrence	6 (11.11)	9 (16.67)	0.697	0.404
Difficulty in defecation	7 (12.96)	13 (24.07)	2.209	0.137

PPH: Procedure for prolapse and hemorrhoids.

patients of both groups. The outcome measures for short-term efficacy and safety are shown in Table 1. The operative time and postoperative hospital stay were significantly shorter in the PPH group than in the control group (both $P < 0.01$). The incidence of postoperative urinary retention was higher in the PPH group than in the control group, but the difference was not statistically significant. The incidence of perianal edema was significantly lower in the PPH group than in the control group ($P < 0.05$). The VAS scores at 24 h after surgery, first defecation, and one week after surgery were significantly lower in the PPH group than in the control group (all $P < 0.01$).

Long-term efficacy and safety

Table 2 shows the outcome measures for short-term efficacy and safety (at one year after surgery). The incidence of anal stenosis was lower in the PPH group than in the control group, but the difference was not significant. The incidences of sensory anal incontinence and anal skin tags (5.56% *vs* 25.93%) were significantly lower in the PPH group than in the control group (both $P < 0.05$). The incidence of anal tenesmus was higher in the PPH group than in the control group, but the difference was not significant. The incidence rates of recurrent internal rectal prolapse and difficulty in defecation were lower in the PPH group than in the control group, but the differences were not significant.

DISCUSSION

Constipation is the most common chronic digestive disease[2]. It is characterized by decreased defecation frequency, dry feces, and difficulty in defecation. Over the past decades, the changes in dietary patterns and the impact of mental and social factors have made constipation a disease that seriously affects people's quality of life. Constipation can lead to digestive system diseases such as colon cancer and hepatic encephalopathy, as well as acute myocardial infarction, cerebrovascular accident, and even death. Therefore, early prevention and reasonable treatment will greatly reduce the potential serious consequences of constipation.

This study shows that compared with the traditional surgery, PPH is associated with less postoperative pain and shorter operative and hospitalization times. There are several possible explanations for this. First, PPH is associated with less trauma and faster recovery[3-9]. Second, PPH is simple and can manage internal rectal prolapse and hemorrhoids simultaneously in one procedure. Finally, PPH is conducted 3-4 cm above the dentate line, and the mucosa above the dentate line is controlled by the plant nerve and is not sensitive to pain. Thus, PPH results in milder postoperative pain. Beattie *et al*[10] reported that approximately 51% of patients undergoing PPH were completely free from postoperative pain. The advantages of PPH have been verified by many clinical trials. In contrast, the traditional surgery consists of two operative procedures, is relatively complex, and has the disadvantages of more trauma, longer operative time, and slower wound recovery, which lead to prolonged hospital stay. Moreover, the conventional surgery is conducted in the area close to the dentate line and tends to damage the pain-sensitive pudendal nerve, thus resulting in more intense postoperative pain.

The advantages of PPH over the traditional surgery lie not only in the short-term curative effects, but also in the long-term curative effects. The incidences of postoperative anal stenosis, sensory anal incontinence, and recurrence were significantly lower in PPH-treated patients, which is consistent with the results of other

studies[11,12]. Ganio *et al*[13] followed patients receiving either PPH or traditional surgery for 87 mo and found that there was no significant difference in postoperative recurrence between the two groups. During PPH, the mucosa is resected, the anal sphincter is not injured, and the anal cushion and anal transitional zone epithelium are retained. Thus, the intact anal canal is preserved and has good postoperative defecation reflex and fine feeling, and the anal function is not affected. In the conventional surgery, too much skin mucosa is removed and insufficient skin and mucosal bridge is retained, and often results in scar stricture[8,14-22]. Of note, if the anastomotic position is too low in PPH, anal stricture often occurs near the dentate line.

In conclusion, PPH is superior to the traditional surgery in the management of outlet obstructive constipation caused by internal rectal prolapse with circumferential hemorrhoids in terms of both short- and long-term efficacies and safety. PPH is associated with less trauma and postoperative pain, shorter operative time, faster recovery, lower recurrence rate, and fewer postoperative complications[11,12,23-27], representing a better choice for treatment of outlet obstructive constipation caused by internal rectal prolapse with circumferential hemorrhoids.

COMMENTS

Background

Constipation is the most common chronic digestive symptom of many causes. It is characterized by decreased defecation frequency, decreased amount of feces, dry feces, and difficulty in defecation. The incidence of constipation is associated with many factors including sex, age, dietary habit, and occupation. Currently, there are multiple surgical procedures available for the treatment of outlet obstructive constipation caused by internal rectal prolapse with circumferential hemorrhoids, with traditional ligation of prolapsed rectal mucosa and hemorrhoids and procedure for prolapse and hemorrhoids (PPH) being the most commonly used.

Research frontiers

The present study was conducted to assess whether PPH is superior to the traditional surgery in the management of outlet obstructive constipation caused by internal rectal prolapse with circumferential hemorrhoids.

Innovations and breakthroughs

The authors found that PPH is superior to the traditional surgery in the management of outlet obstructive constipation caused by internal rectal prolapse with circumferential hemorrhoids.

Peer-review

This is an interesting manuscript about procedure for prolapse and hemorrhoids and conventional surgery.

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ORIGINAL ARTICLE

Randomized Controlled Trial

Intraprocedural bowel cleansing with the JetPrep cleansing system improves adenoma detection

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Author contributions: Hoffman A designed and performed the research, wrote the manuscript, and contributed to data analysis, interpretation, and provision of study material and patients; Murthy S performed the research and collected and/or assembled the data; Pompetzki L analyzed, collected and/or assembled the data; Tresch A and Rey JW analyzed and interpreted the data; Goetz M analyzed the data; Galle PR and Kiesslich R performed the research and gave administrative support.

Institutional review board statement: The study was reviewed and approved by the Ethical Review Committee of the University of Mainz.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: The authors declare no conflict of interest.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at ahoff66286@aol.com.

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Abstract

AIM: To investigate the impact of JetPrep cleansing on adenoma detection rates.

METHODS: In this prospective, randomized, cross-over trial, patients were blindly randomized to an intervention arm or a control arm. In accordance with the risk profile for the development of colorectal carcinoma, the study participants were divided into high-risk and low-risk groups. Individuals with just one criterion (age > 70 years, adenoma in medical history, and first-degree relative with colorectal cancer) were regarded as high-risk patients. Bowel preparation was

performed in a standardized manner one day before the procedure. Participants in the intervention arm underwent an initial colonoscopy with standard bowel cleansing using a 250-mL syringe followed by a second colonoscopy that included irrigation by the use of the JetPrep cleansing system. The reverse sequence was used in the control arm. The study participants were divided into a high-risk group and a low-risk group according to their respective risk profiles for the development of colorectal carcinoma.

RESULTS: A total of 64 patients (34 men and 30 women) were included in the study; 22 were included in the high-risk group. After randomization, 30 patients were assigned to the control group (group A) and 34 to the intervention group (group B). The average Boston Bowel Preparation Scale score was 5.15 ± 2.04. The withdrawal time needed for the first step was significantly longer in group A using the JetPrep system (9.41 ± 3.34 min) compared to group B (7.5 ± 1.92 min). A total of 163 polyps were discovered in 64 study participants who underwent both investigation steps. In group A, 49.4% of the polyps were detected during the step of standard bowel cleansing while the miss rate constituted 50.7%. Group B underwent cleansing with the JetPrep system during the first examination step, and as many as 73.9% of polyps were identified during this step. Thus, the miss rate in group B was a mere 26.1% ($P < 0.001$). When considering only the right side of the colon, the miss rate in group A during the first examination was 60.6%, in contrast to a miss rate of 26.4% in group B ($P < 0.001$).

CONCLUSION: JetPrep is recommended for use during colonoscopy because a better prepared bowel enables a better adenoma detection, particularly in the proximal colon.

Key words: Colon preparation; Adenoma detection rate; Adenoma miss rate; Interval cancer; Boston Bowel Preparation Scale; Right sided colon; Flat adenoma

Core tip: Stool tends to hinder visibility during colonoscopy, and its presence therefore increases the risk that lesions will be overlooked. The JetPrep system is an irrigation system that was designed for intraprocedural colon cleansing. The aim of this randomized, prospective study was to investigate the impact of JetPrep cleansing on detection rates of adenomas. The JetPrep system enabled better cleansing of the colon, which increased the detection of polyps throughout the entire colon and especially on its right side ($P < 0.001$). Based on the results of this study, the JetPrep flushing device may be broadly recommended for use during screening colonoscopy to improve bowel preparation and to increase polyp detection rates.

Hoffman A, Murthy S, Pompetzki L, Rey JW, Goetz M, Tresch A, Galle PR, Kiesslich R. Intraprocedural bowel cleansing with the JetPrep cleansing system improves adenoma detection. *World J Gastroenterol* 2015; 21(26): 8184-8194 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i26/8184.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i26.8184

INTRODUCTION

Colonoscopy is considered the gold standard for colon cancer screening and the removal of precancerous lesions of the colon. However, colonoscopy appears to be less effective at preventing disease (and therefore preventing mortality) when used to examine right-sided colonic lesions *vs* those that develop on the left side[1-3]. Even in cases in which the bowel was properly prepared by the patient prior to colonoscopy, stool remnants and mucus residues can hinder visibility during the procedure. This reduced visibility may result in premalignant or malignant lesions being overlooked during screening colonoscopy and might therefore increase the risk of interval cancers, as it has been shown that better visibility during the procedure (as a result of optimal preparation of the colon) increases the likelihood of discovering precancerous lesions in the colon[1-3]. This may further reduce the incidence of colon cancer and the mortality associated with it. However, despite that there are numerous available options for bowel cleansing and laxative regimens, suboptimal colonic preparation is observed in approximately 20% of all patients[4-8]. Suboptimal bowel preparation leads to reduced cecal intubation rates, longer examination times, and lower polyp detection rates[4-8].

Furthermore, only a few endoscopic options exist that can improve bowel cleansing during colonoscopy. One such example is the irrigation of the bowel using either a water-filled syringe or a peristaltic pump placed through the working channel of the endoscope; unfortunately, this method rarely results in adequate improvements to visibility.

Many novel techniques have been proposed to improve the visualization of the proximal aspects of colonic folds and flexures, with the collective goal of increasing the rate of adenoma detection. However, the majority of these techniques still require further evaluation before being translated into a clinical setting[9,10].

Targeted irrigation of the colon by the use of cleansing systems may serve as one important alternative that can enhance adenoma detection rates and improve the overall quality of colonoscopy. The JetPrep system (MedJet Ltd. Tel Aviv, Israel) is a newly introduced irrigation system designed for intraprocedural colon cleansing. The JetPrep operates in a similar manner to a showerhead and can be introduced into

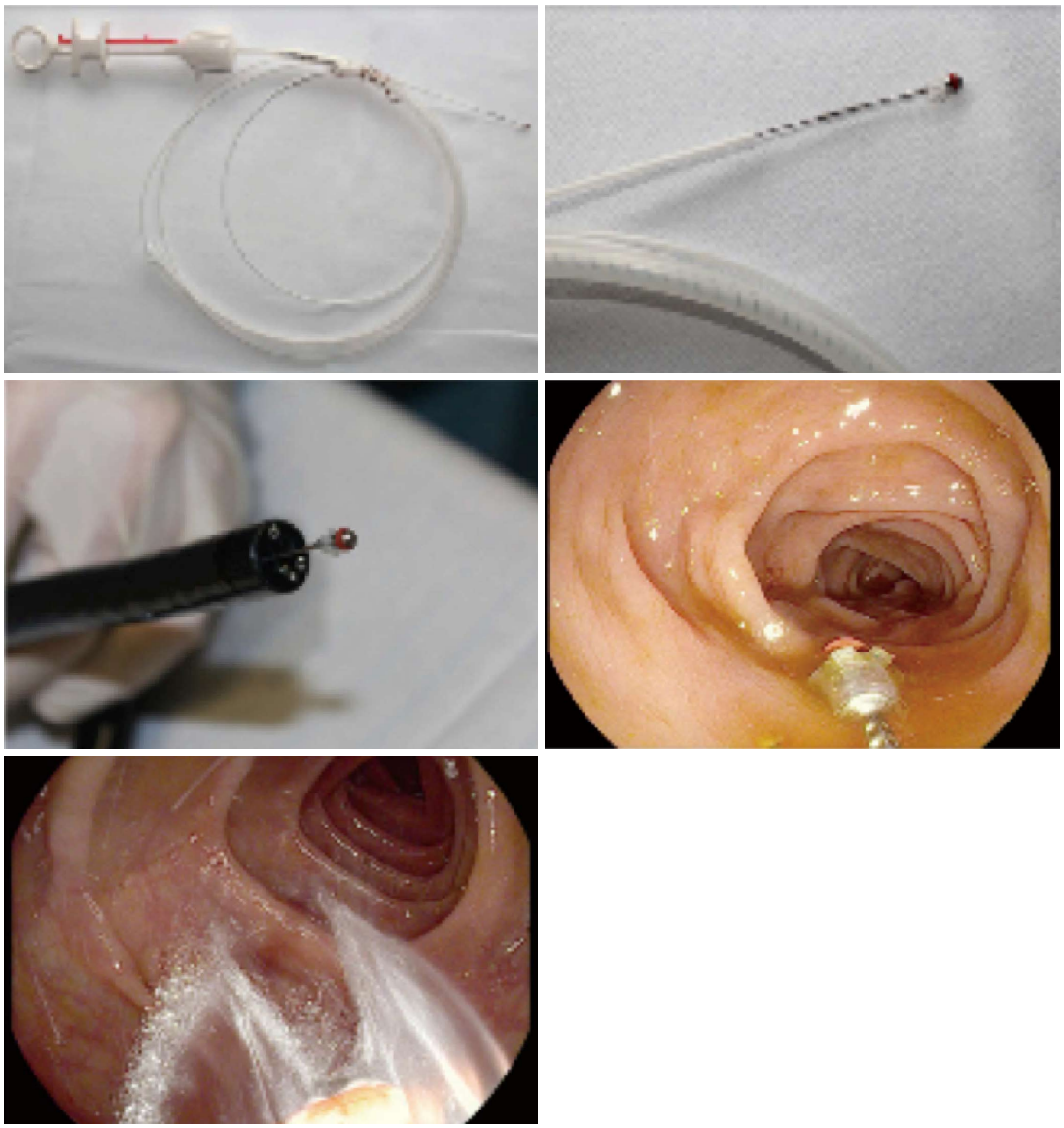


Figure 1 JetPrep system.

the colon through the working channel of an endoscope. Earlier studies have demonstrated significant benefits associated with using JetPrep cleansing compared to standard cleansing (*e.g.*, fixing a 50-mL syringe on the working channel).

Therefore, the aim of the current randomized, prospective study was to investigate the impact of JetPrep cleansing on the detection rates of adenomas and serrated lesions, particularly on the right side of the colon[11-13].

MATERIALS AND METHODS

The JetPrep device (MedJet Ltd. Tel Aviv, Israel) is a CE-certified [39000162CN], sterile, disposable, catheter-based product. It is introduced into the colon through the working channel (3.8 mm) of an endoscope to cleanse the mucosal surface during colonoscopy. To ensure simultaneous suctioning of fluid and stool while the JetPrep device is in the working channel, the spray nozzle at the tip of the device can be pushed out of the working channel by 1-2 cm with a single movement of the hand. This prevents blockage of the working channel. The spray nozzle itself is made of silicone, which minimizes the risk of traumatic injury to the colonic mucosa (Figure 1).

Sterile saline can be introduced into the bowel through the spray nozzle of the device using a commercially available pump. This allows the mucosa to be irrigated with a broad spray of liquid rather than a narrow stream and facilitates the cleaning of the margins of the field of vision (Figure 1).

In preliminary feasibility studies, the JetPrep system proved to be an effective and safe method for cleansing the bowel, although the withdrawal time of the system (11.4 min) reflects that the cleansing of a suboptimally

Table 1 Inclusion and Exclusion criteria

Inclusion criteria
All patients ⩾ 50 yr of age who reported for a screening or surveillance colonoscopy and had a history of removed adenomas were included in the study.
Exclusion criteria
Patients who were unable to sign the informed consent form
Patients who had undergone previous (partial) resection of the large bowel, except for appendectomy
Patients with known or pre-existing colorectal carcinoma
Patients with chronic inflammatory bowel disease
Patients with known FAP or HNPCC syndromes in the family
Patients with a Quick score < 50%, pTT > 50 s, or thrombocytes < 50000/μL who had received no specific measures for the improvement of their coagulation (FFP, TK) before the examination
Patients suffering from a severe underlying disease (ASA > II°)
Patients who were determined to have a cleanliness score of 3 on the Boston Bowel Preparation Scale for the proximal portion of the colon during the examination
Patients in whom a complete colonoscopy could not be performed in the first or second step of the investigation
Patients in whom the first step of the investigation took ⩾ 45 min

prepared colon can be time-consuming[14,15].

Methods

We performed standard high-definition colonoscopies to investigate the efficacy of the JetPrep cleansing system (intervention arm) for improving the detection of adenomas and serrated lesions in the colon compared to standard cleansing procedures that use a 250-mL syringe attached to the working channel of the endoscope (control arm).

This study was approved by the Ethics Committee of the Medical University of Mainz. Our subjects included a cohort of 50-year-old patients who were referred for either screening or surveillance colonoscopy (after previous polypectomy) at the interdisciplinary endoscopy department of the University Hospital. All patients gave consent to participate in the study. Table 1 lists the inclusion and exclusion criteria. Bowel preparation was performed in a standardized manner before the procedure *via* a Moviprep (polyethylene glycol solution, Norgine, Netherland) regimen that consisted of either the consumption of 1 liter of Moviprep during the evening before the investigation and another liter in the morning before the investigation or of 2 litres during the evening before the investigation. In the latter case, each liter of Moviprep had to be taken within the same 1 to 2 h span and the drinking of an additional liter of any clear liquid was required at this time.

