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[1]Passed away on October 20, 2007
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EDITORIAL

Could quantitative liver function tests gain wide acceptance among hepatologists?

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Abstract

It has been emphasized that the assessment of residual liver function is of paramount importance to determine the following: severity of acute or chronic liver diseases independent of etiology; long-term prognosis; step-by-step disease progression; surgical risk; and efficacy of antiviral treatment. The most frequently used tools are the galactose elimination capacity to asses hepatocyte cytosol activity, plasma clearance of indocyanine green to assess excretory function, and antipyrine clearance to estimate microsomal activity. However, a widely accepted liver test (not necessarily a laboratory one) to assess quantitative functional hepatic reserve still needs to be established, although there have been various proposals. Furthermore, who are the operators that should order these tests? Advances in analytic methods are expected to allow quantitative liver function tests to be used in clinical practice.

Key words: Chronic diseases; Fatty liver; Hepatitis, viral; Liver cirrhosis; Liver function tests; Prognosis

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INTRODUCTION

Liver biopsy is the first-line method for evaluating liver injury. As a result of its limitations and risks, alternative methods have been developed. Many markers and several imaging methods have been evaluated in studies published in peer-reviewed journals. Although the methodological rigor of the design, execution and analysis of the studies proposing new tests has always been ascertained, only their regular use can establish their acceptance among physicians. Probably, nervousness-based medicine plays a role. Fear of litigation is a powerful stimulus to over-investigation, and in an atmosphere of litigation phobia, the only bad test is the test that the physician did not think of ordering[1].

The assessment of liver function has always been considered as important for estimating such things as the severity of any acute or chronic liver disease, and its prognosis and treatment efficacy (Table 1). Generally, methods used to determine cellular damage and related consequences consist of noninvasive and rapid tests, which are easy to perform, e.g. serum enzymes, surrogate serum fibrosis markers, or transient elastography and ultrasound imaging. The first quantitative liver function tests (QLFTs) were suggested almost 50 years ago[2]. Their rationale was based on the fact that a drug or a foreign compound is metabolized primarily by the liver cytochrome P450 system, through sequential oxidative processes, and the major metabolite can be detected. The main characteristic of the proposed drug is its relatively high hepatic extraction ratio, but a QLFT depends not only on hepatic metabolic capacity, but also on hepatic blood flow. A more recent study has addressed the clinical utility of measuring galactose elimination capacity (GEC), aminopyrine breath test (ABT) and indocyanine green (ICG) retention test. The results have shown that all QLFTs are predictors of survival in cirrhosis, and that GEC adds new prognostic information to that already available using the Child-Pugh classification[3]. In spite of this, relatively few centers perform these tests. Hence, they are currently performed only in very few specialized institutions and usually within the setting of clinical research projects.

More recently, preoperative assessment of liver function and prediction of residual postoperative functional liver parenchymal mass and reserve has been ascertained to be of paramount importance to minimize surgical risk, especially in patients with hepatocellular carcinoma, the

Table 1 Frequently used QLFTs in patients with liver cirrhosis, chronic viral hepatitis and non-alcoholic liver disease

Type	Methods/drawbacks
GEC	Intravenous administration of galactose; blood samples at 5, 25 and 45 min; urine collected for 5 h
ICG	Measurement of liver plasma flow; injection of ICG must be performed quickly; blood samples at 3, 6 and 49 min
MEGX	Blood samples at 15 and 30 min after i.v. lidocaine administration; allergy to anesthetics
ABT	Resting period of at least 30 min before the breath test that should be repeated three times at 10-min intervals
13C-C breath test	Subjects ingested 2 mg/kg of [3-methyl-13C]-caffeine sitting quietly for 15 min before and throughout the test; breath samples were collected immediately prior to, and 60 min after, caffeine ingestion
13C-M breath test	Measurement of breathed CO_2 by laser-based technology
SCl	Measurement of liver plasma flow. Sorbitol (500 g/L) was administered *via* a perfusor at 7.5 mL/h. Serum and urinary concentrations of sorbitol were determined at the beginning of the perfusion and after reaching steady-state
TOSCA	Unique sample of saliva to be collected in the morning; rare complaiance to drinks containing caffeine by some patients
LURs	Volumetric information as well as functional assessment; expensive
MRI	Expensive; to be validated

QLFTs: Quantitative liver function tests; GEC: Galactose elimination capacity; ICG: Indocyanine green; MEGX: Monoethylglycinexylidide; ABT: Aminopyrine breath test; MRI: Magnetic resonance imaging; LURs: Liver uptake ratios.

majority of whom have liver cirrhosis as a complication. Incorporation of ICG into the decision tree has enabled patients conventionally classified into Child-Pugh class A to be subdivided into several groups in which various hepatectomy procedures are feasible. Applying this choice to 685 patients, during 10 years, only a single fatality was encountered[4]. Probably, this is the most important area of application of QLFTs. Indeed, new limits have been established to decrease mortality and morbidity rates after liver resection in cirrhotic and non-cirrhotic patients.

Various laboratory data and imaging techniques have been used to complement the Child-Pugh score to predict liver failure after hepatectomy and to assess functional hepatic reserve. The greatest experience has been made so far with the ABT and GEC, which are decreased among hepatic failure patients after liver resection. However, absence of these changes does not totally exclude hepatic failure. The ICG retention test is the most widely used clearance test. Nevertheless, it remains imperfect because it depends on hepatic blood flow and on the functional capacity of the liver.

Nuclear imaging plays a determinant role in assessing liver function, although it is expensive. Nuclear imaging of the asialoglycoprotein receptors with radiolabeled synthetic asialoglycoproteins provides volumetric information as well functional assessment of the liver[5]. Single photon emission computed tomography (SPECT) is superior to the planar method for determining liver uptake ratios (LURs). Evaluation of LURs is a suitable indicator of 99mTc-galactosyl serum albumin clearance from the blood pool and of binding to the asialoglycoprotein receptor, which is a simple and clinically useful indicator for the assessment of hepatic functional reserve in chronic liver diseases[6].

Recent interest toward QLFTs comes from emergency medicine and intensive care physicians. There is no ideal real-time and bedside technique for assessing liver function in critically ill patients. Dynamic tests such as ICG plasma disappearance rate and lidocaine metabolism [monoethylglycinexylidide (MEGX) test], are superior to static tests such as prothrombin time and bilirubin measurement. Recently, the ICG plasma disappearance rate, which nowadays can be measured reliably by a transcutaneous system in critically ill patients at the bedside and provides results within a few minutes, has been confirmed to correlate well with ICG clearance. In general, the ICG plasma disappearance rate is superior to bilirubin, and comparable or even superior to complex intensive care scoring systems in terms of outcome prediction. Furthermore, ICG plasma disappearance rate is more sensitive than serum enzyme tests for assessing liver dysfunction, and early improvement in the ICG plasma disappearance rate after onset of septic shock is associated with better outcome[7].

SOME APPLICATIONS OF QLFTs

Liver cirrhosis

The aim of this report is not to review QLFTs, but to provide a critical appraisal of their extremely selective use. Widespread application of QLFTs as a prognostic tool is controversial. In a recent study, the predictive value of serial evaluations of GEC and MEGX on survival in a cohort of 35 patients was assessed, and secondarily, these tests were compared to Child-Pugh and Model for End Stage Liver Disease (MELD) scores. The end points were patient death or liver transplantation. Statistically significant differences between dead/transplanted patients and survivors were found for basal values of GEC, MEGX, Child-Pugh class and MELD score. Surprisingly, receiver operating characteristic (ROC) curves of Child-Pugh class and MELD score showed a higher prognostic accuracy than GEC and MEGX. On multivariate analysis, neither GEC nor MEGX were independent predictors of survival. Repeated-measures analysis of GEC and MEGX did not increase the prognostic accuracy of these tests, and did not add useful prognostic information on patient outcome during the following 6 mo. These data suggest that neither single nor repeated determinations of GEC and MEGX are superior to Child-Pugh class and MELD score in predicting prognosis of patients with viral cirrhosis[8].

Blood galactose clearance after an intravenous galactose load has been used widely as a QLFT. A novel

QLFT, the galactose single point (GSP) method, has been developed to assess residual liver function in various diseases[9]. The goal of that study was to evaluate the influence of non-hepatic factors such as hyperglycemia on GSP and GEC in rats. Four groups of animal studies were carried out, i.e. normal control (NC), streptozotocin-induced diabetes mellitus (DM), CCl_4-induced hepatotoxicity (CCl_4), and streptozotocin-induced diabetes with CCl_4-induced hepatotoxicity (DM + CCl_4). The serum glucose levels in the diabetic groups (DM and DM + CCl_4) were significantly increased compared with those in the NC and CCl_4 groups. A significant increase of aspartate aminotransferase and alanine aminotransferase was observed in the CCl_4-treated groups (CCl_4 and DM + CCl_4) compared with those in the NC and DM groups. In comparison with the NC group, the values of GSP and GEC in the diabetic groups (DM and DM + CCl_4) were significantly reduced and increased, respectively. GSP had highly significant correlations with GEC. These results suggest that galactose metabolism tests should be interpreted with caution under conditions of significant hyperglycemia[9].