The study participants were divided into a high-risk and a low-risk group in accordance with the risk profile for the development of colorectal carcinoma. Individuals with just one criterion (age > 70 years, adenoma in medical history, first-degree relative with colorectal cancer) were regarded as high-risk patients. Patients were blindly randomized into either the intervention arm or the control arm. The group that the patient was classified into was announced only after reaching the cecum.

The patients in the control group (group A) underwent an initial colonoscopy that included standard intraprocedural bowel cleansing (250 mL syringe), which was immediately followed by a second colonoscopy (in a standardized crossover fashion) that included irrigation by the use of the JetPrep cleansing system. The reverse sequence was used for the patients in the intervention group (group B).

Investigation

All procedures included in this study were conducted by two separate investigators (Kiesslich R, Murthy S) who were each experienced in performing colonoscopies using Pentax high-definition endoscopes (Pentax EPKi, Pentax 90i, Pentax Europe).

The length of time that it took to conduct each investigation was measured using a stopwatch and recorded. In agreement with previously published results, the minimal withdrawal time for the detection of polyps per investigation step was set to 6 min; the time was stopped during endoscopic interventions such as polypectomy[16]. During the endoscopic investigation, the patients were sedated with either 1% propofol (Disoprivan, AstraZenca Zug, Switzerland) or midazolam (Dormicum, Roche Pharma AG Basel, Switzerland).

Fluid stool residues were suctioned when inserting the endoscope into the cecum, and the baseline value for bowel preparation was determined using the Boston Bowel Preparation Scale to better objectify the degree of subsequent cleansing (Table 2).

Based on an examination of the right colon, patients who were found to have a Boston Bowel Preparation Scale (BBPS) of 3 were excluded from intraprocedural bowel prep cleansing.

In both investigation groups, the removal of stool deposits from the mucosa was performed only when withdrawing from the cecum.

The cleansing time was included when calculating the withdrawal time; the amount of time taken to set up the JetPrep device was recorded separately and did not influence the withdrawal time. All polyps were removed during withdrawal with either a biopsy

Table 2 Bowel preparation scale

0 = Unprepared colon segment. Due to solid stool that cannot be cleared, the mucosa cannot be observed
1 = Some portions of the mucosa of the colon segment can be observed, but other areas are covered by residual staining consisting of residual stool or opaque fluid
2 = Minor amount of residual staining. No stool fragments or small quantities of opaque fluid, but the mucosal surface of the colon segment can be observed well
3 = The entire mucosa of the colon segment can be observed well and has no residual staining

For the purposes of this scale the bowel is divided into 3 portions, including the ascending colon, the transverse colon, and the descending colon. Each region is assigned a value from 0 to 3. The segment scores are summed to yield a total BBPS score ranging from 0 to 9. The minimum BBPS score was 0 and indicated either the poorest quality of bowel preparation or an unprepared colon while a perfectly clean colon without any residual liquid was scored 9 (47).

Table 3 Secondary endpoint analysis

Miss rate for the entire colon
Polyp miss rates and detection rates for the entire colon and the right side of the colon
Colon cleanliness after JetPrep and standard cleaning (based on the Boston Bowel Preparation Scale)
Rate of adverse events resulting from the use of JetPrep

forceps (< 5 mm) or with an electric loop (> 5 mm), except for small (< 5 mm) hyperplastic polyps of the rectum and the sigmoid colon that presented no evident malignant potential according to the pit pattern classification. Every polyp was graded before removal according to both pit pattern and the Paris classification. The precise quantity of water required for cleansing was recorded for both examination arms. The Boston Bowel Preparation Scale (BBPS) was determined after both the first and second cleansing steps[17]. The proximal portion of the colon was then examined, and all patients who were considered to have a cleanliness score of 3 on the Boston Bowel Preparation Scale were excluded.

After the investigation, all patients were monitored in a standardized manner and were discharged the same day. The study participants were either called the following day or were questioned at the ward about the occurrence of adverse events.

Statistical analysis

Patient data were recorded on a case report form, and relevant details concerning medical history were included.

During the procedure, the time points corresponding to the commencement and conclusion of each examination step were recorded in addition to the length of time that was spent during cleansing and intervention. Every polyp that was discovered during the investigation was registered in a table and the respective histological findings were subsequently recorded.

For both intervention arms, the primary endpoint of the study was the percentage miss rate of adenomas and serrated lesions on the right side of the colon during the first examination step. The secondary endpoints of the study are summarized in Table 3.

Case numbers were calculated based on previously published studies concerning tandem colonoscopy, which reported miss rates of 27%-37% for adenomas on the right side of the colon under standard conditions[12].

Assuming that irrigation with the JetPrep flushing device would reveal not only small adenomas but also adenomas coated with mucus and flat serrated ones, we presumed an absolute risk reduction of $\geqslant$ 30% in the present study, with an effective miss rate of 40% in the control group and 10% in the intervention group. We determined that the detection of at least 64 lesions was necessary to achieve a statistically significant absolute risk reduction when employing a one-sided Fisher's exact test for statistical analysis.

Because the majority of previous studies have assumed an average of one neoplastic lesion per patient, the value described above can be considered equivalent to a sample size of 64 patients or 32 patients per study arm. The χ^2 test was used to compare the categorical variables of the secondary endpoints amongst the various groups. Version 18.0 of the SPSS program was used to evaluate the data.

RESULTS

A total of 73 patients were recruited for the study between March and July of 2012. Six patients were subsequently excluded from the study as a result of having BBPS scores of 3 in the ascending colon. It was not possible to perform a complete colonoscopy in one of the study subjects due to the presence of adhesions.

Two additional patients had to be excluded after the first step of the study due to the respective reasons of an excessively long examination time in one and the presence of stool residues in the bowel that could not be suctioned through the working channel of the endoscope (and thus interfered with the examination) in the other.

A final count of 64 patients (34 men and 30 women) were included in the study, of whom 22 had at least one risk factor for developing colorectal carcinoma (*e.g.*, a first-degree relative with colorectal cancer, a positive medical history for adenoma, or an age > 70 years; Table 4). Thus, 42 patients were assigned to the low-risk group. Following our randomization process a total of 30 patients were

Table 4 Patient characteristics

Original cohort of patients	*n* = 73
Dropouts	9/73 (12.3%)
Number of included patients	64/73 (87.7%)
Group stratification	Group A (first standard), *n* = 30 Group B (first JetPrep), *n* = 34
Age (yr)	63.53 ± 8.03
Sex	M: 34; F: 30
Risk of developing CRC	High: *n* = 22; Low: *n* = 42
BBPS (baseline values) per protocol (*n* = 64)	4.84 ± 1.81
BBPS (baseline values) of original patient cohort[1] (*n* = 71)	5.15 ± 2.04

[1]A complete colonoscopy could not be performed in 2 patients. CRC: Colorectal cancer; BBPS: Boston Bowel Preparation Scale.

Table 5 Investigation-specific characteristics

	Group A (first step: standard cleansing)	Group B (first step: JetPrep)	*P* value
Propofol (mg)	366.33 ± 123.83	421.1 ± 151.05	0.116[1]
Investigator			0.223[2]
Kiesslich R	*n* = 22	*n* = 20	
Murthy S	*n* = 8	*n* = 14	
Withdrawal time (min)			
First step	7.5 ± 1.92	9.41 ± 3.34	0.009[3]
Second step	8.22 ± 2.25	7.60 ± 1.71	0.224[1]
Total	15.72 ± 4.00	17.0 ± 4.70	0.215[3]
Withdrawal time with JetPrep (min)	8.22 ± 2.25	9.41 ± 3.34	0.188[3]
Total duration (min)	36.23 ± 20.31	42.0 ± 18.91	0.073[3]
Intervention time (s)			
First step	218.77 ± 530.21	317.47 ± 638.62	0.289[3]
Second step	122.47 ± 188.31	162.94 ± 361.21	0.376[3]
BBPS basic value	4.9 ± 1.9	4.79 ± 1.79	0.818[2]

[1]Independent *t*-test; [2]χ^2 test; [3]Mann-Whitney *U*-test. BBPS: Boston Bowel Preparation Scale.

assigned to the control group (group A) and 34 to the intervention group (group B).

To estimate the mean quality of bowel preparations, the mean score of the Boston Bowel Preparation Scale was calculated for all patients. The mean BBPS score was found to be 5.15 ± 2.04 (Table 5; *n* = 71, 2 patients did not undergo a complete colonoscopy).

Thus, on a scale from 0-9 (0 = poorest bowel preparation; 9 = best possible bowel preparation), the mean score was found to be in the middle range. The initial degree of cleansing was similar in both groups. Notably, baseline values for the proximal portions of the bowel were on average lower relative to the rest of the colon and increased progressively as one moved further distally into the colon.

Table 5 provides a direct comparison of additional characteristics that were found during investigation.

Table 6 Comparison of water consumption

	Group A (first step: standard cleansing)	Group B (first step: JetPrep)	*P* value
Water consumption (mL)			
First examination	308.70 ± 159.3	900 ± 588.18	< 0.001[2]
Second examination	658.70 ± 272.47	1141.81 ± 102.67	< 0.001[1]
Total water consumption (mL)	967.39 ± 341.99	1014.81 ± 660.54	0.559[2]

[1]Independent *t*-test; [2]Mann-Whitney *U*-test.

The withdrawal time needed for the first step of the procedure was significantly longer in group A (9.41 ± 3.34 min), in which the JetPrep system was used, compared to group B (7.5 ± 1.92 min). However, the total withdrawal time did not differ significantly between the two groups.

Large differences were found between the control group and the intervention group with regard to the quantity of water used during cleansing (Table 6). During both steps of the investigation a greater quantity of water was used to cleanse the bowel when the JetPrep system was employed. Because cleansing was stopped after the first examination step when it produced no further positive effect, the difference between groups for the first step was significant ($P < 0.001$).

This finding was confirmed by the significantly greater quantity of water that was required when using the JetPrep system in the second examination step. However, the overall quantity of water used for both colonoscopies did not differ between groups (Table 6).

Despite differences in water consumption rates none of the 64 study patients experienced complications and all of them could be discharged to go home on the day that they underwent colonoscopy; patients that were already hospitalized were instead sent to the ward for further treatment. A total of 47 (73.4%) out of the 64 patients could be queried about adverse events on the day of the examination; of these, none reported a serious adverse event.

Primary endpoint analysis

A total of 163 discovered polyps were found amongst the 64 study participants who underwent both investigation steps, of these a total of 103 polyps were found during the first investigation step. In group A, 49.4% of the polyps were detected during the standard bowel cleansing procedure and the miss rate (*i.e.*, polyps discovered during the second step of the procedure) of this group was 50.7%. Group B underwent cleansing with the JetPrep system during the first step of the examination and up to 73.9% of polyps were identified during this step. Thus, the miss

Table 7 Overview of detection rates and miss rates

	Group A	Group B	*P* value
Polyps, total found	First step: 35 Second step: 36	First step: 68 Second step: 24	< 0.001
Miss rates for polyps, total	50.70%	26.10%	
Polyps on the right side	First step: 13 Second step: 20	First step: 39 Second step: 14	< 0.001
Miss rate for polyps	60.6%	26.4%	
Adenomas, SSA total	First step: 17 Second step: 13	First step: 42 Second step: 13	0.035
Miss rate for adenomas, SSA total	43.3%	23.7%	
Adenomas, SSA on the right side	First step: 7 Second step: 9	First step: 27 Second step: 11	0.043
Miss rate for adenomas, SSA on the right side	56.3%	29.0%	
Adenomas total	First step: 13 Second step: 11	First step: 32 Second step: 12	0.101
Miss rate for adenomas, total	45.8%	27.3%	
Adenomas on the right side	First step: 3 Second step: 7	First step: 17 Second step: 10	0.064
Miss rate for adenomas on the right side	70.0%	37.0%	
SSA	First step: 4 Second step: 2	First step: 10 Second step: 1	0.243
Miss rate for SSA	33.3%	9.1%	

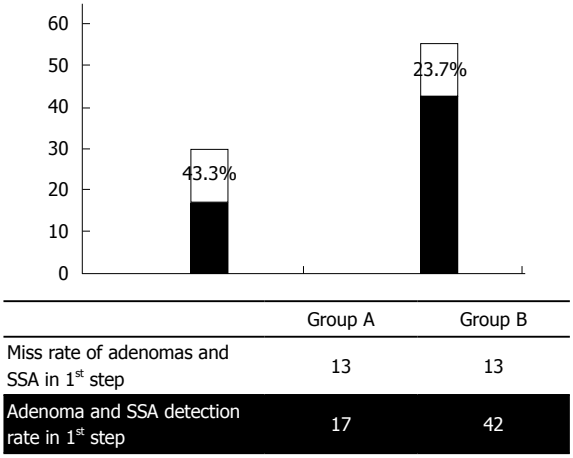
SSA: Sessile serrated adenomas.

rate in group B was only 26.1% ($P < 0.001$; Table 7).

When considering only the investigations that were performed on the right side of the colon, the miss rate in group A during the first examination was 60.6% compared to 26.4% in group B ($P < 0.001$).

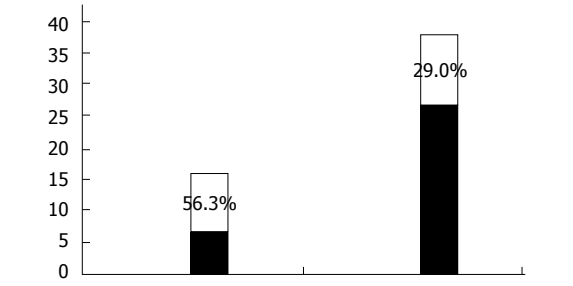
When including the presence of dysplastic lesions such as LGD, HGD and serrated adenomas in the statistical evaluation, the JetPrep system was proven to be significantly more advantageous with respect to both the entire colon and the proximal portions of the colon (Figure 2).

The graphical presentation of miss rates in the first investigation, shown in Figure 3, reveals higher miss rates for lesions on the right side of the colon *vs* the remainder of the colon with respect to both overall lesion numbers and adenomas and sessile serrated adenomas (SSAs). The results show that there was a significant increase in the adenoma detection rates that were measured for both group A ($n = 7$) and group B ($n = 27$), concurrent with a significant decrease in the miss rates that were calculated for each group (group A: 56.3% *vs* group B: 29.0%). When comparing the miss rates for polyps that were distributed throughout the colon *vs* those that were found only on the right side of the colon (Table 7), clear differences were found within the control group (Group A), and virtually no differences were found within the intervention group (Group B). In group A, the miss rate for the detection of all polyps was 50.7%; in group B, this rate was 26.1%. When evaluating only the right side of the colon, the miss rate was found to be 70.0% in group



	Group A	Group B
Miss rate of adenomas and SSA in 1st step	13	13
Adenoma and SSA detection rate in 1st step	17	42

Figure 2 Miss rates for polyps, adenomas and sessile serrated adenomas. SSA: Sessile serrated adenoma.



	Group A	Group B
Miss rate of right-sided colon adenomas and SSA in 1st step	9	11
Adenoma and SSA detection rate on the right side of the colon in 1st step	7	27

Figure 3 Miss rates for polyps, adenomas and sessile serrated adenomas on the right side of the colon. SSA: Sessile serrated adenomas.

A and 37.0% in group B. Table 7 provides a clear overview of the detection and miss rates between the two groups.

DISCUSSION

Colonoscopy has become an integral part of disease prevention, and its use is established in many countries. In Germany, it is common that all insured persons that are ⩾ 50 years in age undergo a screening colonoscopy for the early detection of colorectal cancer (CRC)[18-21]. Furthermore, a collection of respected editorials advocate colonoscopy as a preferred screening strategy, despite the well-known issues of overlooked adenomas and interval cancers[22].

The results of a cohort study that followed 88902 patients over a period of 22 years found screening colonoscopy to be associated with a reduced incidence of cancer in the distal colorectum; however, only a modest reduction in the incidence of proximal colon cancer was found[23]. As of the time of this writing,

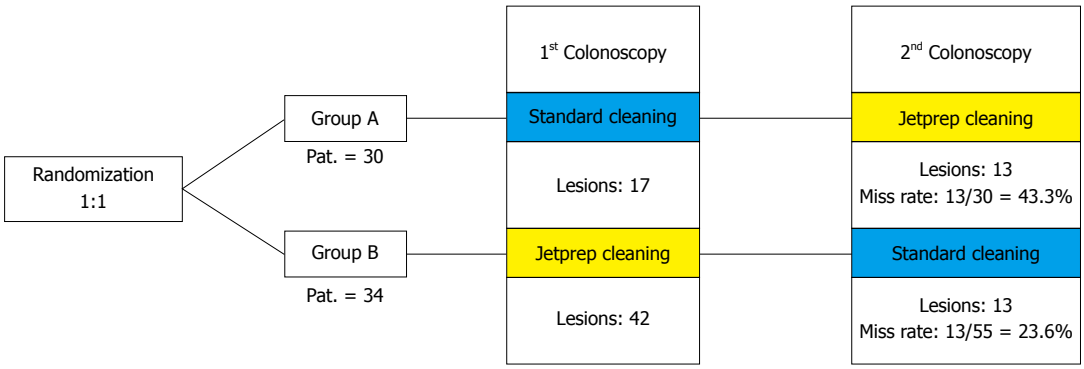


Figure 4 Total number of found adenomas and sessile serrated adenomas.

screening colonoscopies that are used to identify lesions on the left side of the colon are associated with a significantly reduced risk of mortality (OR = 0.33), however, this association does not hold true with respect to the identification of lesions on the right side of the colon (OR = 0.99).

Previously conducted studies have postulated several reasons to explain why the efficacy of screening colonoscopy is so widely different when used on the left side of the colon *vs* the right side; it has been unanimously agreed that no single factor can explain this phenomenon with sufficient clarity. The difficulty associated with the preparation of the right side of the colon during bowel cleansing should certainly be considered an important factor when trying to understand why the detection of small flat polyps in this region of the colon is still a challenge[7,23]. In addition to oral laxatives, a variety of intraprocedural cleaning measures can be used to achieve better visibility in the proximal colon. Considering that rinsing the colon with syringes through the working channel of the endoscope is a time-consuming and ineffective process, many other systems are currently being tested to improve visibility during colonoscopy[11-14].