Transplantation

The unique ability of the liver to regenerate quickly after resection makes living donor liver transplantation (LDLT) possible. However, the quality and course of this regeneration process in humans are still unexplored. In a recent study, GEC, ICG and lidocaine half-life as markers for the quality of liver regeneration in the first 3 mo after LDLT were investigated. Twenty-two consecutive living liver donors and their corresponding recipients were analyzed at baseline and at 10 and 90 d after LDLT. Six recipients lost their grafts during the study period. We compared donors and recipients at the different time points. After LDLT, GEC decreased (-42.6%) and ICG increased (+50.6%) significantly in donors. ICG and GEC remained significantly altered over 3 mo in donors with an improvement between days 10 and 90. ICG and GEC improved significantly in recipients between days 10 and 90. The lidocaine half-life showed no significant changes. The donors had better test results and recovered faster than the recipients. In conclusion, after LDLT, the parameters for liver capacity and flow remain altered in donors and recipients despite rapid volume growth[10].

Timing

The key point of the whole problem is not how to test residual liver function but when. The ^{13}C-methacetin (13C-M) breath test enables the quantitative evaluation of cytochrome-P450-dependent liver function. 13C-M is metabolized in the liver by O-demethylation to $^{13}CO_2$ and acetaminophen. The aim of a previous study was to evaluate the 13C-M breath test in comparison to the Child-Pugh class and other QLFTs (MEGX and ICG). 13C-M (2 mg/kg) was given orally to 31 patients with histologically proven liver cirrhosis of different etiology and severity (nine Child-Pugh class A, 13 class B, and nine class C). The increase of exhaled $^{13}CO_2$ was expressed as delta over baseline (DOB; delta/1000). All breath test parameters analyzed provided an excellent discrimination between cirrhotic and non-cirrhotic individuals. The DOB value at 20 min showed a superior correlation with the Child-Pugh class than did MEGX or ICG clearance results. With a cut-off value of $\leqslant$ 25 delta/1000 at 20 min, sensitivity and specificity to discriminate between cirrhotic and non-cirrhotic individuals was 93.5% and 95%, respectively[11].

Pre-cirrhotic stage

To find out whether this breath test is sensitive in non-cirrhotic patients who also have chronic hepatitis C in the early stages of fibrosis, the following study was carried out. Eighty-one patients with chronic hepatitis C underwent a 13C-M breath test. In all patients, a liver biopsy was performed. The liver histology was classified according to the histology activity index-Knodell score. Patients with early fibrosis did not differ in DOB values from patients at 15 min (23.2 ± 7.9 per thousand *vs* 22.6 ± 7.2 per thousand; $P = 0.61$), or cumulative recovery (13.6% ± 3.7% *vs* 13.2% ± 3.8%; $P = 0.45$) from patients with more advanced fibrosis. Conclusively, the noninvasive 13C-M breath test fails to detect early stages of fibrosis in patients with chronic hepatitis C[12].

The 13C-caffeine (13C-C) breath test is a noninvasive, QLFT that is considered to be a valid tool by many authorities. The utility of the 13C-C breath test was measured in 48 patients with chronic hepatitis B and 24 controls, along with its ability to monitor response to lamivudine. In 28 patients on lamivudine, 13C-C breath tests were performed at 1 wk and 1 year after therapy. Patients with Metavir F0-1 fibrosis had a 13C-C breath test similar to the controls. However, patients with F2-3 fibrosis and cirrhosis patients had a decreased 13C-C breath test. Fibrosis correlated best with the 13C-C breath test. The 13C-C breath test independently predicted significant ($F \geqslant 2$) and advanced ($F \geqslant 3$) fibrosis and yielded the greatest area under the ROC curve (0.91 ± 0.04) for predicting advanced fibrosis. The 13C-C breath test was unaltered by 1 wk of lamivudine but improved by 61% ($P < 0.001$) in responders to long-term lamivudine, whereas in those with viremia and elevated alanine aminotransferase, values remained stable or deteriorated. The 13C-C breath test distinguishes chronic hepatitis-B-virus-related fibrosis and detects improvement in liver function in response to long-term lamivudine[13].

Survival studies

Caffeine clearance (CCl) has been suggested as a more exact method than those commonly used. The aim of the following study was to assess the usefulness of CCl in survival prediction of patients with liver cirrhosis. Thirty-four patients with cirrhosis of varying etiology were included: 19 were Child-Pugh class A or B and 15 were class C. CCl was determined from saliva samples. The mean length of follow-up was 33.8 mo. A bivariant

survival analysis was carried out following the Kaplan-Meier method, together with a multivariate analysis using the Cox proportional hazards model. Twelve patients died during follow-up. CCl values < 0.24 mL/kg per minutes, age > 60 years, and non-alcoholic cause of cirrhosis were factors predicting lower survival. CCl was the only independent predictive factor in the multivariate analysis. The authors concluded that that CCl enables hepatologists to predict survival in cirrhotic patients and, considering its harmlessness, simplicity and cost, it can be used as a routine procedure in the assessment of these patients[14].

A simplification of this test, the so-called Total Overnight Salivary Caffeine Assessment (TOSCA), comes from an other study[15] with a further application (patients divided into rapid and slow metabolizers). Furthermore, TOSCA shows near complete safety (patients drink one or two cups of coffee according their habit in the morning). One drawback of QLFTs is the possible occurrence of severe side effects that are sometimes life-threatening (e.g. anaphylaxis).

Magnetic resonance imaging (MRI) offers several advantages. Gadolinium methoxybenzyl diethylenetriamine penta-acetic acid is a newly developed MR contrast agent. Its hepatic extraction fraction is a direct, noninvasive technique for the quantitative evaluation of liver function. It may be a promising alternative, although expensive, for the determination of noninvasive hepatic function in patients with liver disease[16].

Antiviral therapy

Whether and to what extent does antiviral therapy for chronic hepatitis C influence a broad panel of QLFTs? Fifty patients with chronic hepatitis C were treated with interferon (n = 8), interferon/ribavirin (n = 19) or peg-interferon/ribavirin (n = 23). Quantitative testing of liver function, including ABT, GEC, sorbitol clearance (SCl) and ICG clearance was performed before and 3 mo after initiation of antiviral therapy. After 3 mo, 36 patients showed normal transaminases and were negative for hepatitis C virus RNA, and 14 patients did not respond to therapy. ABT and GEC as parameters of microsomal and cytosolic liver function were reduced in all patients before therapy initiation and returned to normal values in the 36 therapy responders after 3 mo. Parameters of liver perfusion (SCl and ICG) were not affected by antiviral therapy. In the 14 non-responders, no changes in QLFT values were observed during the treatment period. ICG and SCl remained unaffected in patients with chronic hepatitis C, while ABT and GEC were significantly compromised. ABT and GEC normalized in responders to antiviral therapy. Early determination of ABT and GEC may differentiate responders from non-responders to antiviral treatment in hepatitis C[17].

Assessing liver regeneration

Improvement of nitrogen balance is desirable in patients with acute or chronic illness. Both growth hormone and insulin-like growth factor-I are promising anabolic agents, and their combined administration has been shown to reverse catabolism more efficiently than each of the peptides alone. The capacity of urea-nitrogen synthesis [μmol/(min × 100 g body weight)] was evaluated in rats, unravelling a neglected QLFT, based on mitochondrial-cytosolic metabolic capacity (M-CMC) for conversion of amino-nitrogen[18]. Following this approach, the authors used GEC to assess hepatocyte cytosol activity, plasma clearance of ICG to assess excretory function, antipyrine clearance to estimate microsomal activity, and functional hepatic nitrogen clearance to assess M-CMC in females with Turner syndrome[19].

Non-alcoholic steatohepatitis (NASH)

Finally, 13C-C, a noninvasive tool for the evaluation of the cytochrome P450 system, which is implicated in the development of NASH, has been proposed in patients with metabolic dysfunction. Up-to-date research has demonstrated that 13C-C can predict reliably severe hepatic fibrosis in patients with the most severe form of non-alcoholic fatty liver disease (NAFLD). Although this test does not quantify the residual functional liver mass, it is safe and easy to perform. Further large-scale studies are needed to verify its reliability[20]. A previous study that tested 13C-M and ^{13}C-ketoisocaproate for microsomal and mitochondrial liver function has demonstrated its usefulness for better and noninvasive characterization of patients with NAFLD[21]. It is worth stressing that every breath test can be affected badly by severe restrictive lung disease, and in elderly patients with chronic heart failure[22].

Unfortunately, MRI findings of liver steatosis and fibrosis in NASH show moderate correlations with histopathological grade of steatosis and stage of fibrosis, but MRI findings of NASH do not demonstrate any significant correlations with MELD score[23]. Liver scintigraphy (SPECT) might be a promising noninvasive tool in the follow-up of NASH patients in therapeutic trials[24].

CONCLUSION

While liver function is absolutely complex, a widely accepted test to assess quantitative functional hepatic reserve still needs to be established, although there are various tests currently available. The diagnostic and prognostic gain has been quantified as modest. A new condition in which it may be useful to test residual liver function is acute liver disease[25]. Focusing on some aspects of controversial conclusions, or those not supported by very positive results, in the context of the current doctrine is always provocative, although it provides scientists and physicians with responsible and balanced information to support experimental and clinical decisions. Future technical advances may lead to a decrease in time, cost and the number of subjects required to perform QLFTs, therefore, their use in clinical practice is expected to increase.

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TOPIC HIGHLIGHT

Kostas Pantopoulos, Associate Professor, *Series Editor*

Alcoholic liver disease and hepatitis C: A frequently underestimated combination

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Abstract

Alcoholic liver disease (ALD) and hepatitis C virus (HCV) infection represent, either alone or in combination, more than two thirds of all patients with liver disease in the Western world. This review discusses the epidemiology and combined impact of ALD and HCV on the progression of liver disease. ALD and HCV affect the progression of liver disease to liver cirrhosis and hepatocellular carcinoma (HCC) in a synergistic manner. Thus, the risk for HCC increases five times with a daily alcohol consumption of 80 g; in the presence of HCV it is increased 20-fold, and a combination of both risk factors leads to a more than 100-fold risk for HCC development. Alcohol consumption also decreases the response to interferon treatment which is probably due to a lack of compliance than a direct effect on HCV replication. Several molecular mechanisms are discussed that could explain the synergistic interaction of alcohol and HCV on disease progression. They include modulation of the immune response and apoptosis, increased oxidative stress *via* induction of CYP2E1 and the hepatic accumulation of iron. Thus, both HCV and alcohol independently cause hepatic iron accumulation in > 50% of patients probably due to suppression of the liver-secreted systemic iron hormone hepcidin. A better understanding of hepcidin regulation could help in developing novel therapeutic approaches to treat the chronic disease in the future. For now, it can be generally concluded that HCV-infected patients should abstain from alcohol and alcoholics should be encouraged to participate in detoxification programs.

Key words: Alcoholic liver disease; Chronic hepatitis C; Steatosis; Steatohepatitis; Fibrosis; Cirrhosis; Reactive oxygen species; Hepatocellular carcinoma; Iron accumulation

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INTRODUCTION

Together, alcoholic liver disease (ALD) and chronic hepatitis C virus (HCV) infection are the most frequent chronic liver diseases in the Western world. In addition, they frequently coexist in the same individual. While both diseases alone have a similar progression sequence leading to cirrhosis in circa 15% of patients within 10-20 years, their coexistence dramatically enhances disease progression in a so-called synergistic manner. This synergism affects both fibrosis progression and the development of hepatocellular carcinoma (HCC). The basic molecular mechanisms of this synergism are far from being understood but may include increased production of reactive oxygen species (ROS) and deposition of iron. In the present article, we review and discuss the epidemiology of ALD and HCV infection, the synergistic impact of combined alcohol and HCV on the progression of liver disease, viral replication and response to anti-HCV treatment. We finally analyze potentially

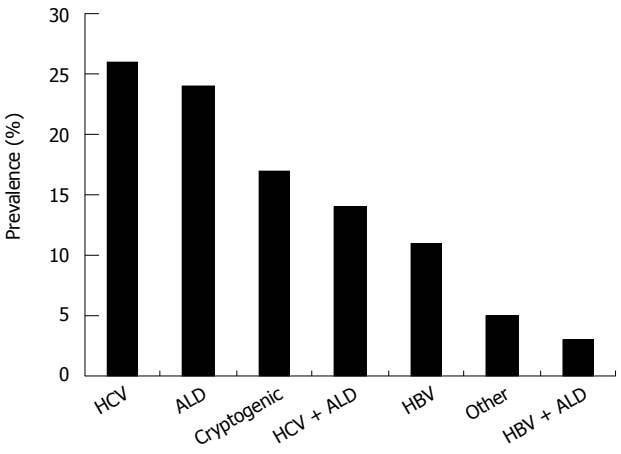

Figure 1 HCV and ALD, either in combination or alone, represent the majority of liver diseases (data from the US Centers of Disease Control and Prevention 2007). HCV: Hepatitis C virus; ALD: Alcoholic liver disease; HBV: Hepatitis B virus.

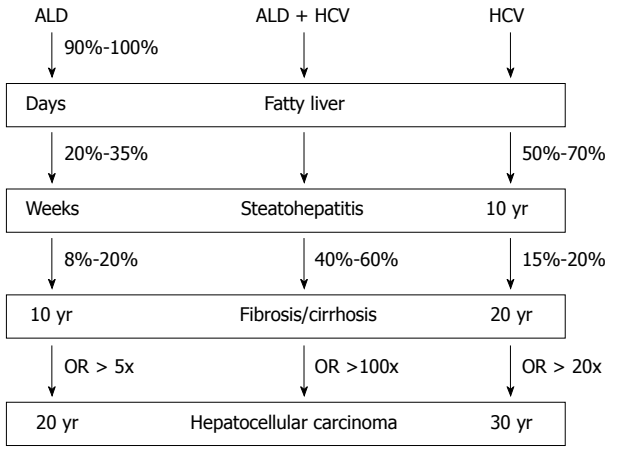

Figure 2 Natural course of ALD and HCV alone or in combination. Estimated risk and time interval for disease states are indicated (for more details see text).

underlying mechanisms that may explain the interaction between alcohol and HCV and offer novel molecular strategies for future therapeutic interventions.

EPIDEMIOLOGY OF ALD AND HCV

Chronic alcohol consumption causes approximately 50% of the chronic liver disease burden in Germany and the death of more than 18000 inhabitants per year[1]. In the US alcohol is also responsible for more than 50% of liver related deaths, and ALD is a major health care cost expenditure, accounting for nearly $3 billion annually[2]. At present, the country with the fastest increase in alcohol associated health problems is the Peoples Republic of China with an annual per capita increase in alcohol consumption of 400% and more in some geographic regions[3]. The exact number of alcohol related deaths is difficult to obtain due to inaccurate reporting of ethanol use. Since patients with compensated liver cirrhosis may often die by causes not obviously related to liver disease e.g. infectious complications, official mortality tables most likely underestimate the true prevalence of ALD. If the relationship between alcohol intake and prevalence of ALD is examined on a population basis, the risk of developing ALD starts at 20-30 g ethanol per day. Liver cirrhosis develops only in 10%-20% of people consuming more than 80 g of ethanol daily[2]. Approximately 5% of the whole population in the US meet diagnostic criteria for alcoholism[4]. In Germany, more than 17.8% of the population > 18 years drink more than 20-30 g of alcohol per day[5], and a comparable number of 5% show high risk drinking behavior (> 80 g/d)[5].

In contrast to ALD, the prevalence of HCV is easier to determine based on serological studies. The worldwide seroprevalence of HCV antibodies is estimated to be 3% with marked geographic variations from 1% in North America to 10% in North Africa[6]. The prevalence is higher in males than in females (2.5% *vs* 1.2%) and is highest in the 30-49 years old age group[7]. Taken together, there is an estimated prevalence of high risk drinking and HCV of 1%-5% in the Western world. According to recent data from the Center of Disease Control and Prevention, the prevalence of HCV and ALD is relatively similar at 26% and 24%, respectively. Although there is a selection bias, these prevalence data are somehow reflected by large transplant centers. In our transplant center at the University of Heidelberg, liver cirrhosis due to HCV and ALD are leading causes for liver transplantation accounting for 32% and 24%, respectively, of all liver transplantations. In summary, HCV and ALD represent either alone or in combination, more than two thirds of all patients with liver disease in the Western world[8] (Figure 1).