The JetPrep cleansing device is a sterile, disposable system that functions through a shower like spray mechanism while simultaneously permitting stool residues to be suctioned through the working channel of an endoscope. Preliminary studies on the JetPrep system have shown that it achieves significantly better cleansing compared to alternative methods[14].

The prospective crossover study described here demonstrated that significantly better cleansing of the colon was achieved with the JetPrep system than by the standard method using a syringe, especially in poorly prepared portions of the proximal colon ($P < 0.001$).

In addition to better cleansing, the methodology presented in this study led to higher detection rates for polypoid lesions in the colon and a significant enhancement of lesion detection rates with respect to the right side of the colon ($P < 0.001$). However, the more relevant feature of this method from the viewpoint of the patient is the enhanced detection of adenomas and dysplastic polyps, as these factors alone constitute a quality criterion that demonstrates the efficacy of the new method and dictates both the intervals of treatment and the prognosis of the patient. Currently, based on the knowledge of the "adenoma-carcinoma sequence", in some cases the next control investigation is recommended after a rather long period of 10 years[21]. However, recent data have revealed a new serrated pathway that encompasses both SSA and the conventional adenoma-carcinoma pathway[8,24-33].

This type of polyp is usually found on the right side of the colon and because it is shallow, its growth pattern is difficult to detect[34,35]; such polyps tend to be easily overlooked and are therefore responsible for increasing the rate of interval carcinomas associated with screening colonoscopy in addition to having a greater potential of transforming into colorectal cancer[36].

In the present study, we found the JetPrep cleansing device to be significantly superior in facilitating the detection of adenomas and serrated adenomas throughout the colon as compared to standard cleansing with a 50-mL syringe (Fisher's exact test, $P < 0.035$) (Figure 4). It is also notable that significantly more adenomas and SSAs were found on the right side of the colon following the use of the JetPrep device ($P = 0.043$) (Figure 5). A large case-control study that was recently published by Baxter reported a 22% average miss rate for the detection of adenoma (range, 15%-32%) when using tandem colonoscopy and further emphasized the benefit of screening colonoscopy in reducing the rate of mortality caused by interval cancers that arise on the right side of the colon[37].

A similar outcome was achieved in a study performed by Rex; tandem colonoscopy in conjunction with a standard cleansing procedure yielded a miss rate of 25%. Lesions less than 5 mm in size were found to be overlooked more frequently in the first examination step whereas larger lesions were more rarely missed[38].

In contrast to the Rex study, in our present investigation we switched between two cleansing procedures. Significantly more colon adenomas and

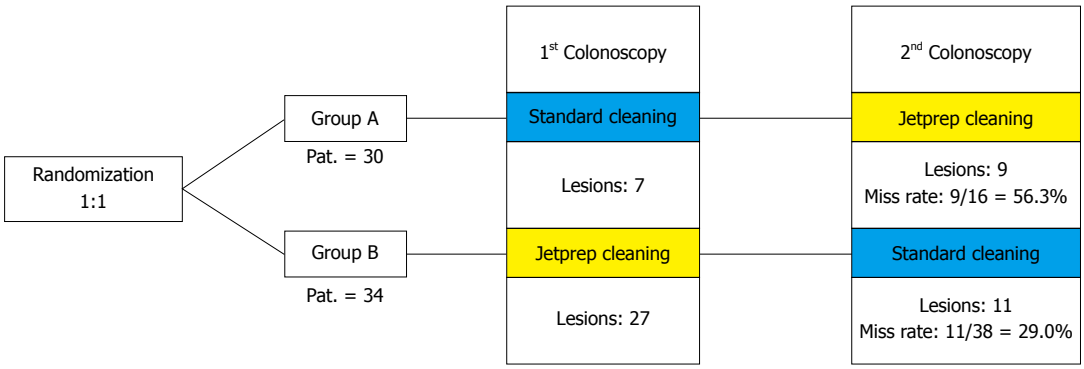


Figure 5 Number of right-sided adenomas and sessile serrated adenomas.

SSAs were overlooked in patients who underwent the first procedure when a standard cleansing method was employed (43.3% miss rate) (Figure 4). The rate of missed right-sided colonic adenomas and SSAs was even more pronounced (56.3%) (Figure 5).

In patients who underwent cleansing with the JetPrep device during the initial colonoscopy, we registered an overall miss rate of 23.7%, with a 29.0% miss rate for right-sided adenomas. Although our results generally agree with other published studies, detection rates tended to be higher and miss rates correspondingly lower when using the JetPrep flushing device[39]. These results might be explained by the superior cleansing that can be achieved with the JetPrep device. Furthermore, the rate at which we detected adenoma is comparable to that obtained when using white light endoscopy in conjunction with newer add-on devices (*e.g.*, Third Eye Retroscope, cap-assisted colonoscopy). However, one potential bias of our approach that must be noted is the fact that we divided our patient cohort into a high-risk and a low-risk group and that the endoscopist performing the procedure was not blinded as to which group each patient fell within; this knowledge could influence the degree to which the colon was inspected on withdrawal.

The relevance of improved adenoma detection rates (and corresponding reductions in miss rates) from the perspective of the patient is reflected by the high rate of interval carcinomas and by the use of different recommendations for the frequency of screening colonoscopy that is necessary for adenoma detection. In 7 out of 30 patients in group A at least one adenoma or SSA was discovered during the second examination when using the JetPrep system that was not detected during the first standard examination. Thus, in the absence of a second examination, the initial colonoscopy would have been performed too late in 23.3% of patients. With regard to the corresponding values for group B, fewer patients (8.8%) would have been subjected to an incorrect screening interval after the first examination step with JetPrep (3 of 34 patients). With regard to withdrawal times, a significantly longer withdrawal time was necessary when using the JetPrep system during the first examination (9.41 ± 3.34 min *vs* 7.5 ± 1.92 min, $P < 0.009$); the withdrawal period for the second examination was nearly just as long (8.22 ± 2.25 min *vs* 7.6 ± 1.71 min, $P = 0.224$). To rule out systematic errors, we calculated the difference in total investigation times for both groups as well as any differences caused by the JetPrep steps and no significant difference was found for either of these parameters between the two groups.

Based on the collective findings detailed above, we can conclude that patients with relevant residual staining are subject to a much higher risk of missed dysplastic lesions. However, it should be noted that the colonoscopies performed in our study were conducted by only two highly experienced endoscopists and that every back-to-back examination was performed by the same investigator, who was not blinded to the results of the first screen.

Furthermore, even advanced cleansing procedures are limited in their ability to enhance the detection of polyps that are concealed behind folds.

In addition to new options becoming available for bowel cleansing, there has also been an increase in the development of new endoscopic techniques, all of which are aimed at enhancing imaging techniques to improve the detection of adenomas and/or combating interval carcinoma.

These new techniques utilize items such as a retro-viewing device (Third Eye Retroscope, Avantis Medical, Sunnyvale, CA), a colonoscope equipped with an integrated balloon at its distal tip (NaviAidTM G-EYE, Smart Medical Systems, Israel), or a full spectrum endoscope (FUSE, EndoChoice, Alpharetta, GA, United States) that can provide the endoscopist with a 330-degree field of vision. Each of these techniques enables the inspection of the proximal surfaces of haustral folds, which are not in the line of vision of the endoscope's forward-viewing optics. Thus far the results obtained from using such devices have been associated with enhanced adenoma detection rates compared with standard colonoscopy[40-43].

Nevertheless, the techniques discussed above should not be considered as direct competitors that

will rule out the use of the JetPrep system; rather, they emphasize the fact that the problem of overlooked adenomas is a persistent one. In fact, the JetPrep system may be used to complement the new technical procedures because of its universal ability to be applied through the working channel of the endoscope. In fact it would even be desirable to explore the combination of JetPrep and these new technologies; such investigations would likely yield even more favorable results than those achieved so far.

In summary, the JetPrep flushing device is safe to use and is the first intraprocedural cleansing system that significantly increases detection rates of right-sided neoplastic lesions (adenomas/SSA). Although based on a relatively small single-center, prospective, randomized clinical study, the JetPrep flushing device may be recommended for use in screening colonoscopies to improve the preparation of the bowel and therefore increase the rate of polyp detection.

COMMENTS

Background

Although screening colonoscopy is considered the gold standard for identifying lesions within the bowel, the problem of overlooked adenomas and interval cancers is increasing.

Research frontiers

The literature postulates several reasons to explain why the efficacy of screening colonoscopy differs widely between the left and right sides of the colon; it is unanimously agreed that no single factor can explain this phenomenon with sufficient clarity.

Innovations and breakthroughs

Flat and sessile polyps are shallow and difficult to detect. These polyps tend to be easily overlooked and possess a greater potential for transformation into colorectal cancer. They may therefore be responsible for interval carcinomas after screening. In the present study the authors found that the JetPrep cleansing system is superior to standard cleansing with respect to facilitating the detection of both adenomas and serrated adenomas throughout the colon.

Applications

Any improvement that can be made to the detection rate of adenoma is highly relevant for patients with regard to recommended control intervals for colonoscopies performed to detect adenomas.

Terminology

The JetPrep system is a new irrigation system for intraprocedural colon cleansing. The system functions in a similar way to a showerhead and is introduced through the working channel of the endoscope.

Peer-review

The authors have investigated an important issue concerning bowel preparation in what appears to be the first randomized, prospective, crossover study designed to examine intraprocedural bowel cleansing with the newly developed JetPrep system during colonoscopy. Quality outcomes such as bowel preparation and adenoma detection rates were examined.

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ORIGINAL ARTICLE

Randomized Controlled Trial

Safety and efficacy of carbon dioxide insufflation during gastric endoscopic submucosal dissection

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Author contributions: Takada J and Araki H contributed to the study design, acquisition and interpretation of data, and in the writing of the manuscript; Onogi F and Nakanishi T acquired the data; Kubota M and Ibuka T analyzed and interpreted the data; Shimizu M wrote the manuscript; and Moriwaki H approved the final contents of the manuscript.

Institutional review board statement: The study was reviewed and approved by the ethics committee for clinical research at Gifu University Hospital.

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Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

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Data sharing statement: The technical appendix, statistical code, and dataset are available from the corresponding author at araara@gifu-u.ac.jp. Participants gave informed consent for data sharing.

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Abstract

AIM: To compare the safety and efficacy of carbon dioxide (CO_2) and air insufflation during gastric endoscopic submucosal dissection (ESD).

METHODS: This study involved 116 patients who underwent gastric ESD between January and December 2009. After eliminating 29 patients who fit the exclusion criteria, 87 patients, without known pulmonary dysfunction, were randomized into the CO_2 insufflation (n = 36) or air insufflation (n = 51) groups. Standard ESD was performed with a CO_2 regulation unit (constant rate of 1.4 L/min) used for patients undergoing CO_2 insufflation. Patients received diazepam for conscious sedation and pentazocine for analgesia. Transcutaneous CO_2 tension ($PtcCO_2$) was recorded 15 min before, during, and after ESD with insufflation. $PtcCO_2$, the correlation between $PtcCO_2$ and procedure time, and ESD-related complications were compared between the two groups. Arterial blood gases were analyzed after ESD in the first 30 patients (12 with CO_2 and 18 with air insufflation) to assess the correlation between arterial blood CO_2 partial pressure ($PaCO_2$) and $PtcCO_2$.

RESULTS: There were no differences in respiratory

functions, median sedative doses, or median procedure times between the groups. Similarly, there was no significant difference in post-ESD blood gas parameters, including $PaCO_2$, between the CO_2 and air groups (44.6 mmHg *vs* 45 mmHg). Both groups demonstrated median pH values of 7.36, and none of the patients exhibited acidemia. No significant differences were observed between the CO_2 and air groups with respect to baseline $PtcCO_2$ (39 mmHg *vs* 40 mmHg), peak $PtcCO_2$ during ESD (52 mmHg *vs* 51 mmHg), or median $PtcCO_2$ after ESD (50 mmHg *vs* 50 mmHg). There was a strong correlation between $PaCO_2$ and $PtcCO_2$ (r = 0.66; P < 0.001). The incidence of Mallory-Weiss tears was significantly lower with CO_2 insufflation than with air insufflation (0% *vs* 15.6%, P = 0.013). CO_2 insufflation did not cause any adverse events, such as CO_2 narcosis or gas embolisms.

CONCLUSION: CO_2 insufflation during gastric ESD results in similar blood gas levels as air insufflation, and also reduces the incidence of Mallory-Weiss tears.

Key words: Carbon dioxide; Gastric endoscopic submucosal dissection; Insufflation; Mallory-Weiss tear; Randomized controlled trial

Core tip: The safety and efficacy of carbon dioxide (CO_2) and air insufflation during gastric endoscopic submucosal dissection (ESD) were compared in a randomized controlled trial. The transcutaneous CO_2 tension and the partial pressure of CO_2 in the arterial blood were measured to directly evaluate CO_2 retention or acidemia. The findings strongly suggest that CO_2 insufflation is as safe as air insufflation with regard to blood gas levels. The present study is the first randomized controlled trial to demonstrate the benefit of CO_2 insufflation in reducing the risk of Mallory-Weiss tears during ESD.

Takada J, Araki H, Onogi F, Nakanishi T, Kubota M, Ibuka T, Shimizu M, Moriwaki H. Safety and efficacy of carbon dioxide insufflation during gastric endoscopic submucosal dissection. *World J Gastroenterol* 2015; 21(26): 8195-8202 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i26/8195.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i26.8195

INTRODUCTION

Endoscopic submucosal dissection (ESD) for gastric neoplasms enables *en bloc* resection of even an extensive superficial lesion[1-8]. However, gastric ESD is technically difficult and time consuming, and therefore, extensive gas insufflation is required to maintain adequate visualization during the procedure. Although air is commonly used for insufflation, it results in the retention of a large amount of residual gas after ESD. Residual gas in the gastrointestinal tract can induce post-ESD pain or discomfort, and in rare cases can give rise to life-threatening complications such as air embolism and tension pneumothorax[9-17].

It is well known that carbon dioxide (CO_2) is absorbed faster in the body than air and is also rapidly excreted through the lungs, except in cases of pulmonary dysfunction. Therefore, CO_2 insufflation is expected to reduce the pain and abdominal discomfort associated with endoscopic examination and therapy[18-25].

Perforation and major bleeding are severe complications of ESD. The reported incidence of perforation in ESD ranges from 1% to 6.1%[1-5], and subsequent peritonitis or mediastinitis could be fatal. CO_2 insufflation reportedly minimizes these ESD-related complications[26]. The safety and efficacy of CO_2 insufflation during ESD for lesions of the esophagus, stomach, and colorectum have been demonstrated in randomized controlled trials (RCTs)[27,28] and prospective studies[29-31]. However, these RCTs measured only transcutaneous CO_2 tension ($PtcCO_2$) or end-tidal CO_2 pressure, not partial pressure of CO_2 in the arterial blood ($PaCO_2$).

The aim of the present prospective RCT was to assess the safety and efficacy of CO_2 insufflation during ESD for gastric neoplasms in patients under conscious sedation. Both $PtcCO_2$ and $PaCO_2$ were measured in order to directly evaluate CO_2 retention or acidemia. Furthermore, a continuous $PtcCO_2$ measuring system to monitor patient safety during CO_2 insufflation was validated.

MATERIALS AND METHODS

Study design and participants

This study was designed as a single-center RCT. Between January 2009 and December 2009, all consecutive patients undergoing ESD for gastric neoplasms at Gifu University Hospital in Japan were screened for this study. Gastric ESD was indicated for differentiated adenocarcinoma that was confined to the mucosa with no risk of lymph node metastasis, and for adenoma, regardless of its size or the presence of ulceration.

Patients were excluded if: (1) they had chronic pulmonary dysfunction defined as a forced expiratory volume in 1.0 second/forced vital capacity ($FEV_{1.0}$%) of < 70% or a vital capacity (%VC) of < 80%; (2) they were unable to understand the consent information required for participation; or (3) they declined participation. The study design was approved by the ethics committee for clinical research at Gifu University Hospital. All eligible individuals provided written informed consent prior to study enrollment. Randomization was conducted using sealed envelopes and patients were divided into two groups: the CO_2 insufflation group (CO_2 group) and the air insufflation group (Air group).

Examination schedule for study events before and after ESD

ESD was conducted during the first afternoon after hospital admission. On the second day in the hospital, blood tests, esophagogastroduodenoscopy, and CT of the chest and abdomen were performed. Blood tests for leukocyte count and C-reactive protein levels were repeated on the third hospital day. Axillary temperature was assessed 1 h after ESD and daily thereafter, at 06:00, 14:00, and 20:00 h.

ESD procedure and conscious sedation method

The standard ESD procedure was performed using a gastroscope with a single working channel and water jet function (GIF-Q260J; Olympus Optical Co., Tokyo, Japan) and a cap attachment (D-201-11804; Olympus). The gastric lesion was resected using either the DualKnife (KD-630L; Olympus) or the ITKnife2 (KD-611L; Olympus), depending on its location. A 0.4% high-molecular-weight hyaluronic acid solution containing epinephrine was injected into the submucosal layer to raise the lesion. Incision of the mucosal layer around the circumferential markings and subsequent direct dissection of the submucosal layer were performed with the DualKnife and ITKnife2.

Patients received diazepam for conscious sedation and pentazocine for analgesia. At the start of the ESD procedure, 5-10 mg of diazepam and 7.5-15.0 mg of pentazocine were injected intravenously for induction of anesthesia and analgesia, with an additional 5 mg of diazepam or 7.5 mg of pentazocine administered repeatedly as necessary. When the combination of diazepam and pentazocine did not achieve conscious sedation, intravenous midazolam was administered. Oxygen was administered nasally at 2.0 L/min during ESD, and the flow volume was adjusted by monitoring transcutaneous oxygen saturation (SpO_2). Arterial blood samples were immediately analyzed using a blood gas analyzer (ABL700; Radiometer Medical, Copenhagen, Denmark) after the ESD procedure, for the first 30 consecutive patients.