NATURAL COURSE OF ALD AND HCV AND IMPORTANT PROGRESSION FACTORS

ALD is the most important organ manifestation of chronic alcohol consumption. Ninety percent to one hundred percent of heavy drinkers develop alcoholic fatty liver. Ten percent to thirty-five percent of them develop alcoholic steatohepatitis and 8%-20% develop alcoholic liver cirrhosis within 10-20 years[9]. The natural course of ALD and HCV is given in Figure 2. Due to better treatment options for complications of liver cirrhosis e.g. variceal bleeding, the prevalence of HCC is increasing with an annual risk of 1%-2%. HCC represents the most common cause of death in patients with ALD. HCV shows a similar progression pattern. In a US study, the mean interval between HCV infection, chronic hepatitis, cirrhosis and HCC was circa 10, 20 and 30 years, respectively[10]. A large cohort study with long-term follow up showed that 75% of HCV-infected patients develop persistent infection while severe progressive liver disease occurred in 15%-20%[11].

Factors that contribute to progression of ALD and HCV are summarized in Table 1. For ALD, these include the amount of alcohol consumed over a life time[12,13], drinking patterns, and nutritional status. Both malnutrition and obesity are associated with an increased risk for alcoholic cirrhosis[14-16]. This is especially relevant with the endemic occurrence of non-alcoholic fatty liver disease

Table 1 Comparison of risk factors for ALD and HCV

HCV	ALD
Male gender	Female gender
	Amount and duration of alcohol consumption
	Continuous drinking (*vs* sporadic drinking)
	Overweight/malnutrition
Hepatic iron deposition age > 40	Hepatic iron deposition
Immunosuppression	Vitamin A, co-medication

ALD: Alcoholic liver disease; HCV: Hepatitis C virus.

(NAFLD) in the Western World due to obesity and being overweight associated with diabetes mellitus type Ⅱ and peripheral insulin resistance. Co-medication of certain drugs together with ethanol may also harm the liver by increased conversion to toxic metabolites due to induced enzyme systems. This is well known for acetaminophen[17], methotrexate[18] and the tuberculostatic drug isoniazid[17] but also occurs with retinoids such as β-carotin and vitamin A[19]. Males and females show different courses of ALD and HCV. While females are more susceptible to alcoholic damage, they progress slower in chronic HCV infection. Other important factors that contribute to disease progression in ALD are co-morbidities such as HBV, hemochromatosis, and Wilson's disease. Factors associated with HCV progression are co-infection with HBV, HIV, schistosomiasis or conditions of immunosuppression. Finally, iron accumulation has been recognized both in ALD and HCV as an independent risk factor for the development of HCC. Pathological hepatocellular iron deposits are found in more than 50% of patients with either HCV or ALD. Underlying mechanisms and potential therapeutic options are still under investigation.

EFFECT OF ALCOHOL ON THE PREVALENCE OF HCV INFECTION

Chronic alcoholics have an increased prevalence of HCV infection, increasing with the severity of the ALD. Takase *et al*[20] showed that HCV prevalence demonstrated by anti-HCV positivity increases with the severity of ALD, having a prevalence of approximately 5% in alcoholic fibrosis, almost 40% in alcoholic cirrhosis and almost in 80% in HCC due to alcohol. This could be due to the lifestyle of chronic alcoholics, since many of them are also intravenous drug abusers, which is a high risk for HCV infection. It could also be due to the immunosuppressive effect of alcohol decreasing the HCV-clearance rate after infection since it has been shown that alcohol suppresses the function of various immune components including natural killer cells, neutrophils, monocytes and others[21].

ALCOHOL CONSUMPTION, HCV REPLICATION AND RESPONSE TO HCV THERAPY

A great number of studies emphasize the fact that alcoholics respond poorly to interferon therapy. More than ten years ago, Mochida *et al*[22] showed that almost 30% of non-alcoholics responded biochemically and virologically to interferon therapy compared to less than 10% of alcoholics. The question remained open whether this is due to a direct inhibitory effect of alcohol on interferon response or due to poor compliance of these patients. Pessione *et al*[23] studied serum HCV RNA in HCV patients with increasing alcohol intake (reported in gram per week). In this study a significant dose-dependent increase in serum HCV RNA was noted starting from 70 g alcohol per week. In line with this observation, a decrease of alcohol consumption prior treatment of hepatitis C significantly reduced viral load. In addition, Cromie *et al*[24] showed that viral load decreased highly significantly within 4 mo when patients cut down on alcohol consumption from 39-100 g/d to 0-50 g/d. More recent data, however, clearly suggest that the poor response of alcoholics towards interferon therapy is more likely due to reduced compliance. In this study, the recorded alcohol consumption during the months before HCV treatment was associated with an increased rate of therapy drop out (3% *vs* 26%, $P = 0.002$)[25] while the response rate was comparable (25% *vs* 23%) after correction for this confounding factor. In conclusion, poor compliance of alcoholics is probably the major cause for poor antiviral response to HCV therapy.

COMBINED EFFECTS OF ALCOHOL AND HCV ON FIBROSIS PROGRESSION

A huge number of studies have shown that concomitant alcohol consumption in the presence of HCV increases progression of fibrosis[23,26-54]. This means that fibrosis occurs at an earlier time point and its development is accelerated. A summary of selected studies on alcohol consumption and fibrosis progression is given in Table 2. Thus, it has been shown in more than 2000 HCV patients that fibrosis progression was significant ($P < 0.001$) if more than 50 g/d alcohol is consumed[26]. Similar results were obtained by Roudot-Thoraval *et al*[27] with a prevalence of cirrhosis of 35 % *vs* 18 % ($P < 0.001$). Pessione showed in more than 200 HCV patients that weekly alcohol consumption correlated significantly with fibrosis score[23]. He also showed that the relative risk for decompensated cirrhosis correlated with alcohol intake. Alcohol-driven fibrogenesis in HCV patients is dose-dependent and starts at less than 30 g/d. Overweight and obese patients as well as type Ⅱ diabetics are especially sensitive to fibrosis progression[55]. HCV patients with excessive alcohol abuse have a 2-3 fold increased risk of severe liver disease compared with HCV patients without a history of drinking[56]. So far it is still unclear how long a patient has to abstain from alcohol before the negative effect of alcohol is abolished[57]. Alcoholics with HCV infection seem to stop drinking more frequently compared to alcoholics without HCV infection. This is possibly due to a higher awareness in these patients that liver disease can lead to cirrhosis and death without a change in lifestyle[58]. Finally, it has been a

Table 2 Fibrosis progression in HCV and alcohol consumption from selected studies

No. of patients	Alcohol consumption	Results	Ref.
2235	0 g, < 50 g, > 50 g	> 50 g independent risk factor for fibrosis progression ($P < 0.001$)	[26]
6664	> 5/6 drinks (female/male), > 1 year	Higher risk of cirrhosis (35% *vs* 18 %)	[27]
176	> 40/60 g (female/male), > 5 years	Faster cirrhosis progression (58 % *vs* 10 %), 2-3 fold increased risk of developing cirrhosis	[29]
168	Low < 30, medium 30-80; high > 80 g/d, > 5 J	Alcohol consumption low/medium/high significantly different between non-cirrhotics (58%/27%/16%) and cirrhotics (76%/15%/9%) ($P < 0.05$)	[28]
234	Lifetime alcohol consumption	Cirrhotics have greater alcohol consumption than patients with hepatitis (240 g/wk *vs* 146 g/wk) ($P = 0.02$)	[30]
233	0, 25, 50, 75, 100, > 125 g	Weekly alcohol consumption correlates with serum HCV RNA levels and fibrosis score ($P < 0.001$)	[23]
702	0/175 g/d	HCV increases OR for cirrhosis from 1 to 15 (0 g), 9.2 to 147.2 (175 g)	[141]
1667	Subgroup: > 260 g/wk *vs* < 90 g/wk	Risk for cirrhosis increases by 3.6	[31]
636	> 80	RR for cirrhosis: HCV 7.8, HCV + alcohol 31.1	[32]

continuous debate whether small amounts of alcohol (< 20-30 g/d) alter progression in HCV infection. An answer may come from a Scandinavian study by Westin *et al*[59]. These authors investigated 78 patients with hepatitis C infection who underwent two liver biopsies in a mean interval of 6.3 years. Alcohol consumption was less than 40 g/d. The authors found progressive fibrosis with (a) increased total alcohol consumption (15.4 kg *vs* 3.9 kg; $P = 0.007$), (b) increased daily alcohol consumption (5.7 g *vs* 2.6 g/d; $P = 0.03$) and (c) increased frequency of drinking occasions (35 *vs* 8 d per year; $P = 0.006$). These results underscore that even small amounts of alcohol may increase fibrosis progression in HCV. Confirmation comes from another prospective study by Hezode *et al*[60] who showed an impact of mild alcohol consumption on histological activity and fibrosis starting as low as 20 g/d.