CO_2 insufflation and transcutaneous gas analysis

CO_2 was delivered using a CO_2 regulation unit (Olympus UCR; Olympus). The TOSCA measurement system and TOSCA 500 monitor (Linde Medical Sensors, Basel, Switzerland) were used to measure the $PtcCO_2$ noninvasively and continuously with an earlobe sensor attached by a low-pressure clip. We used a default temperature setting of 42 ℃ for the sensor and recalibrated the system before each ESD. The low-flow gas tube (MAJ-1742; Olympus) of the Olympus UCR was set at a constant rate of 1.4 L/min for CO_2 insufflation in all patients.

Definitions of outcome parameters and complications

Operation time was measured from the start of circumferential marking to the completion of resection.

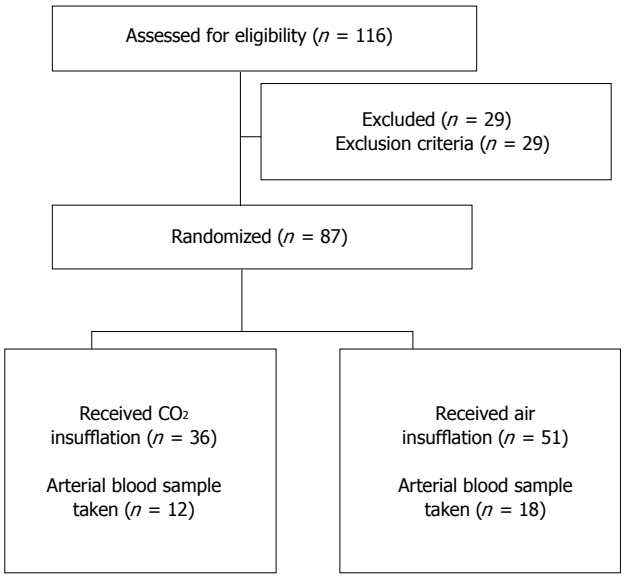

Figure 1 Flow chart of patient enrollment in the study and allocation into groups.

A diagnosis of perforation was made by direct endoscopic observation of visceral organs during ESD or by the presence of free air on follow-up plain chest radiography. Evidence of aspiration pneumonia was determined by the appearance of an obvious pneumonia shadow on a plain chest CT one day after ESD. Bleeding was defined as clinical evidence of bleeding after ESD, such as hematemesis or melena that required endoscopic treatment. A Mallory-Weiss tear (MWT) was defined as a mucosal tear or laceration adjacent to the esophagogastric junction with active bleeding, either spurting or oozing, during ESD.

Statistical analysis

Values are expressed as the number and percentage of patients or median (range). Differences in distribution of categorical variables between the two groups were analyzed by χ^2 or by Fisher's exact tests when required. The nonparametric Mann-Whitney *U* test was used for comparing continuous variables. A $P < 0.05$ was considered significant. All statistical analyses were conducted with JMP version 10 (SAS Institute, Cary, NC, United States).

RESULTS

Patient enrollment and group allocation

Of the 116 candidate patients for gastric ESD, 87 were enrolled in the trial and randomized. Among them, 36 received CO_2 insufflation and 51 received air insufflation. Twenty-nine patients were excluded due to impaired respiratory function ($n = 24$), severe chronic obstructive pulmonary disease requiring oxygen ($n = 3$), and inability to understand the consent information required for participation ($n = 2$).

Table 1 Patients and examination characteristics

Characteristic	CO_2 group (n = 36)	Air group (n = 51)	P value
Age, yr	74 (52-87)	70 (45-93)	NS
Sex, male/female	22/14	36/15	NS
$FEV_{1.0}$%	72 (70-89)	73 (70-93)	NS
%VC	103 (80-102)	109 (80-152)	NS
Location of lesion, n: upper/middle/lower	5/22/9	12/15/24	0.012
En bloc resection, n (%)	36 (100)	51 (100)	NS
Histopathologic type, n: tub1/tub2/por/sig/adenoma	18/3/0/0/15	36/6/2/1/6	0.020
Histologic depth, n: M/SM1/SM2	32/2/2	42/1/8	NS
Histopathologically curative resection, n (%)	31 (86.1)	43 (84.3)	NS
Tumor size, mm	18 (4-75)	17 (3-47)	NS
Resection size, mm	35 (22-110)	37 (23-95)	NS
Procedure time, min	46 (18-194)	48 (15-145)	NS
Dose of diazepam, mg	20 (5-30)	20 (5-30)	NS
Dose of pentazocine, mg	18.8 (7.5-45)	22.5 (7.5-45)	NS
Patients receiving midazolam, n (%)	3 (8.3)	5 (9.8)	NS
Dose of midazolam, mg	10.0 (2.5-20)	7.5 (2.5-10)	NS

Data are presented as n, n (%), or median (range). $FEV_{1.0}$%: Forced expiratory volume 1.0 second/forced vital capacity; %VC: Vital capacity; tub1: Well-differentiated tubular adenocarcinoma; tub2: Moderately differentiated tubular adenocarcinoma; por: Poorly differentiated adenocarcinoma; sig: Signet ring cell carcinoma; M: Tumor confined to the mucosa; SM1: Tumor confined to the submucosa and tumor invasion within 0.5 mm of the muscularis mucosae; SM2: Tumor confined to the submucosa and tumor invasion of 0.5 mm or more into the muscularis mucosae; NS: Not significant.

Table 2 Patient characteristics and parameters of arterial blood analysis

Characteristic	CO_2 group (n = 12)	Air group (n = 18)	P value
Age, yr	73 (63-82)	70 (45-87)	NS
Procedure time, min	66 (26-156)	56 (23-107)	NS
Dose of diazepam, mg	17.5 (10.0-22.5)	20.0 (5.0-30.0)	NS
Dose of pentazocine, mg	15.0 (15.0-30.0)	22.5 (15.0-37.5)	NS
Patients receiving midazolam, n (%)	1 (8.3)	1 (5.6)	NS
Dose of midazolam, mg	2.5	5	
pH value	7.36 (7.34-7.39)	7.36 (7.33-7.40)	NS
$PaCO_2$, mmHg	44.6 (39-53)	45 (40-50)	NS
PaO_2, mmHg	168 (68-203)	143 (78-259)	NS
HCO_3^-, mEq/L	25.1 (23.0-30.0)	25.5 (22.0-27.0)	NS
Base excess, mEq/L	-0.05 (-2-4)	0.3 (-3.1-2.6)	NS

Data are presented as median (range) unless otherwise indicated. PaO_2: Partial pressure of oxygen in arterial blood; $PaCO_2$: Partial pressure of carbon dioxide in arterial blood; NS: Not significant.

The first 30 participants (12 from the CO_2 group and 18 from the Air group) underwent arterial blood gas analysis (Figure 1).

Baseline characteristics

Baseline characteristics for each treatment group are shown in Table 1. The location (P = 0.012) and histopathology (P = 0.020) of the gastric lesions were significantly different between the groups. The median procedure time was 46 min in the CO_2 group and 48 min in the Air group (not significant). There were no differences in respiratory function ($FEV_{1.0}$% and %VC) between the groups. No significant differences were observed in the median dose of sedative drugs administered to the patients in each group.

Arterial blood gas analysis

No significant differences were observed between the two groups that received blood gas analysis after ESD with respect to the median procedure time and the median dose of sedative drugs (Table 2). There was no significant difference between the CO_2 group and the Air group in any blood gas parameters, including $PaCO_2$ (44.6 mmHg *vs* 45 mmHg). The median pH values were 7.36 in both groups, and there were no patients with acidemia. As shown in Figure 2, $PtcCO_2$ was significantly correlated with $PaCO_2$ (r = 0.66; P < 0.001). The median difference between $PaCO_2$ and $PtcCO_2$ was 4.8 mmHg.

$PtcCO_2$ and SpO_2 before and after ESD

The median $PtcCO_2$ before (baseline) and after ESD was 39 mmHg (28-52 mmHg) and 50 mmHg (41-68 mmHg), respectively, in the CO_2 group, and 40 mmHg (22-51 mmHg) and 50 mmHg (40-64 mmHg), respectively, in the Air group. The $PtcCO_2$ increased significantly (P < 0.001) after the procedure in both groups, though there was no significant difference between the groups. The median peak $PtcCO_2$ during the procedure was 52 mmHg (43-68 mmHg) in the

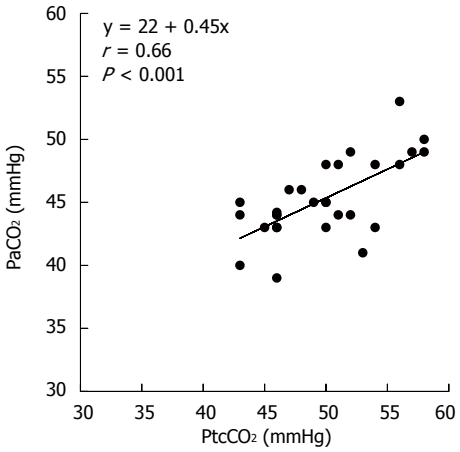

Figure 2 Correlation between partial pressure of carbon dioxide in arterial blood ($PaCO_2$) and transcutaneous carbon dioxide tension ($PtcCO_2$) after endoscopic submucosal dissection.

CO_2 group and 51 mmHg (40-64 mmHg) in the Air group (not significant, Table 3). There was no correlation between the procedure time and $PtcCO_2$ elevation in either the CO_2 group or the Air group (Figure 3). The median minimum SpO_2 level and oxygen flow rate were similar between the groups (98% and 2.0 L/min, respectively).

Incidence of complications and duration of hospital stay

ESD-related complications and the duration of the hospital stay are listed in Table 4. CO_2 insufflation did not cause any adverse events such as CO_2 narcosis or gas embolism. No significant difference was observed between the two groups with respect to the incidence of fever (body temperature > 37.5 ℃), pneumonia, perforation, or post-ESD hemorrhage. The incidence of MWTs was significantly lower in the CO_2 group than in the Air group (P = 0.013). Serum C-reactive protein levels and white blood cell counts on days 1 and 3 after ESD were not significantly different between the groups, and the median hospital stay was equivalent at 7 d for each group.

DISCUSSION

The safety and efficacy of insufflation using CO_2 as an alternative to air has been demonstrated in several RCTs for various kinds of endoscopic procedures[18-20,22-24,27,28]. In gastric ESD, Maeda *et al*[28] reported that CO_2 insufflation significantly reduced the volume of residual gas in the digestive tract compared with air insufflation. In the present study, under similar ESD conditions with regard to procedure time, respiratory function, sedative drug doses, and minimum SpO_2, neither the post-procedure $PaCO_2$ nor the median $PtcCO_2$ differed between the CO_2 group and the Air group. The peak $PtcCO_2$ during ESD also did not differ between the two groups. Furthermore, we confirmed a strong correlation between $PaCO_2$ and $PtcCO_2$. Therefore, the $PtcCO_2$ value can be used as a surrogate marker of CO_2 retention in patients who received CO_2 insufflation during ESD.

In this study, the maximum $PtcCO_2$ and $PaCO_2$ reached 68 mmHg and 53 mmHg in the CO_2 group, and 64 mmHg and 50 mmHg in the Air group, respectively. However, no adverse events such as acidemia, CO_2 narcosis, or SpO_2 depression were reported in either group. The elevated $PtcCO_2$ in both groups after the procedures is likely due to respiratory depression associated with conscious sedation. Several studies have demonstrated that such respiratory depression is involved in the elevation of $PaCO_2$ or $PtcCO_2$ in patients undergoing endoscopic treatment[27,29-31]. There was no correlation between procedure time and $PtcCO_2$ elevation in the present study. These results indicate that CO_2 insufflation is as safe as air insufflation for gastric ESD when the CO_2 insufflation rate is 1.4 L/min and the median procedure time is 48 min.

With regard to complications, the incidence of MWTs was significantly lower in the CO_2 group than in the Air group. This may be due to the rapid absorption of CO_2 by the body compared to air. Indeed, CO_2 insufflation in esophagogastroduodenoscopy efficiently reduces MWTs by lowering the tension of the gastric mucosa caused by residual gas in the stomach[32]. To our knowledge, the present study is the first RCT to demonstrate the benefit of CO_2 insufflation in reducing the risk of MWTs during ESD.

Because respiratory depression due to conscious sedation may lead to CO_2 retention, arterial CO_2 monitoring during lengthy endoscopic procedures is important, even if the patient's respiratory function is normal. However, arterial blood sampling is invasive and it is not practical to measure $PaCO_2$ serially in all ESD patients. Instead, $PtcCO_2$, which correlates well with $PaCO_2$, can be measured noninvasively and continuously. $PtcCO_2$ is usually greater than $PaCO_2$ by 5-6 mmHg[33,34]. Indeed, in the present study, the median difference between these values was 4.8 mmHg. Because of the strong correlation between $PtcCO_2$ and $PaCO_2$, a $PtcCO_2$ monitoring system is considered a reliable and efficient alternative to $PaCO_2$ measuring. Thus, arterial blood analysis was not continued after the first 30 patients.

This study has some limitations. First, 27 patients (23.3%) who had chronic pulmonary dysfunction were excluded, as the safety of CO_2 insufflation during gastric ESD has not been established for these patients. We recently reported the safety of CO_2 insufflation during gastric ESD in patients with pulmonary dysfunction ($FEV_{1.0}$% < 70% or %VC < 80%) under conscious sedation[35]. However, in patients with severe obstructive pulmonary disease, a longer procedure time may increase the risk of CO_2 retention because there is a significant correlation between $PtcCO_2$ elevation and ESD procedure time in

Table 3 Transcutaneous carbon dioxide tension values, transcutaneous oxygen saturation values, and oxygen flow rate

Variable	CO_2 group (n = 36)	Air group (n = 51)	P value
Baseline $PtcCO_2$, mmHg	39 (28-52)[1]	40 (22-51)[1]	NS
$PtcCO_2$ after ESD, mmHg	50 (41-68)[1]	50 (40-64)[1]	NS
Peak $PtcCO_2$, mmHg	52 (43-68)	51 (40-64)	NS
$PtcCO_2$ > 60 mmHg during ESD	3 (8.3)	2 (3.9)	NS
Minimum SpO_2, %	98 (90-100)	98 (89-100)	NS
Oxygen flow rate, L/min	2 (1-5)	2 (2-4)	NS

[1]The $PtcCO_2$ increased significantly (P < 0.001) after the procedure. Data are presented as n (%) or median (range). ESD: Endoscopic submucosal dissection; $PtcCO_2$: Transcutaneous carbon dioxide tension; SpO_2: Transcutaneous oxygen saturation.

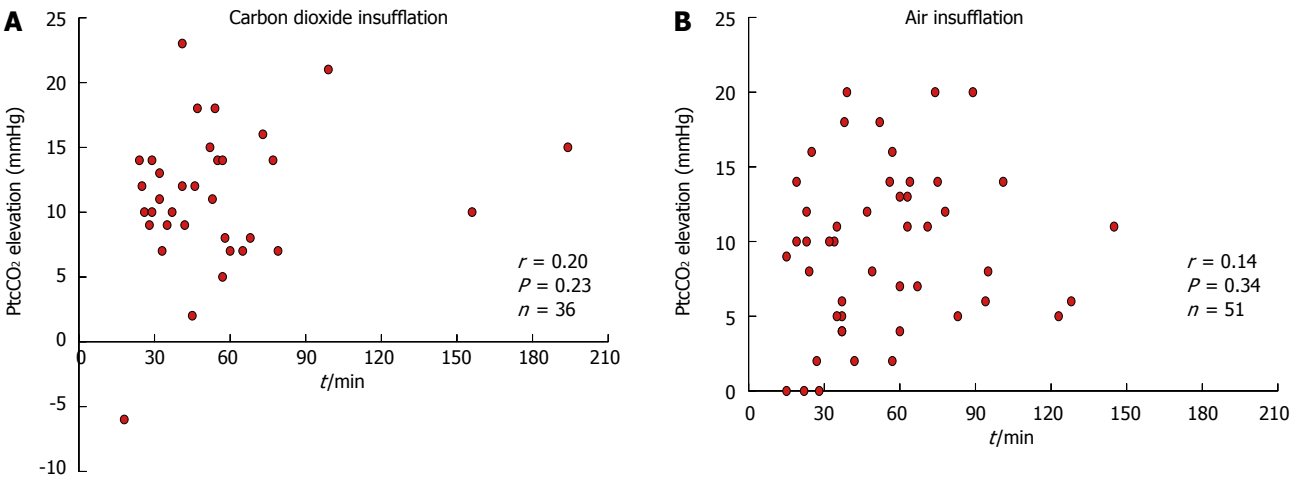

Figure 3 Elevation of transcutaneous carbon dioxide tension ($PtcCO_2$) in the carbon dioxide and air insufflation groups. There was no significant correlation between the procedure time and $PtcCO_2$ elevation in either group.

Table 4 Complications from endoscopic submucosal dissection, blood parameters, and duration of hospital stay

Variable	CO_2 group (n = 36)	Air group (n = 51)	P value
Fever (body temperature > 37.5 ℃)	9 (25.0)	9 (17.6)	NS
Pneumonia	3 (8.3)	5 (9.8)	NS
Perforation	1 (2.7)	1 (1.9)	NS
Post-procedure hemorrhage	0	4 (7.8)	NS
Mallory-Weiss tears	0	8 (9.8)	0.013
CRP on day 1 after ESD, mg/dL	0.30 (0.09-6.19)	0.40 (0.04-3.62)	NS
CRP on day 3 after ESD, mg/dL	2.00 (0.18-7.83)	2.00 (0.08-14.20)	NS
WBC on day 1 after ESD, n/μL	9020 (3730-15680)	8090 (4510-13450)	NS
WBC on day 3 after ESD, n/μL	6310 (2560-11200)	6260 (3100-10660)	NS
Hospital stay, d	7 (7-16)	7 (7-20)	NS

Data are presented as n (%) or median (range). ESD: Endoscopic submucosal dissection; CRP: C-reactive protein; WBC: White blood cell.

patients with pulmonary dysfunction[35]. Therefore, $PtcCO_2$ should be carefully monitored in these patients to avoid severe complications such as CO_2 narcosis and acidemia. Second, the number of patients who underwent arterial blood gas analysis in the present study may be too small. The present study was also a single-center trial. Therefore, further larger prospective multicenter studies are required to confirm the safety and efficacy of CO_2 insufflation for gastric ESD by evaluating both $PtcCO_2$ and $PaCO_2$ values.

In conclusion, this study strongly suggests that CO_2 insufflation is safe and effective during gastric ESD under conscious sedation in patients without pulmonary dysfunction. Furthermore, CO_2 insufflation reduces the incidence of MWTs compared to air insufflation. However, conscious sedation might increase the risk of CO_2 retention and the $PtcCO_2$ should be monitored carefully in these cases.

COMMENTS

Background

The safety and efficacy of insufflation using carbon dioxide (CO_2) as an alternative to air has been demonstrated in several randomized controlled trials for various kinds of endoscopic procedure. However, there has been no report on the safety and efficacy of CO_2 insufflation for gastric endoscopic submucosal

dissection (ESD) based on the measurement of both the partial pressure of CO_2 in the arterial blood and transcutaneous CO_2 tension.

Research frontiers

Based on the observation that CO_2 is rapidly absorbed from the bowel, this study investigated the effect of CO_2 insufflation on patients undergoing gastric ESD.

Innovations and breakthroughs

CO_2 insufflation remarkably reduced the incidence of Mallory-Weiss tears without any adverse events.

Applications

The safety and efficacy of CO_2 insufflation during gastric ESD in patients under conscious sedation were demonstrated in this study. Further investigation in patients with pulmonary dysfunction is necessary.

Peer-review

The present study is the first randomized controlled trial to demonstrate that CO_2 insufflation can reduce the risk of Mallory-Weiss tears during ESD, and represents a major contribution to this field.

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CASE REPORT

Anti-inflammatory effect of recombinant thrombomodulin for fulminant hepatic failure

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Author contributions: Kurokohchi K managed the patient's case, contributed to the literature search, and wrote the manuscript; Kurokohchi K made substantial contributions to the concept and design of the report; Imataki O qualified the patient's data, suggested important intellectual content, and reviewed the manuscript; Kurokohchi K and Imataki O contributed equally to this work; Kubo F was involved in the drafting and supervision of the manuscript and took part in critical discussions; all authors read and approved the final version of the manuscript.

Informed consent statement: All study participants, or their legal guardian, provided written informed consent prior to study enrollment.

Conflict-of-interest statement: The authors declare that they have no competing interests.

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Abstract

Fulminant hepatic failure (FHF) is a critical illness that can be comorbid to primary liver damage. FHF shows a high mortality rate, and patients with FHF require intensive therapy, including plasma apheresis. However, intensive care at the present is not enough to restore the severe liver damage or promote hepatocellular reproduction, and a standard therapy for the treatment of FHF has not been established. An 86-year-old female with FHF was admitted to our hospital. Her manifestation demonstrated a clinical situation of systemic inflammatory response syndrome (SIRS) and disseminated intravascular coagulation. A diagnosis of fulminant hepatitis was made according to the definition given in the position paper of the American Association for the Study of Liver Diseases. Her serum hepatocyte growth factor (HGF) level had increased to 11.84 ng/mL. The HGF level indicated massive liver damage as seen in FHF. Recombinant thrombomodulin (rTM) was administered daily from the admission day for 1 wk at 380 U/kg. The patient's white blood cells and C-reactive protein responded to the rTM treatment within a few days. The HGF level and PT recovered to the normal range. The levels of proinflammatory cytokines (tumor necrosis factor-α and interleukin-1β) were suppressed by the administration of rTM. The patient's hepatic function (*e.g.*, PT and albumin) completely recovered without plasma exchange. rTM may modulate the over-response of SIRS with the improvement of proinflammatory cytokines. The underlying mechanism is thought to be the inhibitory effect of rTM on high-mobility group box 1 (HMBG1). The pathogenesis of HMBG1 protein in fulminant hepatic failure has been

already known. A novel favorable effect of rTM for SIRS would be promising for FHF, and the wide application of rTM for SIRS should be considered.

Key words: Fulminant hepatic failure; Disseminated intravascular coagulation; Thrombomodulin; Hepatocyte growth factor; Systemic inflammatory response syndrome

Core tip: Fulminant hepatic failure (FHF) is a critical illness that can be comorbid to primary liver damage. However, no standard therapy for the treatment of FHF has been established. We experienced a fatal FHF case with systemic inflammatory response syndrome followed by disseminated intravascular coagulopathy (DIC). We administered recombinant thrombomodulin (rTM) for the treatment of DIC, which ameliorated all lethal conditions (coagulopathy and inflammation). Monitoring of proinflammatory cytokines, hepatocyte growth factor, and prothrombin time revealed the response of FHF to rTM. We hypothesized an anti-inflammatory effect of rTM-enhanced hepatocyte regeneration through inactivation of high-mobility group box 1.

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INTRODUCTION

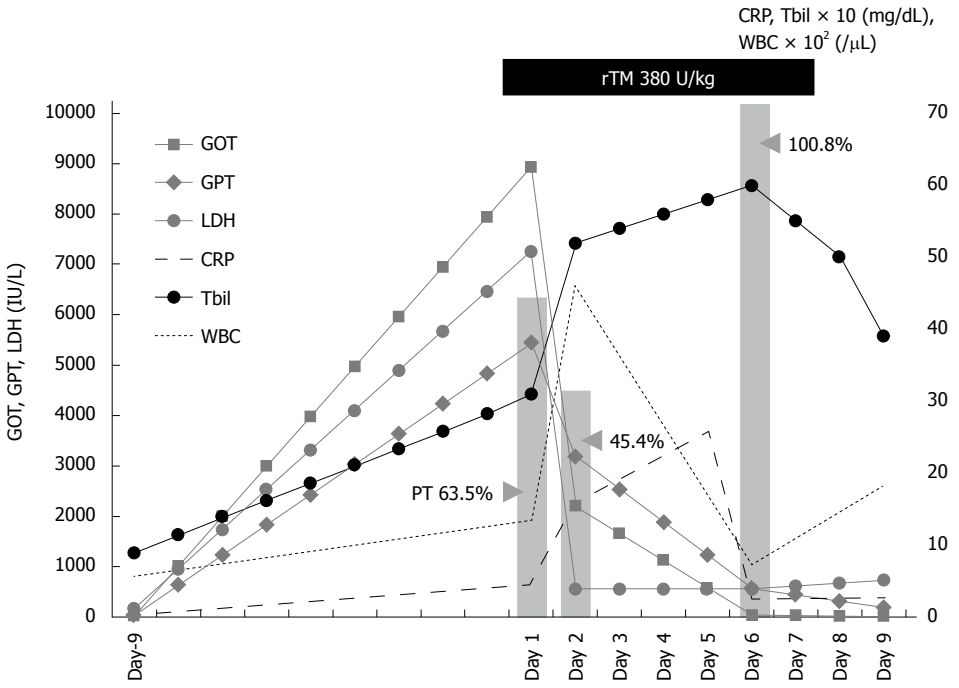
Fulminant hepatic failure (FHF) is a critical illness that can be comorbid to primary liver damage[1]. FHF shows a high mortality rate, and patients with FHF require intensive therapy[2]. However, intensive care at the present is not enough to restore the severe liver damage or promote hepatocellular reproduction, and a standard therapy for the treatment of FHF has not been established[1]. FHF is produced from any background of liver injury, such as those due to drugs, autoimmune disorders, and acute or chronic viral infections[3]. Severe FHF is fatal, and the survival rate of FHF patients in the Japanese population has been estimated as 11.5%-24.4%[4]. Almost all patients with FHF are at risk of multi-organ failure. The only effective therapy for FHF is liver transplantation, but the indications for this are limited[2]. To rescue FHF patients, non-transplantation therapy, corticosteroids, and plasma apheresis can be applied, but clinical evidence of the efficacy of these non-transplantation therapies is scarce. Several risk factors for fulminant hepatitis are known. FHF patients with systemic inflammatory response syndrome (SIRS) also show poor prognoses. The concomitance of any complication also significantly decreases the survival rate of FHF patients[2,4].

Thrombomodulin (TM) is a physiological anti-coagulation factor that acts as a direct inactivator of thrombin and a suppressor of coagulation factors Va and Ⅷa *via* activated protein C (APC)[5]. Recombinant thrombomodulin (rTM) was developed as a therapeutic agent for disseminated intravascular coagulation (DIC) syndrome, and it is widely used in a variety of clinical situations[6]. A loss or lack of TM disrupts the protein C anticoagulant pathway and causes thrombosis[7]. Some clinical situations associated with SIRS (including severe infection, sepsis, trauma, and organ inflammation) may cause a decrease of TM, which is a substantial pathogenesis of DIC syndrome[8]. Additionally, a novel anti-inflammatory effect of TM and the APC pathway has been gradually unveiled[5], and aggregated knowledge has established the dual mechanisms of TM in DIC and SIRS by the modulation of aberrant coagulopathy and the attenuation of inflammatory milieu. However, the anti-inflammatory effect of rTM in FHF has not been elucidated, and the greater involvement of rTM in SIRS, a non-specific inflammatory disease, is not yet understood.

CASE REPORT

An 86-year-old female visited an outpatient clinic of our hospital due to increased fever, diarrhea, and decreased blood pressure. Her laboratory data indicated intensely impaired liver function, bilirubinemia, and renal dysfunction: aspartate transaminase 8929 U/L, alanine transaminase 5449 U/L, lactate dehydrogenase (LDH) 7248 U/L, total bilirubin (Tbil) 3.1 mg/dL, blood urea nitrogen (BUN) 34.8 mg/dL, Cr 1.9 mg/dL, and PT 42%. She was diagnosed as having concomitant DIC based on the coagulation test: PT 63.5%, PT-INR 1.23, APTT 26.0 s, fibrinogen 273 mg/dL, FDP > 80 μg/dL, and D-dimer > 5.00 μg/mL. She did not have a history of viral hepatitis or autoimmune liver disease, such as primary biliary cirrhosis (PBC) or autoimmune hepatitis (AIH). Radiological findings on admission and on previous examinations revealed no fatty liver or splenomegaly. A diagnosis of fulminant hepatitis was made according to the definition given in the position paper of the American Association for the Study of Liver Diseases (AASLD)[2]. The cause of the acute hepatic failure was not specified, but we suspected that it was associated with her treatment with a non-steroidal anti-inflammatory drug (NSAID), diclofenac suppository 25 mg/d, that was administered for lumbago starting 2 mo prior[3].

On the day of the patient's admission to our hospital, corticosteroid pulse therapy was initiated (500 mg/d, × 3 d). At the same time, we started treatment with biological agents derived from human blood in order to conserve the patient's liver function. We transfused fresh frozen plasma (FFP) 2 units/d every



	Day 1	Day 6	Day 9	
HGF	11.84	0.59	1.36	ng/mL
TNF-α	14.90	7.10	12.10	ng/mL
IL-1b	11.00	< 10	< 10	ng/mL

Figure 1 Clinical course of the patient, an 80-year-old Japanese female. White blood cells (WBC) and C-reactive protein (CRP) recovered within a few days after the initiation of recombinant thrombomodulin (rTM) treatment. PT (indicated by a bar) rose to 100.8% from 54.4%. WBC, CRP, and transaminase (GOT and GPT) all recovered, corresponding to the rTM administration. Tbil: Total bilirubin; TNF: Tumor necrosis factor; IL: Interleukin.

day until the end of treatment due to the patient's death on the ninth day after her admission. We also administered rTM 380 U/kg per day for 6 d. Empirical treatment for infection was concomitantly driven by ceftriaxone (1 g × 1/d), cefozopran (1 g × 2/d), and cefoperazone/sulbactam (1 g × 2/d) appropriately dose-adjusted to the patient's organ function (Figure 1). The clinical and laboratory alternation of the patient's clinical course is illustrated in Figure 1, demonstrating a rapid decrease of liver function test values (GOT, GPT, LDH, ALP, and Tbil) immediately after the initiation of the treatment with rTM. The patient's white blood cells and inflammatory reactive protein [C-reactive protein (CRP)] gradually improved over the next few days. We also found her hepatocyte growth factor (HGF) and inflammatory or proinflammatory cytokines [tumor necrosis factor (TNF)-α and interleukin (IL)-1β] levels were all diminished after the initiation of treatment with rTM (Figure 1).

Proteins reflecting the reproduction of liver cells such as PT and albumin were improved during the patient's treatment, apparently responding to the rTM injection. Her hepatic function completely recovered without plasma exchange or any interventional apheresis. The patient did not exhibit the development of hepatic encephalopathy (*e.g.*, confusion, stupor, and coma). Unfortunately, respiratory failure caused her death on day 9 after the initiation of treatment, but her hepatic failure was not a factor. An autopsy was not performed.

DISCUSSION

Severe FHF is fatal, with the only effective therapy being liver transplantation, but the indications for this are limited[2]. HGF was purified as a hepatocyte proliferation agent from patients with fulminant hepatitis and promotes hepatocyte mitosis[9]. Substantial elevations of HGF in FHF patients have been observed in clinical settings[9]. It was hypothesized that external supplementations of an overdose of HGF may promote the regeneration of hepatocytes and modulate hepatic function[10], but recombinant HGF (rhHGF) administration failed to show efficacy as a treatment for FHF in a Phase Ⅰ/Ⅱ study setting[10]. These observations may indicate that an elevated plasma HGF level corresponds to hepatocyte breakage, explaining why no obvious efficacy of additional HGF treatment has been observed to date. In fact, it is known that the large-scale production of HGF occurs in the onset of fulminant hepatitis irrespective of causes of hepatitis, such as drugs, viral infection, and autoimmune-mediated causes[11].

HGF has also been investigated as a hepatocyte

protective agent for the therapy of hepatitis[12]. However, many clinical observations showed that additional external HGF supplementation was not hepatotropic and did not induce the regeneration of hepatocytes or produce better outcomes in humans[10]. The up-regulation of HGF expression in injured liver is mediated by the pro-inflammatory cytokines IL-1, IL-6, IFN-γ, and TNF-α[12]. We thus suggest that an increase in plasma HGF levels is a reflection of the degree of liver injury[12]. The dramatic improvement of our patient's HGF level suggests that rTM treatment attenuated her liver cell damage. This is substantiated based on the patient's profile of pro-inflammatory cytokines TNF-α and IL-1β, and during her rTM therapy.

The anti-inflammatory role of rTM has recently been highlighted in the field of clinical management for DIC[13]. rTM corrects coagulopathy through the inhibition of thrombin and the activation of protein C. The major pharmacological effect of rTM was demonstrated to be an enhanced physiological effect on APC[5]. Several research groups reported the anti-inflammatory effect of TM itself in the lectin-like domain, which can inactivate high-mobility group box 1 (HMGB1)[14], interfere with complement activation[15], and neutralize endotoxin[16]. HMGB1 is an inflammatory mediator that acts as a nuclear factor, and is secreted by activated monocytes and macrophages[17]. Some clinical articles support the pathogenesis of HMGB1 protein in FHF[18]. We did not define an elevation of HMGB1 in our patient's case, however rTM treatment may have suppressed the activation of HMGB1. Corticosteroid therapy can also modulate the inflammatory response[19]. An inhibitory effect of glucocorticoids on HMGB1-induced TNF-α production has been observed[19]. However, the effect is only for the reduction of extracellular HMGB1 expression. The effect of systemic corticosteroid treatment was confined to a reduction in extracellular HMGB1 expression, but not intracellular expression[20]. The inflammatory cellular responses downstream from HMGB1 are less well understood[19]. Glucocorticoids inhibit HMGB1-induced TNF-α production in the pathway downstream from HMGB1. Moreover, HMGB1 is secreted mainly by monocytes and corticosteroid, and cannot adequately suppress activated monocytes[21]. Thus, other pathways from HMGB1 are assumed. This mechanism can be specifically targeted to therapeutic advantage in sterile SIRS associated with HMGB1 elevation, refractory to corticosteroids[19,21].

Because our patient's clinical status before her admission to our hospital was not fully evaluated, we categorized her case as fulminant hepatitis of the subacute type[9] according to her medication history. Coagulopathy will occur generally in patients with FHF[2]. Beyond the anti-coagulant mechanism, however, the administration of rTM would be preferable to curing inflammatory status, especially SIRS, possibly by inactivating HMBG1. rTM as a novel treatment for FHF could become promising when the inflammatory profile in FHF is identified, but the categorization of inflammation in FHF is not yet underway, to the best of our knowledge.

In the present case, both a variety of biomarkers reflecting hepatocellular damage (including GOT, GPT, LDH, and ALP) and inflammatory cytokines decreased very rapidly immediately after the administration of rTM. In cases of FHF, most liver cells are often necrotized at once by the intense immunological reaction, resulting in the decrease of GOT and GPT levels. In those cases, liver cells are not reproduced, and the levels of PT and hepaplastin test do not recover. However, surprisingly, our patient's decreased PT level was rapidly recovered (to over 100%) immediately after the administration of rTM. We have never experienced a case like this one in which the PT level was so remarkably recovered simply by the administration of FFP or a blood transfusion without plasma apheresis. Thus, rTM may play a critical role and become a new and effective therapeutic agent for the treatment of FHF and other disorders. Other organ failures with systemic or local inflammation may also be treatable with rTM[22]. We propose the necessity of confirming the effectiveness of rTM for the treatment of FHF in further studies.

COMMENTS

Case characteristics

An 86-year-old female came to an ambulatory care facility with fever, diarrhea, and hypotension.

Clinical diagnosis

The patient showed dehydration, which was diagnosed based on fever, tachycardia, and decreased blood pressure.