ROLE OF ALCOHOL AND HCV ON DEVELOPMENT OF HCC

Various studies have shown that there is an increased risk of HCC in patients with HCV and alcohol abuse compared to either HCV or ALD alone[61-68]. Since these studies vary considerably in their definition of alcohol abuse, Table 3 is restricted to comparable studies that tried to identify the independent contribution of HCV and ALD to HCC development. It can be concluded from these data, that a daily uptake of > 80 g alcohol alone increases HCC risk 5-fold while the presence of HCV alone increases HCC 20-fold. A combination of both risk factors increases the risk for HCC development over 100-fold. Thus, HCV and alcohol act truly synergistic on HCC development.

Table 3 Odds ratio for the development of HCC as a function of HCV seroprevalence and amount of alcohol consumption

	Alcohol consumption (in grams alcohol per day)						
	No	Yes	0-40	40-80	> 60	> 80	Ref.
Without HCV			1	1.5		7.3	[63]
	1	2.4		1.7		4.5	[68]
					2		[67]
					3		[64]
With HCV			26.1	62.6		126	[63]
	19.1	53.9					
							[64]

POSSIBLE MECHANISMS FOR THE SYNERGISM OF ALCOHOL AND HCV

The underlying molecular mechanisms of alcohol and HCV-mediated liver disease are complex and they are still incompletely understood despite intensive efforts over decades.

Both alcohol and HCV can reproduce the four sequential hallmarks of liver disease: steatosis, steatohepatitis, fibrosis and HCC. Molecular key features of ethanol and HCV mediated liver damage include direct biochemical consequences of alcohol metabolism such as the production of acetaldehyde, generation of reactive oxygen species (ROS) and oxidative damage, epigenetic modifications such as hypomethylation of histones and modulation of the signaling machinery. Some of these events lead to similar downstream effects such as fatty liver, ROS and iron accumulation but are based on different mechanisms which could well explain the synergistic effects of alcohol and HCV on the liver. Thus, steatosis in HCV is mainly caused by impairment of mitochondria preventing mitochondrial metabolism of fatty acids, while ethanol primarily stimulates lipogenesis. On the other hand, HCV and ethanol both stimulate ROS generation *via* distinct mechanisms and they both lead to hepatic iron accumulation, one of the most pro-fibrogenic and pro-tumorigenic factors in liver disease. For this reason, ROS generation and iron accumulation are discussed separately below. It should also be mentioned that alcohol may have direct molecular effects on HCV infection since it exerts stimulatory effects on HCV replication probably *via* signaling pathways[69]. The enhanced quasispecies complexity in the hypervariable region 1 of HCV in alcoholics may be one major cause that sensitizes for faster disease progression[70].

Alcohol, HCV and liver damage

Ethanol biochemically leads to a shift towards NADH which ultimately stimulates lipogenesis. In addition,

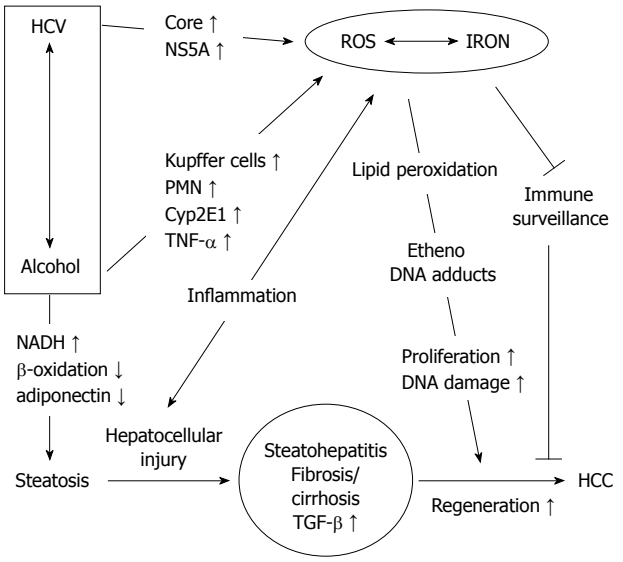

Figure 3 Potential molecular mechanisms that explain the synergistic effect of alcohol and HCV on the progression of liver disease. Reactive oxygen species (ROS) and iron accumulation seem to be key features of both diseases.

ethanol is metabolized to the mutagenic metabolite acetaldehyde and during that reaction ROS are produced mainly as a byproduct of CYP2E1. Additional mechanisms include the release of cytokines such as TNF-α, which increases free fatty acid release from adipocytes in the periphery of the liver[71], stimulates lipogenesis in hepatocytes[72], and inhibits β-oxidation of fatty acids[73]. Chronic ethanol consumption also impairs transport and secretion of triglycerides as VLDL[74] which again leads to an increased hepatic fat accumulation. Activation of macrophages by lipopolysaccharides *via* the toll-like receptor 4 (TLR-4) leads to the production of a variety of inflammatory mediators, such as TNF-α and ROS. HCV also leads to steatosis but in contrast to ALD mainly *via* a decreased mitochondrial β-oxidation with ultrastructural alterations of hepatocyte mitochondria in more than half of the patients. This means that HCV and alcohol stimulate fat accumulation in the liver *via* distinct mechanisms. In addition to its role in steatosis, abnormal production of TNF-α is also a critical inflammatory component in the liver induced by chronic ethanol exposure[75,76]. Although direct exposure of macrophages in culture can mimic some of the effects of ethanol[77,78], there seem to be multiple hepatic and extra-hepatic consequences of ethanol that finally render Kupffer cells more reactive to LPS, leading to generation of ROS and ROS-modulated signal transduction cascades[79,80]. The fat regulating hormone adiponectin also seems to be involved in ethanol mediated steatohepatitis[81-83]. Some data indicate that ethanol directly drives fibrosis progression: acetaldehyde is supposed to increase TGF-β1 secretion[84] and both ethanol and acetaldehyde induce accumulation of collagen[85]. Similar findings have been shown for HCV-replicating hepatoma cells[86].

HCC pathogenesis by ethanol seems to require several factors[87] including the presence of cirrhosis, oxidative stress, altered methyl transfer resulting in DNA hypomethylation, and a decrease in retinoic acid. In addition, co-morbidities such as viral hepatitis, diabetes mellitus and obesity are known to accelerate HCC development in patients with ALD. ROS play an important role in hepatocarcinogenesis[87,88]. Chronic ethanol consumption results in the generation of ROS *via* multiple pathways leading to lipid peroxidation (LPO) and LPO-byproducts such as 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA). These DNA-reactive aldehydes in turn form mutagenic exocyclic DNA adducts including 1, N6-ethenodeoxyadenosine (εdA) and 3, N4-ethenodeoxycytidine[89,90].

Role of ROS in HCV and ALD

The generation of ROS seems to be a hallmark of both ALD and HCV[91-95] (Figure 3). While location and mechanisms of their generation differ markedly between ALD and HCV, downstream events of oxidative damage are similar due to the high but rather unspecific reactivity of species such as hydroxyl radicals or lipid peroxidation products. Hepatocyte mitochondria are structurally altered in more than 50% of HCV patients and these conditions are accompanied by a significant depletion of hepatocellular and lymphocyte glutathione (GSH), an increase of oxidized GSH (GSSG) and the lipid peroxidation marker malondialdehyde[91]. ROS are either induced directly by the virus or indirectly through activation of inflammatory cells. HCV core[96] and NS5A[96,97] have been implicated in generating ROS *via* mitochondrial damage and calcium release.

ROS also play an important role in alcohol-induced liver injury and in hepatocarcinogenesis[87-90]. Several enzyme systems are capable of producing ROS including the mitochondrial respiratory chain, the cytosolic enzymes xanthine oxidase and aldehyde oxidase, as well as the microsomal cytochrome P450-dependent mono-oxygenases[88]. One member of the latter system, cytochrome P450 2E1 (CYP2E1), is involved in the major pathway by which ethanol generates oxidative stress. Expression of CYP2E1 has been shown to correlate well with the generation of hydroxyethyl radicals and with LPO products such as 4-HNE and MDA[98]. CYP2E1 is induced by chronic alcohol consumption within a week even at a relatively low ethanol dose (40 g/d), but the degree of CYP2E1 induction shows high variations between individuals[99]. Inhibition of CYP2E1 by chlormethiazole, a specific CYP2E1 inhibitor, improved ALD as shown in the Tsukamoto-French rat model[100]. An increase of oxidative DNA adducts and of mutagenic apurinic and apyrimidinic DNA sites has been found in chronically ethanol-treated wild- type mice but not in mice that lack functional CYP2E1[101] further stressing the importance of CYP2E1 in the generation of DNA damage following ethanol ingestion. Increased levels of Cyp2E1 also potentiate pro-apoptotic effects of TGF-β resulting in increased cell death of hepatocytes[102]. Recently, we have been able to detect etheno-DNA adducts such as εdA in the livers of patients with ALD[89,103].

Kupffer cells are also an important source of ROS

during ethanol exposure[93] and in response to LPS[104]. NADPH oxidase-dependent production of ROS is implicated in ethanol-induced liver injury since p47phox -/- mice which are deficient in this regulatory subunit of NADPH oxidase are resistant to chronic ethanol-induced injury[105]. Chronic ethanol feeding increases the LPS-stimulated production of ROS by Kupffer cells; this increase is primarily due to an increase in NADPH oxidase activation after chronic ethanol feeding[81]. Recently, Thakur and colleagues have specifically identified NADPH oxidase-derived ROS as an important contributor to increased TLR-4 mediated signal transduction and TNF-α expression in rat Kupffer cells, particularly after chronic ethanol exposure[81].

Ethanol and HCV lead to hepatic iron accumulation

In contrast to hepatitis B infection, iron deposits are found in more than 50% of patients with HCV infection or chronic ethanol consumption[96,106-109]. Even mild to moderate alcohol consumption has recently been shown to increase the prevalence of iron overload[110]. Iron localization has been reported in Kupffer cells[108] as well as in hepatocytes[111-113]. In our experience, iron accumulation is more common in hepatocytes than Kupffer cells in patients with ALD. Increased hepatic iron content is associated with greater mortality from alcoholic cirrhosis, suggesting a pathogenic role of iron in ALD. Genetic hemochromatosis in conjunction with excessive alcohol consumption exacerbates liver injury[100]. It should be mentioned that iron *per se* is the most profibrogenic and genotoxic factor and 50% of patients with hereditary hemochromatosis develop fibrosis and have a 200-fold increased risk for HCC[114]. On the other hand, immune surveillance can be impaired by iron overload, since it compromises anti-tumor activity of macrophages[115-117].

The underlying mechanisms of iron accumulation observed in ALD and HCV are still poorly understood but seem to involve an inadequate upregulation of the iron hormone hepcidin. Genome wide microarray based screening for candidate genes that could cause iron overload involved several genes not yet linked to iron metabolism[118]. Preliminary data from ALD patients and ethanol-treated mice models suggest that hepatic iron uptake pathways are increased in the liver and potential mechanisms involve an increase of the transferrin receptor (TfR)1 and repression of the systemic iron hormone hepcidin that controls duodenal iron absorption and RES-mediated iron release *via* the iron exporter ferroportin[119,120]. Using novel *in vitro* and *in vivo* models[121,122], we have recently demonstrated that H_2O_2 alone increases TfR1 *via* posttranscriptional and translation mechanisms ultimately leading to cellular accumulation of iron[123,124]. These data show that chronic exposure of cells to non toxic levels of H_2O_2 lead to accumulation of iron *via* distinct regulatory mechanisms promoting Fenton chemistry. We suggest that increased oxidative stress in the form of H_2O_2 is an important regulatory factor that causes continuous iron accumulation and may support ALD progression. Valuable information on the direct interaction of HCV with host metabolism has been gained from studies with genetically modified animals, though with some controversial results[125]. Thus mice transgenic for the total open reading frame of HCV under the murine albumin promoter developed steatosis and liver cancer[126,127], but this association disappeared in later generations of animals, casting doubt on the earlier conclusions that HCV infection alone (in the absence of cirrhosis and iron overload) drives hepatic carcino genesis[109,128,129]. In addition, iron overload induced in mice either through diet[130,131] or genetic deletion of the HFE locus[132] did not lead to advanced fibrosis or HCC. In HCV transgenic mice, hepcidin was found to be suppressed despite iron loading. This is unexpected, since hepcidin inhibits cellular iron efflux by inducing internalization of ferroportin[133], an iron exporter that is expressed in macrophages, hepatocytes and intestinal cells. The mechanism by which hepcidin was downregulated in the present model remains elusive, since cytokines such as TNF-α, IL-1β and IL-6 which can upregulate hepcidin levels[134,135] were not suppressed. Other important players such as iron regulatory proteins (IRP1 and IRP2) which sense iron but also ROS at the cellular level have not been assessed[136].

Finally, it has also been investigated whether iron directly affects HCV replication. In hepatoma cells iron loading promoted reporter expression under the control of regulatory HCV mRNA stem-loop structures by upregulating expression of the translation initiation factor 3eIF3[137]. In contrast, iron was shown to suppress HCV replication by inactivating the RNA polymerase NS5B[138]. Clinical data indicate that iron status does not significantly influence HCV replication *in vivo*, since the response to therapy of patients with β-thalassemia was not influenced by the degree of iron accumulation[139], and venesection did not reduce hepatitis C viral load[140]. Taken together, iron accumulation in patients with HCV and ALD is an important progression factor. The underlying mechanisms are being intensively studied in search for novel therapeutic approaches.

CONCLUSION

ALD and HCV are the most common liver diseases in the Western world either alone or in combination. Coexistence of both diseases has a true synergistic effect on fibrosis progression and HCC development. Thus, a daily consumption of more than 80 g alcohol increases the risk for HCC 5-fold, in the presence of HCV 100-fold while HCV alone increases the risk for HCC 20-fold. Alcohol abusers have an increased prevalence of HCV infection probably due to lifestyle or to immune suppression. Alcoholics also have a decreased response rate to antiviral therapy which is most probably due to poor compliance. There is obviously no safe level of drinking in patients with hepatitis C and it remains unclear how long abstinence is necessary to abolish the negative effect of alcohol on the liver. Potential mechanisms which may explain the synergistic negative effect of alcohol

and HCV infection on liver disease include generation of ROS, iron accumulation, steatosis induction, immune modulation, stimulation of HCV replication and direct DNA damage. Abstaining from drinking in HCV patients who do not respond to antiviral treatment is the sole efficient treatment option to date. A better understanding of the underlying molecular mechanisms could help to develop novel targeted treatment options.

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REVIEW

Targeting host factors: A novel rationale for the management of hepatitis C virus

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Abstract

Hepatitis C is recognized as a major threat to global public health. The current treatment of patients with chronic hepatitis C is the addition of ribavirin to interferon-based therapy which has limited efficacy, poor tolerability, and significant expense. New treatment options that are more potent and less toxic are much needed. Moreover, more effective treatment is an urgent priority for those who relapse or do not respond to current regimens. A major obstacle in combating hepatitis C virus (HCV) infection is that the fidelity of the viral replication machinery is notoriously low, thus enabling the virus to quickly develop mutations that resist compounds targeting viral enzymes. Therefore, an approach targeting the host cofactors, which are indispensable for the propagation of viruses, may be an ideal target for the development of antiviral agents because they have a lower rate of mutation than that of the viral genome, as long as they have no side effects to patients. Drugs targeting, for example, receptors of viral entry, host metabolism or nuclear receptors, which are factors required to complete the HCV life cycle, may be more effective in combating the viral infection. Targeting host cofactors of the HCV life cycle is an attractive concept because it imposes a higher genetic barrier for resistance than direct antiviral compounds. However the principle drawback of this strategy is the greater potential for cellular toxicity.

Key words: Host factors; Hepatitis C virus; Novel treatment; Cell entry; Host metabolism; Nuclear receptors; Insulin resistance

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INTRODUCTION

Chronic hepatitis C virus (CHC) infects approximately 170 million individuals worldwide and is a major cause of mortality and morbidity[1].

Egypt has the highest hepatitis C virus (HCV) prevalence in the world (overall prevalence of HCV is 12% among the general population, reaches 40% in persons above 40 years of age and is more in rural areas)[2,3].

HCV is an RNA virus that belongs to the family *Flaviviridae* with six known genotypes (numbered 1-6) and more than 50 subtypes (e.g. 1a, 1b, 2, *etc*)[4]. In general, the genetic make-up of the HCV genotype varies by about 30%-35% between its different genotypes[5,6], and these differences in genotype are related to response to antiviral treatment.

Current treatment for patients with CHC is interferon-based therapies with ribavirin for 24-48 wk. Unfortunately, a sustained virological response (SVR) is achieved in only 42%-52% of treatment-naïve patients, and the rest of patients either show no response or experience a relapse when therapy is stopped[7], with a wide profile of side effects.

The mechanisms underlying the failure of interferon therapy are not well understood, but evidence indicates that in addition to viral factors, several host factors are also involved[8]. So, CHC patients still need a novel approach for treatment of HCV infection.

A major obstacle in combating HCV infection is that the fidelity of the viral replication machinery is notoriously low, thus enabling the virus to quickly develop mutations that resist compounds targeting viral enzymes[9]. Therefore, an approach targeting the host factors that are indispensable for the propagation of viruses might be an ideal target for the development of antiviral agents because of a lower rate of mutation compared to that of the viral genome, as long as they have no serious side effects to patients.