Differential diagnosis

The cause of dehydration was initially thought to be an infection.

Laboratory diagnosis

The patient's laboratory tests showed severe liver and renal dysfunction with coagulopathy: aspartate transaminase 8929 U/L, alanine transaminase 5449 U/L, lactate dehydrogenase 7248 U/L, total bilirubin 3.1 mg/dL, BUN 34.8 mg/dL, Cr 1.9 mg/dL, and PT 42%.

Imaging diagnosis

Computed tomography imaging did not reveal hepatomegaly or splenomegaly, and did not detect an infection focus.

Treatment

The authors administered recombinant thrombomodulin (rTM), which ameliorated the coagulopathy and hepatic function.

Related reports

The potential role of rTM, the anti-inflammatory effect of which has been the focus in the clinical management of disseminated intravascular coagulopathy (DIC), is now being examined for the pathology of systemic inflammatory response syndrome (SIRS), including fulminant hepatic failure (FHF).

Term explanation

rTM is a novel product of human soluble thrombomodulin fragments that acts as a direct inactivator of thrombin and exerts a powerful inhibitory effect on activated Factors Ⅴ and Ⅷ by activating protein C.

Experiences and lessons

This case suggested that rTM could be a promising agent to treat not only DIC, but also any inflammatory pathogenesis including SIRS, as shown in this case of FHF. The authors hypothesized that the underlying mechanism is an inhibition of the high-mobility group box 1 (HMGB1) pathway, which is known to

be elevated in the sera of patients with FHF.

Peer-review

The anti-inflammatory pharmacological effect of rTM has been attracting more attention for further clinical application to other pathological conditions. In the present case, the clinical effect of rTM in FHF with SIRS was observed, revealing the kinetics of growth factors (hepatocyte growth factor) and inflammatory/proinflammatory cytokines (tumor necrosis factor-α and interleukin-1β).

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CASE REPORT

Gastric adenocarcinoma of fundic gland type: Five cases treated with endoscopic resection

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Institutional review board statement: This study (case report) does not involve human and/or animal subjects.

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Abstract

Recently, a new disease entity termed gastric adenocarcinoma of fundic gland type (GA-FG) was proposed. We treated five cases of GA-FG with endoscopic submucosal dissection. All tumors were small and located in the upper third of the stomach. Four tumors were macroscopically identified as 0-IIa and one was identified as 0-IIb. Narrow-band imaging with magnifying endoscopy showed an irregular microvascular pattern in 2 cases and a regular microvascular pattern in the remainder. All tumors arose from the deep layer of the lamina propria mucosae and showed submucosal invasion. Lymphatic invasion was seen only in one case, while no venous invasion was recognized. All tumors were positive for pepsinogen-I and MUC6 by immunohistochemistry. None showed p53 overexpression, and the labeling index of Ki-67 was low in all cases. All cases have been free from recurrence or metastasis. Herein, we discussed the clinicopathological features of GA-FG in comparison with past reports.

Key words: Gastric adenocarcinoma of fundic gland type; Pepsinogen-I; Chief cell; Endoscopic diagnosis; Narrow-band imaging with magnifying endoscopy; Endoscopic submucosal dissection

Core tip: Recently, a new disease entity termed gastric adenocarcinoma of fundic gland type (GA-FG) was proposed. We treated five cases that were diagnosed as GA-FG with endoscopic submucosal dissection. GA-FG has characteristic findings in endoscopic and pathological examinations. For accurate and early endoscopic diagnosis of GA-FG, careful endoscopic examination and detailed pathological evaluation are important. Herein, we discussed the clinicopathological

features of GA-FG in comparison with past reports.

Miyazawa M, Matsuda M, Yano M, Hara Y, Arihara F, Horita Y, Matsuda K, Sakai A, Noda Y. Gastric adenocarcinoma of fundic gland type: Five cases treated with endoscopic resection. *World J Gastroenterol* 2015; 21(26): 8208-8214 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i26/8208.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i26.8208

INTRODUCTION

According to clinicopathological studies of gastric adenocarcinoma, it has been assumed that differentiated adenocarcinomas arise from intestinal metaplasia involving *Helicobacter pylori* (*H. pylori*) infection, and thus, has an intestinal phenotype while undifferentiated adenocarcinomas develop directly from the proper gastric mucosa without the process of intestinal metaplasia, and therefore, has a gastric phenotype. Recently, immunohistochemical staining for mucin has proven that some differentiated adenocarcinomas have a gastric phenotype[1,2]. Early differentiated adenocarcinomas with a gastric phenotype are thought to be difficult to diagnose by biopsy, which examines only a small amount of specimen, because of their mild histological atypism.

Regarding differentiated gastric adenocarcinomas with a gastric phenotype, Yao *et al*[3] in 2006 reported cases of extremely well-differentiated adenocarcinoma resembling gastric foveolar epithelium, mucous neck cells and pyloric glands. In 2007, Tsukamoto et al reported the first case of adenocarcinoma with differentiation to chief cells that comprise the fundic gland[4]. Furthermore in 2010, Ueyama *et al*[5] reported 10 cases that showed differentiation to chief cells, and proposed a new disease entity termed gastric adenocarcinoma of fundic gland type (GA-FG) based on their clinicopathological features. Since it is assumed that GA-FGs arise from normal gastric mucosa of the fundic gland region without intestinal metaplasia, the percentage of GA-FGs among all gastric adenocarcinomas is expected to increase along with decreasing prevalence of *H. pylori* infection.

Although the concept of GA-FG has been gradually spreading, quite a few cases may still be undiagnosed due to the difficulty in making a correct diagnosis. Since the current general consensus on treatment of early gastric adenocarcinomas is by endoscopic submucosal dissection (ESD), early endoscopic and pathological diagnosis of endoscopically resectable GA-FG is necessary. For accurate and early diagnosis, careful examination using narrow-band imaging with magnifying endoscopy (NBI-ME) in addition to conventional endoscopy and detailed pathological evaluation using immunohistochemical staining are extremely important. Pathological characteristics of GA-FG have occasionally been reported, but to the best of our knowledge, detailed reports on its endoscopic findings are not available. Herein, we examined and discussed the clinicopathological features, especially the endoscopic findings of GA-FG treated with ESD.

CASE REPORT

We studied the clinicopathological features of GA-FG treated with ESD at Toyama Prefectural Central Hospital from 2010 to 2013. We defined GA-FG as neoplastic lesions, which were composed of cells that mimic the fundic gland cells and were positive for pepsinogen-Ⅰ (a marker of chief cells) by immunohistochemical staining. Lesion sites, macroscopic types and histopathological findings were described according to the Japanese classification of gastric carcinoma, 3rd English edition[6]. Before performing ESD, upper gastrointestinal endoscopy was conducted by employing NBI-ME in addition to conventional white light endoscopy. Endoscopic findings obtained by NBI-ME were described according to the VS classification system of Yao *et al*[7] While one biopsy specimen was obtained from a lesion for histological diagnosis, another four specimens were taken from the surrounding mucosa at a 5 mm distance from the margin of the lesion to verify non-malignancy and to assess the status of the background gastric mucosa.

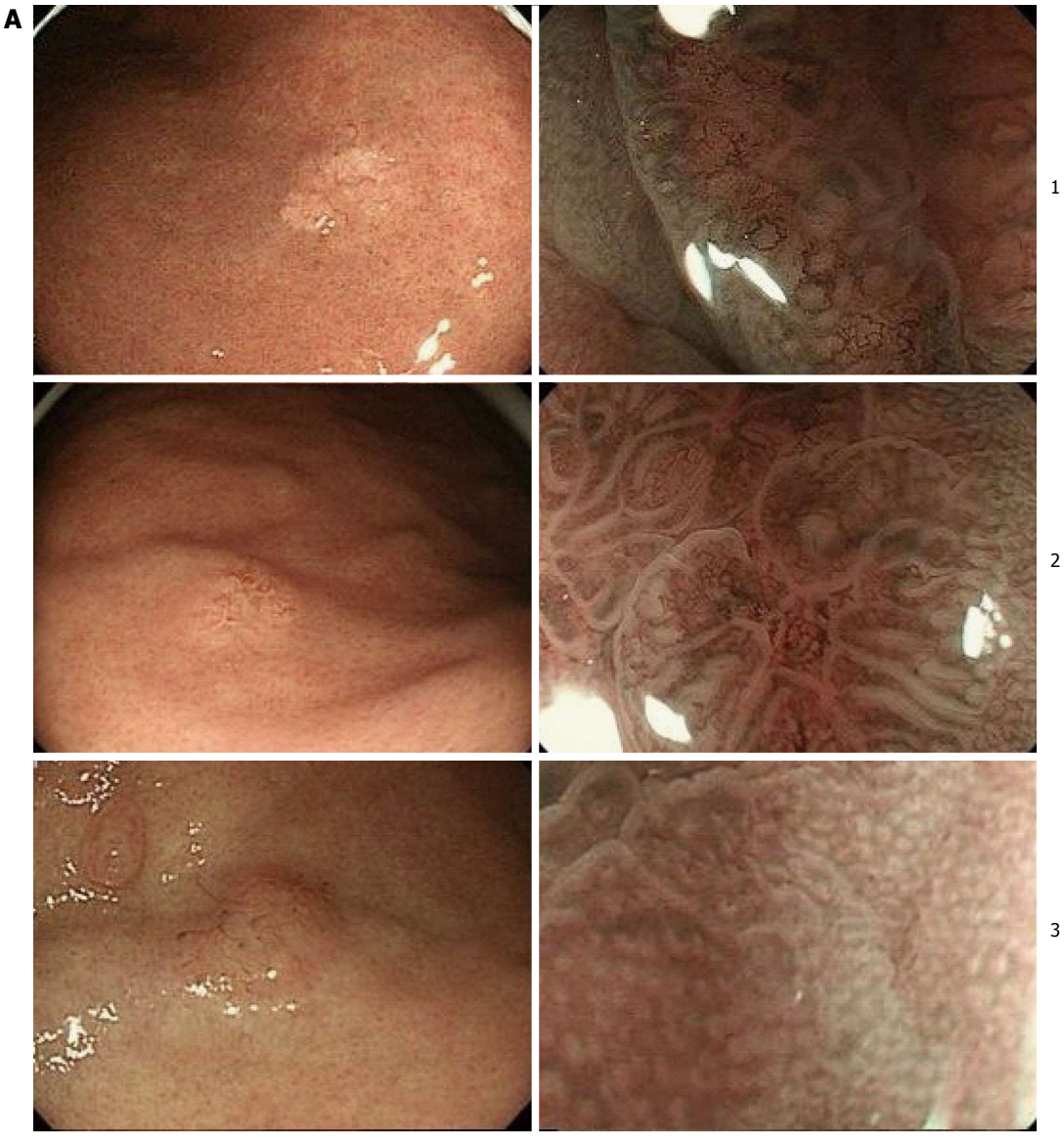
Of 506 early gastric adenocarcinomas that underwent ESD at our institution during a period from May 2010 to March 2013, 5 cases (0.98%) were GA-FG, including 3 males and 2 females with ages ranging from 67 to 78 years (average, 72.2 years). The characteristics of these 5 cases are summarized in Table 1. None of these cases had serum anti-*H. pylori* antibody. Only one case had antral atrophic gastritis while the remainder had no such lesion. Endoscopic findings of all cases are shown in Figure 1. All tumors were located in the upper third of the stomach. Four tumors were macroscopically identified as submucosal tumor (SMT)-like 0-Ⅱa (superficial elevated type) and one was identified as 0-Ⅱb (superficial flat type). They were 5 to 13 mm (average, 7.8 mm) in diameter and covered with normal colored or whitish vasodilated mucosa. NBI-ME showed an irregular microvascular pattern (MVP), which indicated nonuniformity and heterogeneity of microvessels, and an absent micro-surface pattern (MSP), which indicated the lack of microstructure such as marginal crypt epithelium, on the small region of the tumor in 2 cases, while regular MVP and MSP were demonstrated in the remainder. Endoscopic examination prior to pathological diagnosis suspected GA-FG in 1 case and malignant lesions such as adenocarcinoma, SMT or carcinoid in 4 cases.

ESD-resected specimens were subjected to immunohistochemical staining in addition to conventional hematoxylin and eosin staining. Immunohistochemical

Table 1 Characteristics of our 5 cases

	Case 1	Case 2	Case 3	Case 4	Case 5
Age (yr)/sex	78/Female	74/Male	67/Male	72/Male	70/Female
Serum anti-*H. pylori* antibody	Negative	Negative	Negative	Negative	Negative
Chronic gastritis	Antral gastritis	(-)	(-)	(-)	(-)
Location	Upper/Ant	Upper/Gre	Upper/Gre	Upper/Gre	Upper/Post
Size (mm)	13	5	8	5	8
Macroscopic feature	Ⅱa	Ⅱa	Ⅱa	Ⅱb	Ⅱa
Microvascular pattern on NBI-ME	Irregular	Irregular	Regular	Regular	Regular
Endoscopic diagnosis	Adenocarcinoma	SMT	Carcinoid	GA-FG	Adenocarcinoma
Histological classification	tubular	tubular	tubular	tubular	tubular
Depth	SM (700 μm)	SM (80 μm)	SM (980 μm)	SM (110 μm)	SM (1230 μm)
Lymphatic/venous invasion	(+)/(-)	(-)/(-)	(-)/(-)	(-)/(-)	(-)/(-)
MUC2/MUC5AC/MUC6/Pepsinogen-I	(-)/(-)/(+)/(+)	(-)/(-)/(+)/(+)	(-)/(-)/(+)/(+)	(-)/(-)/(+)/(+)	(-)/(-)/(+)/(+)
p53/Ki-67 labeling index	(-)/Low	(-)/Low	(-)/Low	(-)/Low	(-)/Low

Ⅱa: Elevated lesion; Ⅱb: Flat lesion; Ant: Anterior wall; GA-FG: Gastric adenocarcinoma of fundic gland type; Gre: Greater curvature; NBI-ME: Narrow-band imaging with magnifying endoscopy; Post: Posterior wall; SM: Submucosal layer; SMT: Submucosal tumor.



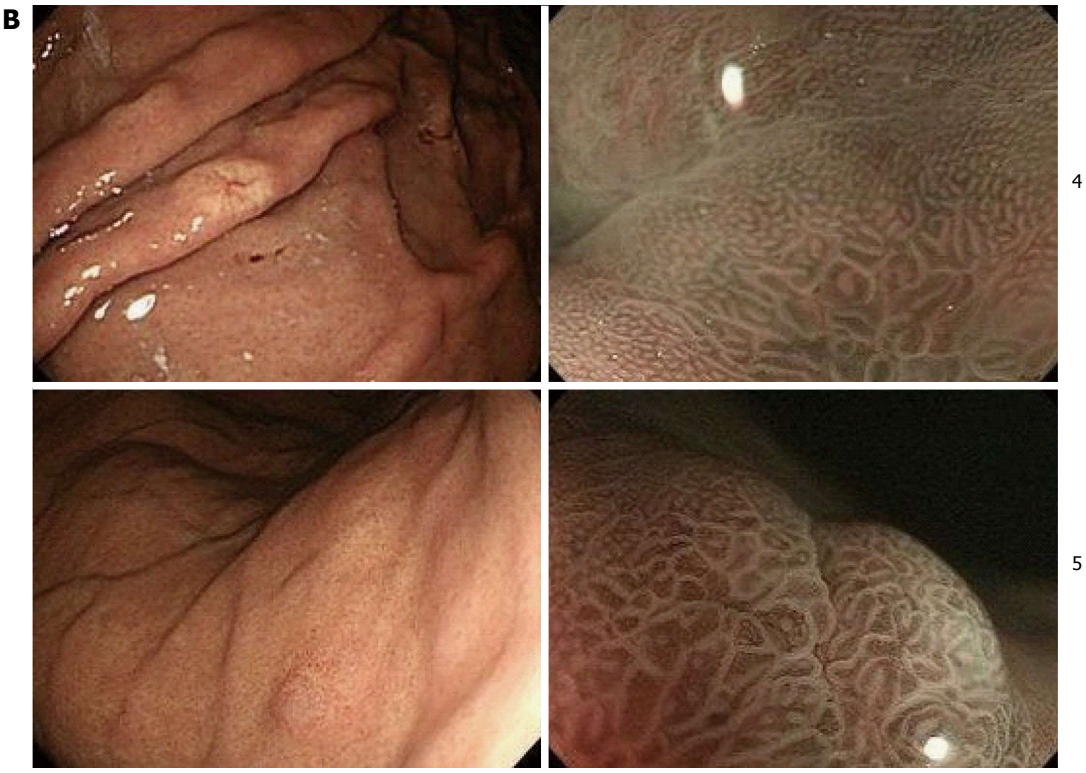


Figure 1 Endoscopic examination of five cases. A: Conventional white light endoscopy showed elevated (type 0-Ⅱa) or flat (type 0-Ⅱb) lesions with dilatation of microvessels on the upper gastric body; B: Narrow-band imaging with magnifying endoscopy showed an irregular microvascular pattern (MVP) and an absent microsurface pattern (MSP) on the small region of the tumor in cases 1 and 2, while regular MVP and MSP were demonstrated in the remainder.

markers used were as follows: MUC5AC for foveolar cells, MUC6 for mucous neck cells or pyloric gland cells, MUC2 for goblet cells, and CD10 for intestinal brush border. The mucin phenotypes were assessed according to the expression of gastric type markers such as MUC5AC and MUC6 and intestinal type markers such as MUC2 and CD10. Furthermore, pepsinogen-Ⅰ was also employed as a marker of differentiation to chief cells. When these markers were expressed in 10% or greater of the cytoplasm, they were considered as positive. Proliferative activity was assessed as a ratio of Ki-67 positive cells to 1000 tumor cells while p53 overexpression was defined as positive when the protein was expressed in 10% or greater of tumor cell nuclei.