A unique aspect of HCV that has not been observed in other viruses is that the entire viral life cycle is associated with cholesterol metabolism in host cells. Thus, drugs that target cholesterol metabolism might be useful for treating HCV infection[10]. Also, drugs targeting the host proteins required for HCV infection, nuclear receptor or anti-

receptor antibodies may be more helpful in combating the viral infection[11] (Table 1).

INHIBITION OF VIRAL ENTRY

Anti-receptor antibodies

HCV circulates in the bloodstream in different forms; either free or in a complex with immunoglobulin or lipoprotein. Implicated lipoproteins are very-low-density lipoprotein (VLDL), intermediate-density lipoprotein, or low-density lipoprotein (LDL)[12]. HCV RNA is always found in at least one of these fractions and represents 8% to 95% of the total plasma HCV RNA[13,14]. Entry into the host cell is the primary step in the HCV life cycle, which makes it an attractive target for antiviral therapies. Inhibition of viral entry can be accomplished at the level of cell receptor(s) or HCV pseudo-particles (HCVpp) but both approaches require an in-depth knowledge of interactions between host and virus[15].

Attachment and cell entry of HCV is pH dependent and is a clathrin-dependent endocytic pathway[16,17]. Although the molecular details regarding how this virus enters a cell are unknown, CD81[18] and scavenger receptor class B type 1[19] seem to be the key receptor components that mediate viral entry. However, other potential receptors play a role in entry of HCV such as LDL receptor[20], negatively charged glycosaminoglycans, and recently, Evans *et al*[21] added another molecule to the list of HCV receptors, namely, the tight junction protein claudin-1 (CLDN1).

The rationale for anti-receptor antibodies as a drug target is based on them not being prone to the problems of viral variability and high density lipoprotein (HDL) attenuation of neutralizing activity[22].

Targeting viral receptors can be accomplished by various methods, including the design of small molecules that bind to proteins and prevent interaction(s) with HCV. The crystal structure of CD81 long extracellular loop enabled the design of small molecules that bind CD81 and prevent its association with HCV E2[23]. A recent presentation by Liu *et al*[24] identified compounds that displayed a dose-dependent inhibition of HCV infection.

Scavenger receptor BI (SR-BI): SR-BI is a lipoprotein receptor with the highest levels found in the liver and adrenal glands, responsible for the selective uptake of cholesteryl ester from HDL[25-27]. HCV particles have been reported to be complexed with lipoproteins; it is possible that HDL interacts with HCVpp, *via* protein/protein or lipid/protein interactions[28], suggesting an indirect interaction of virus with lipoprotein receptors[29,30]. Recent studies have demonstrated the cell culture-derived HCV association with VLDL containing apolipoprotein B (ApoB) and apolipoprotein E, supporting earlier claims of lipo-viral particles in human plasma.

Previous observations implicated SR-B1 as important for infection by different HCV subtypes and support for this hypothesis is the fact that the same SR-B1 protein element is responsible for the recognition of different HCV E2 glycoproteins despite the high level of variability between their amino acid sequences, especially in the HVR1 region previously shown to be involved in interaction with SR-B1[19,31].

Table 1 Summary of the potential treatment options of CHC targeting host factors and their mechanism of action

Drug	Mechanism of action
Nitazoxanide	Induces PKR phosphorylation
HMG-CoA reductase inhibitors	Disruption of HCV replication; depletion of geranylgeranyl lipids
Antisense RNA drugs targeting apoB	Blocks the assembly and secretion of VLDL, inhibits release of HCV particles from infected cells
Microsomal triglyceride protein (MTP) inhibitors	Blocks the assembly and secretion of VLDL, inhibits release of HCV particles from infected cells
Insulin sensitizer	
Metformin	Insulin sensitivity by acting on hepatic AMP-activated protein kinase
Thiazolidindiones (pioglitazone)	Insulin sensitivity by activating peroxisome proliferator-activated receptors (PPARs)
Debio-025	Inhibition of Cyclophilin B
NIM 811	Inhibition of Cyclophilin B
Tamoxifen, other anti-estrogen drugs	Potentially suppresses genome replication
Small molecules (e.g. receptor mimics, soluble intracellular adhesion molecule-1)	Receptor and uptake inhibition
Receptor antibodies (e.g. Anti CD81)	

HCV appears to use SR-BI during cell entry not merely as an additional site for the viral particle entry but also for exploiting its physiological activity, i.e. the capacity to mediate lipid transfer from HDL which is known to facilitate the entry of many different viruses, such as influenza virus, HIV, and HCV[32,33]. However, HCVs are many times more sensitive to HDL-mediated infection enhancement than other cholesterol-sensitive viruses. Therefore, enhancement of viral infection might be dependent on the lipid exchange activity of SR-B1[28]. Recently, a novel function of SR-Bs for viral antigen uptake and recognition has been suggested; SR-BI may represent a cell-surface receptor for the recognition of viral antigens and be implicated in trafficking exogenous viral antigens toward the MHC class I presentation pathway. The SR-BI-viral antigen interaction may represent a novel target for therapeutic or preventive strategies aiming at the induction of efficient antiviral immune responses[34].

Moreover, HDL with SR-BI is the predominant enhancing factor in infectivity and the presence of HVR1 with HDL protects HCV from neutralizing antibodies as HDL can reduce the neutralizing effect of anti-HCV antibodies[35,36]. This phenomenon might be responsible, at least in part, for the limited ability of the humoral immune response to control HCV infection *in vivo*, which raises concerns about the efficacy of anti-HCV antibodies for active or passive immunotherapy[37]. Thus, as an alternative to the development of anti-HCV antibodies, one could consider anti-SR-B1 human MAbs or anti-CD-81 capable of interfering with HCV infection as potential therapeutic leads. Agents involved in modulating the normal hepatocellular processes of lipid transport have been reported to have pleiotropic effects on HCV infectivity. Antibodies to ApoB have been shown to have antiviral activity[13,29-31,38-43].

Recent data show that BLT-4 and other inhibitors of SR-BI-mediated lipid transfer not only inhibit HCV entry but also fully restore the potency of neutralizing antibodies in infection assays conducted in the presence of HS/HDL, indicating an intriguing link between neutralization efficiency and stimulation of cell entry[28,35]. However, it is too early to know whether the potential for vaccines and passive immunotherapy will be realized. Cholesterol-lowering drugs may be beneficial in patients with chronic hepatitis C by exerting effects on cholesterol metabolism and lipoprotein trafficking *via* SR-BI (See below).

CD81: Recently, Meuleman *et al*[11] showed that CD81 is a critical receptor for HCV infection *in vivo*. Prophylactic injection of monoclonal anti-CD81 antibodies prevented infection of human liver-uPA-SCID mice, however once an infection occurred, no significant difference in viremia was observed between anti-CD81-treated and control animals (irrelevant antibody). These results strongly support the use of CD81 as a clinical target for HCV prevention, especially in the context of orthotopic liver transplantation[11].

Modes of virus transmission

Targeting CD81, SR-BI or CLDN1 may be complicated by receptor-independent modes of virus transmission. In general, there are two primary routes of virus transmission: cell-free and cell-to-cell. Cell-free transmission begins when virus is released from an infected cell and enters the extracellular environment. The virion can bind to surface-expressed receptors on naïve or uninfected cells, become internalized, and initiate new rounds of infection. En route from one cell to the next, the virus may encounter neutralising antibodies or other components of the immune response that may limit infection. In the second route of transmission, the virus spreads directly from one cell to another and, in doing so, may bypass receptor-mediated attachment as well as the immune response.

Direct cell-to-cell transmission has been observed with several viruses, including HIV[44], human T-lymphotropic virus type 1[45] and vesicular stomatitis virus[46] and it was recently proposed that HCV may use this route *in vitro*[47]. Whether cell-to-cell HCV transmission occurs *in vivo* remains to be determined. If this mode of transmission exists *in vivo*, targeting cellular receptors alone may not be an effective antiviral therapy[48].

Targeting receptors as antiviral therapy may also be complicated by their ubiquitous expression in human tissue and their essential roles in cell biology.

TARGETING HOST METABOLISM

HCV seems to be not only an infectious hepatotropic virus but also a metabolic disease[49] with a wide area of metabolic disarrangement, including lipid metabolism[50], glucose metabolism[51] and vitamin D metabolism[52,53]. Metabolism refers to all the reactions by which living things break down nutrients to produce energy, along with those reactions by which they rebuild broken-down nutrients into complex molecules (e.g. DNA). Many viruses, including influenza, HIV and hepatitis, dramatically increase cellular metabolism. The fields of metabolomics and fluxomics have emerged to measure these patterns and to provide insight into diseases with a metabolic component, from diabetes to cancer to infectious diseases such as HCV. Many metabolic processes are essential to the survival of human cells, and so are not candidates for research efforts that would shut them down in an attempt to stop viral replication.