Pathological findings of typical GA-FG are shown in Figure 2 (case 1). All tumors showed well-differentiated tubular adenocarcinoma. The tumors arose from the deep layer of the lamina propria mucosae, and most of their surfaces were covered with non-atypical foveolar epithelium. All tumors showed submucosal invasion, and the depth of invasion ranged from 80 to 1230 μm (average, 620 μm). Lymphatic invasion was seen only in case 1, while no venous invasion was recognized. By immunohistochemical examination, it was found that in addition to pepsinogen-Ⅰ positivity, all tumors showed diffuse positivity for MUC6. In contrast, MUC2 and MUC5AC were negative in all cases. The malignant potential of all cases was considered low as none showed overexpression of p53, and the labeling index of Ki-67 was low.

All cases could be followed up after ESD for a period from 9.7 to 27.7 mo. Of the three cases that showed submucosal invasion, the depth of which was greater than 500 μm, case 3 subsequently underwent additional fundusectomy, resulting in neither remnant adenocarcinoma nor lymph node metastasis. All cases have been free from recurrence or metastasis.

DISCUSSION

GA-FGs are characterized by the following: (1) they arise most commonly from normal gastric mucosa of the fundic gland region without intestinal metaplasia; (2) they are recognized as smooth elevated or depressed lesions; (3) they tend to invade the submucosal layer while they rarely show lymphatic and venous invasion; and (4) they show histologically mild atypism.

We reviewed previous reports on GA-FG published in the English literature or the Japanese literature with English abstract, and found that 30 domestic cases were reported until 2013[4,5,8-12], while Singhi *et al*[13] was the first to report 10 non-Japanese cases in 2012. Clinicopathological findings of the previous and the present cases are shown in Table 2. These cases were characterized by common occurring sites such as the upper gastric region, a tendency to take the form of SMT-like elevation and rare lymphatic and venous invasion despite frequent submucosal invasion. Most

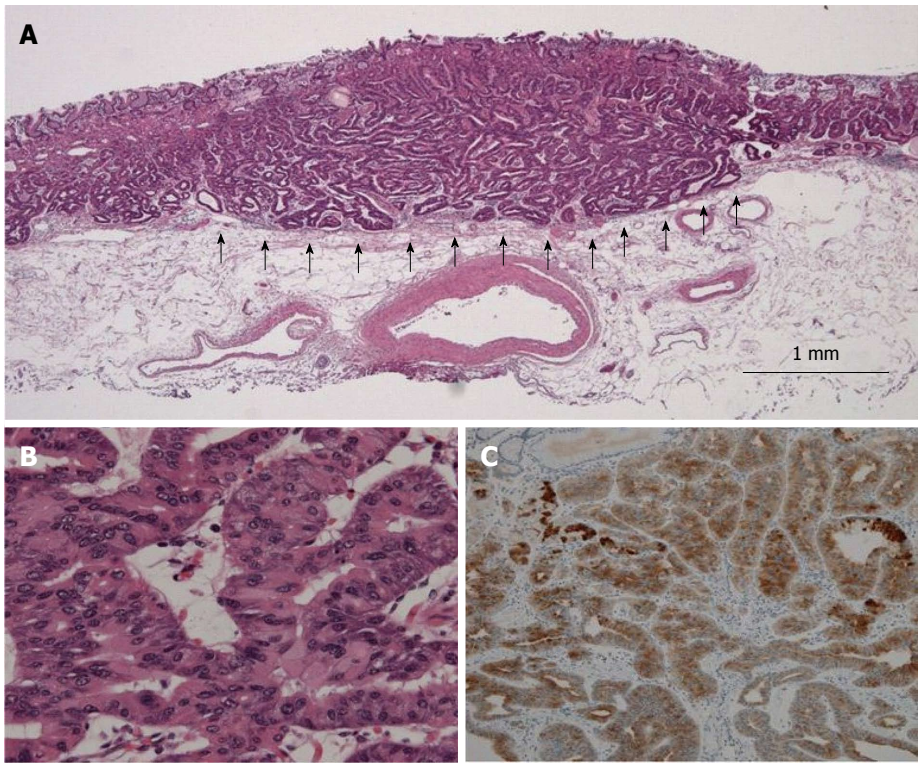


Figure 2 Pathological examination of resected gastric adenocarcinoma of fundic gland type (case 1). A: In low-power view, the tumor arose from the deep layer of the lamina propria mucosae and invaded the submucosal layer at a depth of 980 μm (arrow). Most of the surface was covered with non-atypical foveolar epithelium; B: In high-power view, the tumor was composed of well-differentiated columnar cells mimicking the fundic gland cells with mild nuclear atypism; C: Immunohistological staining showed diffuse positivity for pepsinogen-I.

Table 2 Clinicopathological findings of previous and present cases

Reports	[4]	[5,8]	[9]	[10]	[11]	[12]	[13]	Present cases
Number of patients	1	25 (27 lesions)	1	1	1	1	10	5
Age (yr)	82	67 (average)	59	56	50s	71	64.2 (average)	72.2 (average)
Sex (Male:Female)	Female	16:9	Female	Male	Male	Female	4:6	3:2
Therapy	EMR	Operation: 3 ESD or EMR: 7 ND: 15	ESD	EAM	ESD	EMR	polypectomy	ESD
Location (Upper:Middle:Lower)	Upper	23:4:0	Upper	Upper	Middle	Upper	10:0:0	5:0:0
Size (average, mm)	16	12.2 (3-42)	8	5	42	ND	4.3	7.8 (5-13)
Macroscopic feature (Ⅰ:Ⅱa:Ⅱb:Ⅱc:Ⅱa + Ⅱc)	Ⅰ (protrude)	1:14:2:8:2	Ⅱb	Ⅱa	Ⅱa + Ⅱc	Ⅱa	10:0:0:0:0	0:4:1:0:0
Depth (M:SM)	M	6:21	SM	SM	SM	SM	10:00	0:05
Lymphatic/venous invasion	(-)/(-)	2/1	(-)/(-)	(-)/(-)	(-)/(-)	(-)/(-)	0/0	1/0
Survival time (average, mo)	ND	27 (10-70)	17	12	ND	12	15 (6-39)	10.6 (2-20)
Outcome	ND	NED: 10 ND: 15	NED	NED	ND	NED	NED: 8 Persistence: 1 ND: 1	NED:5

EAM: Endoscopic aspiration mucosectomy; EMR: Endoscopic mucosal resection; ESD: Endoscopic submucosal dissection; M: Mucosal; ND: Not described; NED: No evidence of disease; SM: Submucosal.

of the cases were treated with endoscopic resection, and there was no recurrence or metastasis except for a case that had local residual recurrence.

Endoscopic findings of the present cases were similar to those of previous reports. In particular, the tumors were characteristically identified as whitish SMT-like elevations associated with vasodilation. The whitish coloring seemed to be produced by dimmed reddening of the mucosal surface due to reduced transparency of the submucosal vessels brought about by the tumor located in the deep layer of the mucosa. Vasodilation on the tumor surface is considered ascribable to displacement of the surface vessels by the tumor tissue. A possible reason for

the macroscopic similarity to SMT is that the tumors arose from the deep layer of the gastric mucosa, growing laterally toward the lamina propria. If they had grown straight toward the mucosal surface, they would have been recognized as unsmooth tumors by endoscopy. However, since they were scarcely exposed on the mucosal surface, they were recognized as SMT-like elevations. From the above, careful endoscopic screening is very important for detecting slight differences between GA-FG and the surrounding mucosa even if there is no obvious irregularity in the mucosal surface.

In our study, NBI-ME was also performed in addition to conventional endoscopy. At present, observation of early gastric adenocarcinoma using NBI-ME is assessed based on the worldwide standard diagnostic system of the VS classification, which uses MVP and MSP as indices as proposed by Yao *et al*[7]. Only two of our cases showed a minimal irregular MVP and absent MSP under NBI-ME, which are consistent with the findings of gastric adenocarcinoma. In contrast, the other cases showed regular MVP and MSP probably because these tumors, which were located in the deep layer of the mucosa, hardly showed any inner abnormal microstructure through the thick mucosa.

Despite the detailed endoscopic examination, a definite diagnosis was finally made by histopathological examination. If GA-FG is suspected by endoscopy, a pathologist should be informed of such suspicion in order for immunohistochemical staining to be performed which may lead to a definite diagnosis. Even if not suspected, the lesion should be recognized as malignant that is distinct from a real SMT or a fundic gland polyp and biopsy should be performed. It is important to acquire a sufficient amount of tissue because the tumor location is commonly in the deep layer of the mucosa.

With regard to therapy, the majority of GA-FGs are thought to be indications for endoscopic resection. However, for lesions that show submucosal invasion deeper than 500 μm as in our cases, curative resection may not be possible according to Japanese gastric cancer treatment guidelines 2010 (ver.3)[14]. On the other hand, GA-FGs rarely demonstrate lymphatic and venous invasions despite deep submucosal invasion. Regarding therapeutic approaches following incomplete resection of GA-FG, which is relatively common among the elderly, it will be controversial in the future whether additional treatment such as for differentiated adenocarcinoma of intestinal phenotype should be administered or not.

In conclusion, we examined 5 cases of GA-FG and discussed their characteristics in comparison with previous reports. Even when atrophic mucosa due to *H. pylori* infection is not observed, detailed examination should be done under suspicion of GA-FG if a whitish SMT-like lesion with vasodilation on the surface is found in the upper third of the stomach. Whereas the consensus is for the use of conventional white light endoscopy to facilitate diagnosis, few reports on NBI-ME are available. It is therefore necessary to accumulate more endoscopic data to identify the characteristics of GA-FG. Furthermore, what remains to be done is to explore long-term outcomes of GA-FG such as the natural course, recurrence rate and prognosis after resection, and to elucidate the genetic aberrations involved in carcinogenesis.

COMMENTS

Case characteristics

Patients were 3 males and 2 females with ages ranging from 67 to 78 years.

Clinical diagnosis

All patients had no symptom and no specific physical signs.

Differential diagnosis

The authors cannot make mention of differential diagnosis at this point.

Laboratory diagnosis

None of five cases had serum anti-*Helicobacter pylori* (*H. pylori*) antibody.

Imaging diagnosis

Endoscopically, all tumors were located in the upper third of the stomach and macroscopically identified as superficial elevated type or flat type, which were 5 to 13 mm (average, 7.8 mm) in diameter and covered with normal colored or whitish vasodilated mucosa.

Pathological diagnosis

Pathologically, all tumors showed well-differentiated tubular adenocarcinoma, which arose from the deep layer of the lamina propria mucosae and showed submucosal invasion.

Treatment

All tumors were treated with endoscopic submucosal dissection and no case has experienced recurrence or metastasis.

Related reports

The authors reviewed previous reports and found 40 cases, whose clinicopathological findings were similar to the present cases.

Term explanation

Gastric adenocarcinoma of fundic gland type (GA-FG), which shows differentiation to chief cells and arises from normal gastric mucosa of the fundic gland region without intestinal metaplasia, was first proposed as a new disease entity in 2010.

Experiences and lessons

Even when atrophic mucosa due to *H. pylori* infection is not observed endoscopically, detailed examination should be done under suspicion of GA-FG if a whitish SMT-like lesion with vasodilation findings on the surface is found in the upper third of the stomach.

Peer-review

This article shows detailed endoscopic and pathological findings of GA-FG for accurate diagnosis.

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CASE REPORT

Endoscopic snare papillectomy for a solitary Peutz-Jeghers-type polyp in the duodenum with ingrowth into the common bile duct: Case report

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Author contributions: Suzuki K and Higuchi H participated in endoscopic treatment; Shimizu S was the physician in charge; Morinaga S made the histopathologic diagnosis; Nakano M and Serizawa H contributed as administrators of the endoscopic center; Suzuki K performed data analysis and wrote the manuscript; all authors read and approved the final manuscript.

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Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

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Abstract

Solitary duodenal Peutz-Jeghers (PJ)-type hamartomatous polyps are rare and considered a different disease entity than classic PJ syndrome. We describe the case of an 89-year-old man admitted to our emergency department with symptoms of acute cholangitis, liver dysfunction, and slight jaundice. Magnetic resonance imaging showed multiple signal voids, reflecting choledocholithiasis, and an oval-shaped tumor in the common bile duct (CBD). Following endoscopic retrograde cholangiopancreatography, the patient was diagnosed with a lower CBD tumor 20 mm in diameter. Endoscopic sphincterotomy was performed for choledocholithotomy, resulting in the expulsion of a large tumor with a stalk connected to the papilla of Vater. The tumor was successfully excised *en bloc* by endoscopic snare papillectomy. Histopathologic examination showed that the tumor was a PJ-type hamartomatous polyp. No mucocutaneous pigmentation of the skin was evident and the patient's family history was negative. Solitary duodenal PJ-type hamartomatous polyps are usually diagnosed incidentally during endoscopy for other indications because most of these tumors are asymptomatic or have nonspecific presentations. To our knowledge, this is the first reported solitary PJ-type polyp with intra-CBD growth treated by endoscopic snare papillectomy.

Key words: Acute cholangitis; Hamartoma; Jaundice;

Peutz-Jeghers polyp; Duodenum polyp

Core tip: Solitary duodenal Peutz-Jeghers (PJ)-type hamartomatous polyps are rare. This polyp is usually diagnosed incidentally during endoscopy because its presentation is nonspecific. Thus, it is interesting that acute cholangitis was shown as initial symptoms of PJ-type hamartomatous polyp due to intra-common bile duct growth. This is the first reported solitary duodenal PJ-type hamartomatous polyp treated by endoscopic snare papillectomy. PJ-type polyps should be distinguished as solitary or an incomplete type of PJ syndrome. These polyps should be treated by endoscopic or surgical resection due to their malignant potential.

Suzuki K, Higuchi H, Shimizu S, Nakano M, Serizawa H, Morinaga S. Endoscopic snare papillectomy for a solitary Peutz-Jeghers-type polyp in the duodenum with ingrowth into the common bile duct: Case report. *World J Gastroenterol* 2015; 21(26): 8215-8220 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i26/8215.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i26.8215

INTRODUCTION

Hamartomatous polyps are usually associated with Peutz-Jeghers syndrome (PJS)[1], which is characterized by gastrointestinal polyposis and mucocutaneous pigmentation. Solitary hamartomatous polyps have been considered a variant or a separate disease entity without the features of PJS[2]. Solitary duodenal hamartomas are even less common. These lesions are usually accompanied by nonspecific symptoms, with most diagnosed incidentally during endoscopy. We describe the successful use of endoscopic snare papillectomy to remove a solitary Peutz-Jeghers (PJ)-type polyp on the papilla of Vater. To our knowledge, this is the first patient to be described with a solitary PJ-type hamartomatous polyp showing ingrowth into the common bile duct (CBD).

CASE REPORT

An 89-year-old man with a chief compliant of left brachium paresis was referred to our hospital and admitted to the emergency department. The patient had no significant medical history except for hypertension, atrial fibrillation, and chronic gastritis, and no significant family history. On admission, he showed evidence of an inflammatory reaction, with elevated WBC (10310/μL) and C-reactive protein (4.62 mg/dL). Liver dysfunction and slight jaundice were also observed, with elevated levels of aspartate transaminase (152 IU/L), alanine transaminase (68 IU/L), lactate dehydrogenase (551 IU/L), gamma-glutamyl transpeptidase (84 IU/L), and total bilirubin (2.32 mg/dL). Tumor markers, including carcinoembryonic antigen, cancer antigen 19-9 and alpha-fetoprotein, were within normal limits. Plain abdominal computed tomography (CT) demonstrated choledocholithiasis with multiple calcified stones in the dilated CBD and a tumor in the lower CBD (Figure 1). Magnetic resonance imaging with cholangiopancreatography showed multiple signal voids in the CBD reflecting tiny stones (Figure 2A). An oval-shaped tumor 20 mm in diameter in the lower CBD was detected by high-intensity signals on T2-weighted and diffusion-weighted imaging (Figure 2B). Following a diagnosis of jaundice due to choledocholithiasis and a CBD tumor, endoscopic retrograde cholangiopancreatography was performed. The lateral view showed a slight swelling of the papilla of Vater (Figure 3A), as well as multiple filling defects in the CBD. The largest defect, with a diameter of 4 cm, was located at the inferior extremity of the CBD without flexibility (Figure 3A). Intraductal ultrasound showed soft tissue echogenicity, suggesting that the defect was a neoplasm growing into the CBD (Figure 3B). Endoscopic sphincterotomy was performed and a plastic tube stent was inserted. A second endoscopic retrograde cholangiopancreatography, performed one week later, showed inversion and exclusion of the tumor (Figure 3C). The tumor, which had a stalk

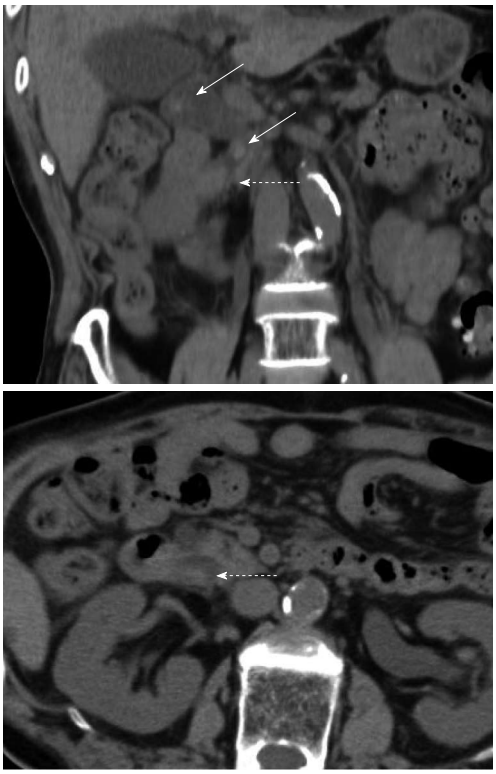


Figure 1 Computed tomography on admission. Plain abdominal computed tomography showing choledocholithiasis with multiple calcified stones (solid arrows) in the dilated common bile duct and a round-shaped tumor in the lower common bile duct (dotted arrows).

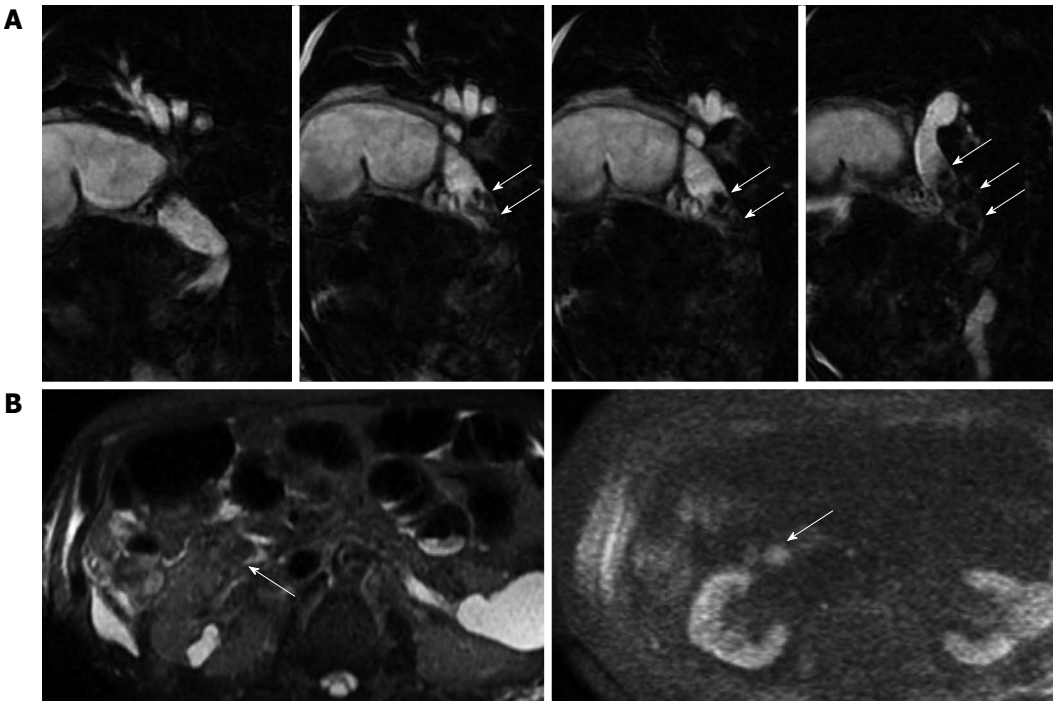
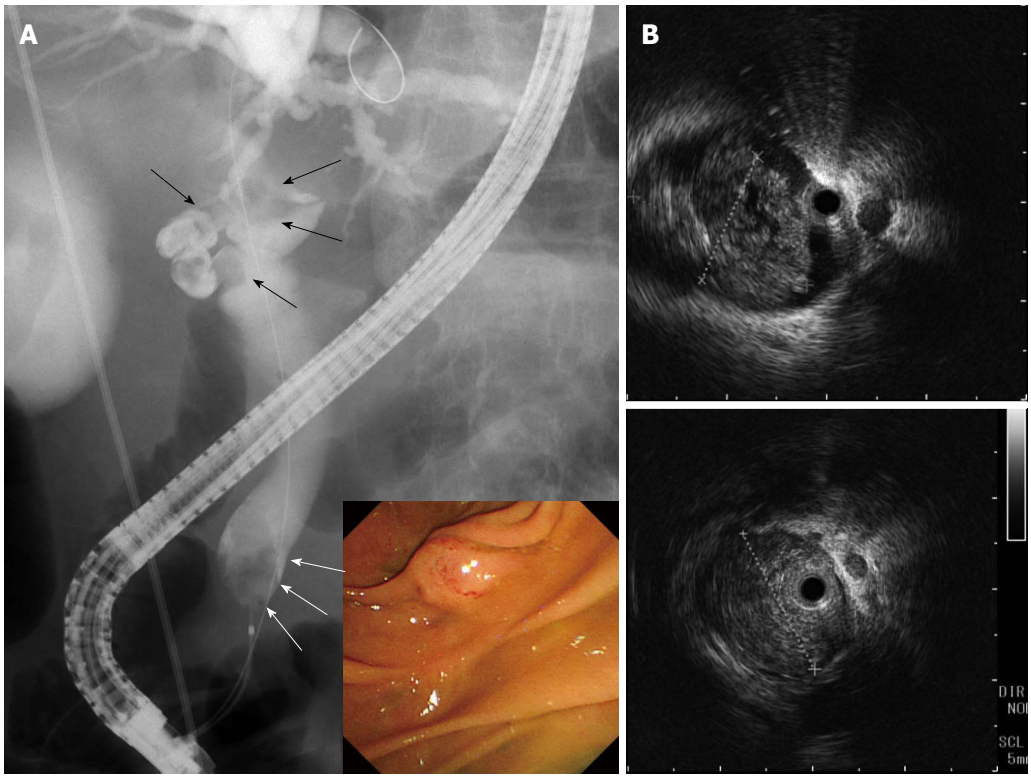


Figure 2 Magnetic resonance imaging showing multiple stones and an oval-shaped tumor in the common bile duct. A: Magnetic resonance imaging (MRI) with cholangiopancreatography showing multiple signal voids in the common bile duct (CBD) reflecting tiny stones; B: T2-weighted MRI image showing an oval-shaped tumor 20 mm in diameter in the lower CBD with high-intensity signal (left row); and a diffusion-weighted image showing a mass lesion in the lower CBD with a round high-intensity signal (right row).



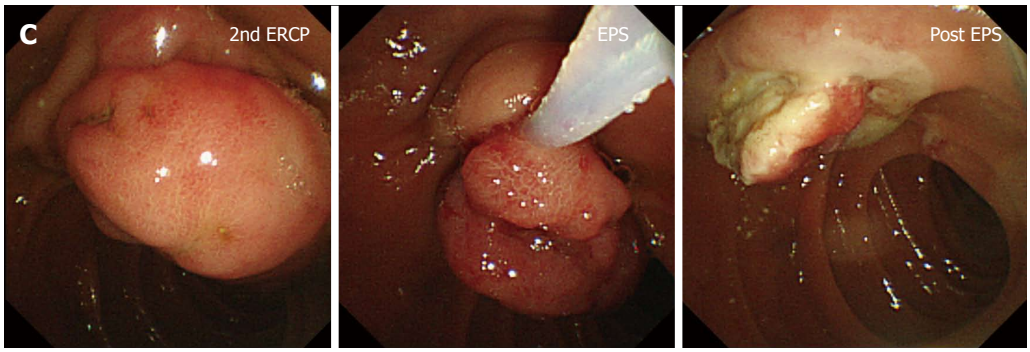


Figure 3 Tumor resection by endoscopic snare papillectomy. A: Endoscopic retrograde cholangiopancreatography (ERCP) showing several filling defects in the upper part of the common bile duct (CBD) (black arrows), and an oval-shaped defect in the intra-pancreatic CBD (white arrows). Endoscopic picture during ERCP did not show any remarkable findings without juxtapapillary duodenal diverticula; B: Intraductal ultrasonography confirming that the lower filling defect was a papillary-growing tumor in the CBD; C: Second ERCP, performed one week later, showing inversion and exclusion of the tumor after endoscopic sphincterotomy. The tumor was a pedunculated polyp, enabling *en bloc* resection by endoscopic snare papillectomy.

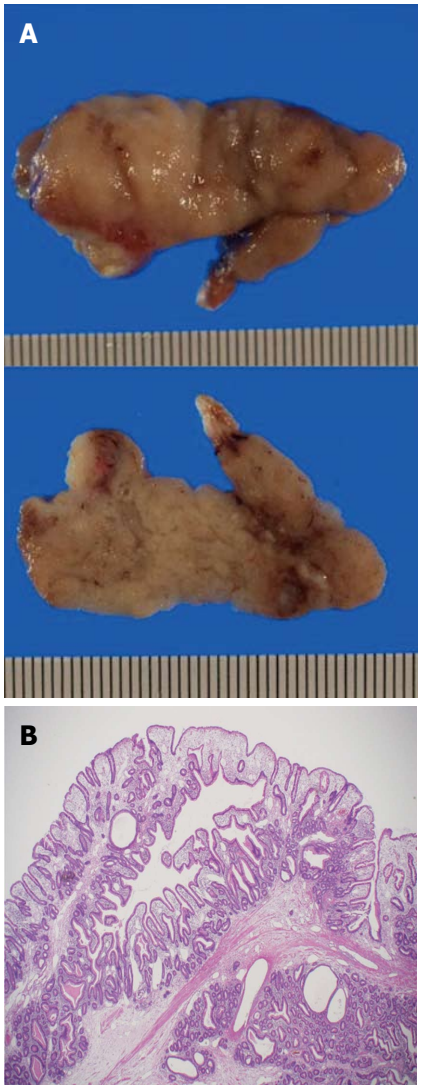


Figure 4 Histopathologic findings. A: View of the surgical specimen, showing that it was 4.8 cm × 1.9 cm × 1.8 cm in diameter with lobular surfaces, which were epithelialized with intestinal type mucosa; B: Histopathologic examination showed features suggesting a hamartoma. For example, the polyp contained irregular hyperplastic crypts with focal cystic dilatation and branching bundles of smooth muscle extending from the muscularis mucosae. The epithelium was hyperplastic.

growing from the major duodenal papilla, was excised *en bloc* by endoscopic snare papillectomy (Figure 3C).

Histopathologic examination of the resected specimen showed a large polypoid neoplasm, 4.8 cm × 1.9 cm × 1.8 cm in size, with a lobular surface and epithelialized with intestinal type mucosa (Figure 4A). The polyp contained irregular hyperplastic crypts with focal cystic dilatation, especially in the lower layer, and branching bundles of smooth muscle extending from the muscularis mucosae. The epithelium was hyperplastic in nature but not dysplastic, resulting in a diagnosis of PJ-type hamartomatous polyp (Figure 4B).

The patient had no mucocutaneous pigmentation or family history of PJS. Upper gastrointestinal endoscopy and colonoscopy did not reveal any other PJ-type polyps. Thus, the final diagnosis was solitary PJ-type hamartomatous polyp of the major duodenal papilla.

DISCUSSION

PJS is a rare autosomal dominant disorder associated with mucocutaneous pigmentation (buccal mucosa, lips, fingers, and toes) and a family history of this condition[3]. Solitary PJ-type polyps, defined as hamartomatous polyps without any of the clinical symptoms of PJS, are characterized histologically by tree-like branching of smooth muscle fibers, with a core of smooth muscle, covered by mucosal tissue of near-normal appearance[4]. It is truly difficult to determine whether these solitary duodenal hamartomatous polyps are an incomplete type or initial clinical manifestation of PJS[2]. This entity may be differentiated from PJS based on information from previous studies, including: the lack of perioral or perianal mucocutaneous pigmentation and lack of family history; symptom onset usually occurring past the sixth and seventh decades of life; and lower risk of local cancer development than hamartomatous polyps in patients with PJS. The findings in our patient are consistent with these characteristics.

A search of case reports on the MEDLINE database

Table 1 Previously reported patients with duodenal solitary Peutz–Jeghers type hamartomatous polyps (English publications only)

Case	Author	Year	Age	Sex	Location	Size (mm)	Symptoms	Treatment	Malignancy
1	Bott	1986	23	M	4th	50 × 40	GI bleeding	Surgery	None
2	Naitoh	1988	56	F	3rd	30 × 15	NA	Endoscopy	None
3	Tanaka	1990	41	M	3rd	25 × 18	Asymptomatic	Endoscopy	None
4	-	-	82	F	2nd	25 × 20	Asymptomatic	Endoscopy	None
5	Acea Nebril	1993	63	F	1st	50 × 35	Anemia	Surgery	None
6	Ichiyoshi	1996	84	F	2nd	25 × 20	GI bleeding	Endoscopy	Yes
7	Oncel	2003	68	F	3rd	25	GI bleeding	Endoscopy	None
8	-	-	53	M	2nd	5	Dyspepsia	Endoscopy	None
9	Kitaoka	2004	22	F	1st	30	Asymptomatic	Endoscopy	None
10	Itaba	2006	87	F	2nd	18	Abdominal pain	Endoscopy	None
11			56	M	2nd	12	CEA elevation	Endoscopy	None
12	Suzuki	2008	59	F	2nd	15 × 15	Epigastric discomfort	Endoscopy	None
13	-	-	68	F	2nd	10 × 8	Epigastralgia	Endoscopy	Yes
14	-	-	60	F	1st	10 × 10	Asymptomatic	Endoscopy	None
15	Jamaludin	2009	46	M	1st	70 × 40	Dyspepsia	Surgery	Yes
16	Kantarciogle	2009	28	F	2nd	25 × 15	Acute pancreatitis	Endoscopy	None
17	Sekino	2011	84	M	1st	14	Asymptomatic	Endoscopy	Yes
18	-	-	76	M	2nd	15	Asymptomatic	Endoscopy	None
19	Our case	2014	89	M	2nd	48 × 19 × 18	Acute cholangitis	Endoscopy	None

NA: Not available; GI: Gastrointestinal.

up to 2014 using the terms "solitary", "duodenum", and "hamartomatous polyp", but without PJS, identified 19 patients in 13 studies published in English with solitary-PJ type polyps in the duodenum, including the patient described here[2,5-15] (Table 1). Age at onset ranged from 23-89 years (mean ± SD: 60.3 ± 20.1 years). The most frequent polyp site was the second portion of the duodenum, followed by the duodenal bulb. Maximum polyp diameter ranged from 5-70 mm (mean ± SD: 26.4 ± 16.6 mm). The predominant presentation was asymptomatic, with other patients having nonspecific symptoms. Most patients underwent endoscopic resection, although some patients with large polyps (> 50 mm) required surgery. Of the 19 polyps, four showed malignant transformation.

Duodenal solitary PJ-type hamartomatous polyp is usually diagnosed incidentally during gastroduodenal endoscopy. Its presentation is nonspecific and resembles common conditions, such as peptic ulcer disease[13]. Our patient was the first to report acute cholangitis and jaundice as the chief complaints. Moreover, this was the first solitary PJ-type hamartomatous polyp showing ingrowth into the CBD, although a solitary hamartomatous polyp in the CBD was reported in a patient without PJS after hepatoduodenostomy[16]. The mechanism of ingrowth into the CBD in our patient is undetermined. However, a polyp that initially developed on the papilla of Vater may have invaginated accidentally into the CBD while still tiny due to peristaltic motion. This polyp may then have grown in the CBD.

The characterization of PJ-type polyps as having a low risk of cancer is unclear. To our knowledge, 4 of 19 such lesions showed malignant transformation. The mean age of patient with malignancy was 70.5 years, higher than that of all 19 patients (60.3 years). In contrast, the maximum diameter may be nonindicative of malignancy, as patient 13 had a small malignant polyp only 10 mm in diameter. There is no definitive guideline for treatment of solitary duodenal PJ-type hamartomatous polyps. In general, as with other common gastroduodenal polyps, solitary PJ-type polyps with features of large size, sessile type, presence of induration, or with suspicion of submucosal invasion should be considered for surgical resection. As the differential diagnosis by morphologic features is difficult, endoscopic biopsies must be performed for the selection of an appropriate therapeutic strategy. When the biopsy is malignant, surgery should be performed according to the depth of tumor invasion. Endoscopic ultrasonography is useful. Meanwhile, comprehensive and cautious decision of surgical indication is required by consideration of patient characteristics, such as age, past history, and general condition.

In conclusion, associated signs and symptoms should be evaluated in patients with duodenal PJ-type hamartomatous polyps. In addition, patients should undergo upper intestinal endoscopy, colonoscopy, and whole-body screening. Solitary PJ-type hamartomatous polyps should be distinguished as solitary or as an incomplete type of PJS. These polyps should be treated by endoscopic or surgical resection.

COMMENTS

Case characteristics

An 89-year-old male with solitary duodenal Peutz-Jeghers (PJ)-type hamartomatous polyp, which had grown into the common bile duct (CBD).

Clinical diagnosis

Expulsion of a large tumor with a stalk connected to the papilla of Vater after endoscopic snare papillectomy.

Differential diagnosis

Papillary-expanding-type cholangiocarcinoma; polypoid type carcinoma of the

papilla of Vater.

Laboratory diagnosis

WBC, 10310/μL; C-reactive protein, 4.62 mg/dL; aspartate transaminase, 152 IU/L; alanine transaminase, 68 IU/L; lactate dehydrogenase, 551 IU/L; gamma-glutamyl transpeptidase, 84 IU/L; total bilirubin, 2.32 mg/dL; inflammatory reaction with liver dysfunction and slight jaundice.

Imaging diagnosis

Computed tomography and magnetic resonance imaging revealed choledocholithiasis with multiple calcified stones in the dilated CBD and a tumor in the lower CBD.

Pathological findings

Histopathologic examination showed a large polypoid neoplasm with a lobular surface and epithelialized with intestinal type mucosa containing irregular hyperplastic crypts with focal cystic dilatation, and branching bundles of smooth muscle extending from the muscularis mucosae.

Treatment

The tumor with a stalk growing from the major duodenal papilla, which was inverted and excluded from the CBD, was excised *en bloc* by endoscopic snare papillectomy.

Related reports

There are a few cases of solitary duodenal PJ-type hamartomatous polyps reported in the literature, however, the present case is the first to report showing ingrowth into the CBD with acute cholangitis and jaundice.

Term explanation

Endoscopic snare papillectomy is an endoscopic excision technique for a benign and/or low-grade malignant tumor of the papilla of the Vater, using a snare loop to enable *en bloc* resection.

Experience and lessons

This case report not only represents the diagnosis and therapeutic strategy for a solitary duodenal PJ-type hamartomatous polyp, but also introduces the usefulness of endoscopic snare papillectomy for a large pedunculated polyp adjacent to the papilla of Vater.

Peer-review

This article presents a rare case of solitary duodenal PJ-type hamartomatous polyp grown from the CBD, and also addresses a critical clinical question by integrating an extended review of literatures.

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