Host lipid biosynthesis inhibitors

Recently, using the new fluxomic techniques, studies revealed that viral infection takes control of cellular metabolism and drives, among other things, marked increases in fatty acid synthesis. Interfering with glucose-to-fatty acid metabolism could stop viral replication, because fatty acid biosynthesis is not essential in adult humans. It does appear, however, to be essential to the ability of HCV to build their envelopes, reproduce and spread. So, targeting of host lipid metabolism by the existing anti-obesity drugs may represent a new way to block these metabolic changes and inhibit viral replication, and may therefore be a potential novel approach that could improve response rates to treatment[54]. There are at least two different molecular mechanisms representing a novel target for management of HCV through the modulation of cellular lipid and cholesterol metabolism. *In vitro* data suggest that statins, the widely used cholesterol-lowering drugs, may inhibit HCV RNA replication by depletion of geranylgeranyl lipids[55,56]. It was recently demonstrated that dose-dependent strong antiviral effects exist for all the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, except for pravastatin, *in vitro*. Fluvastatin exhibited the strongest antiviral activity, followed by atorvastatin and simvastatin[57].

Recently, Bader *et al*[58] reported that fluvastatin inhibits HCV RNA replication in patients with CHC; the study provided evidence that fluvastatin is well tolerated in patients with CHC and at relatively low doses.

These findings, along with other data suggesting synergism with α-interferon, support 'proof-of-concept' for trials combining fluvastatin with standard pegylated interferon plus ribavirin. Cautious, prospective and randomized trials are needed before we can call statin therapy an adjuvant treatment panacea[54].

Another class of drugs designed for treating hypercholesterolemia blocks the assembly and secretion of VLDL. These drugs may also be effective in treating HCV infection because they inhibit release of HCV particles from infected cells[59]. In this regard, antisense RNA drugs targeting apoB[60] and several microsomal triglyceride protein (MTP) inhibitors[61,62] have already been tested in clinical trials because of their ability to block VLDL secretion, thereby lowering the plasma levels of VLDL triglycerides and LDL cholesterol. Long-term treatment with MTP inhibitors led to the toxic accumulation of fat in livers[61,62], thus hampering the approval of these drugs for the treatment of hypercholesterolemia on a long-term basis. However, short-term treatment (up to several weeks) reduced the plasma level of VLDL with only minor adverse effects, which disappeared after drug discontinuation[61]. It will be interesting to examine whether short-term treatment with MTP inhibitors is beneficial in treating HCV infection (Figure 1).

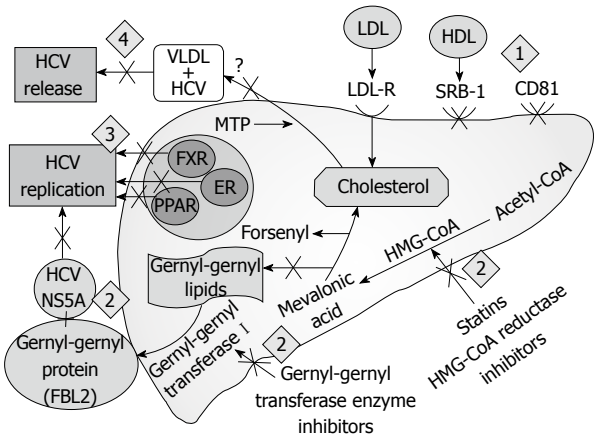

Figure 1 Possible sites targeting host factors as a novel antiviral treatment. (1) Inhibition of HCV entry by anti-receptor antibodies; (2) Interference with the host metabolic factor involved in HCV replication; (3) Modulation of nuclear receptors involved in HCV replication; (4) Inhibition of HCV release. LDL-R: Low density lipoprotein receptor; HDL: High density lipoprotein; VLDL: Very low density lipoprotein; SRB-1: Scavenger receptor B1; FXR: Farnesoid X receptor; ER: Estrogen receptors; MTP: Microsomal triglyceride protein; HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A.

Cyclophilin B inhibitors

Another host cell factor involved in HCV RNA replication is the human protein cyclophilin B protein which interacts with the C-terminal region of NS5B and appears to stimulate its RNA binding activity[63]. The cyclophilin B inhibitor Debio-025 potently suppresses genotype 1 HCV replication *in vivo*[64].

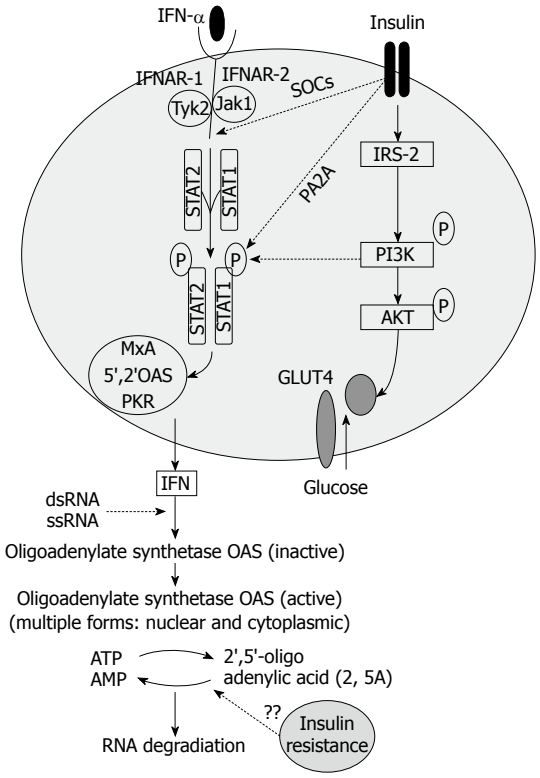

Figure 2 Interaction between insulin and interferon-alfa signaling pathway. SOCs: Suppressor of cytokine signaling; PA2A: Protein phosphatase 2A; PI3K: Phosphatidil-Inositol 3-kinase; JAK: Janus kinase; STAT: Signal transduction and activator of transcription; TYK2: Tyrosine kinase 2; Dotted lines: Represent inhibition; Continuous lines: Represent activation.

Insulin resistance

Insulin resistance emerges as a very important host factor in patients with CHC, mainly because it has been related to steatosis development, fibrosis progression and non-response to peg-interferon plus ribavirin[65]. Insulin resistance is the main pathogenic factor in the development of steatosis in chronic hepatitis C; both viral-induced insulin resistance and metabolic insulin resistance could be implicated in the development of steatosis[66].

Insulin resistance, calculated by the homeostasis model assessment (HOMA), has been found to be one of the most important host factors related to the impermanence of virological response to combined therapy in chronic hepatitis C patients[67].

Recently, obesity has been identified as a modifiable host factor associated with a lower SVR. An elevated BMI is associated with reduced insulin sensitivity and HCV treatment outcome. This observation has led experts to suggest that managing insulin resistance might improve hepatitis treatment outcome and that insulin resistance seems to be a new target in the management of hepatitis C.

The rationale of increasing insulin sensitivity in patients with chronic hepatitis C is based on the premise that insulin resistant state directly or indirectly inhibits the antiviral action of interferon (IFN)-α-β, or increases the viral fitness making it more resistant to therapy, or both[8,68]. Since HCV appears to directly interfere with the glucose homeostasis, several studies have tried to analyze in detail the potential interactions between viral products and insulin signaling. Experimental data suggest a direct interference of HCV with the insulin signaling cascade *via* proteasome degradation of the insulin receptor substrates-1 and -2[69,70]. HCV may also impair insulin signaling transduction indirectly, that is, through increased levels of proinflammatory cytokines such as tumor necrosis factor (TNF)-α[71,72]. The interference with insulin signaling seems to proceed *via* HCV genotype-specific mechanisms and insulin resistance levels vary according to the infecting HCV genotype, although all genotypes induce insulin resistance. Interestingly, intracellular factors dysregulated by HCV and responsible for the insulin resistant phenotype may play promiscuous effects as they are also involved in regulating IFN-α signaling (Figure 2). These factors include some members of the suppressor of cytokine signalling (SOCS) family[69,70,73] and the protein phosphatase 2A[74]. Thus, modulating the levels and/or the activity of these factors may not only reverse hepatic insulin resistance but also help in establishing the IFN-α-induced antiviral state at the site of HCV replication. This is one of the reasons for trying to restore insulin sensitivity in chronic hepatitis C patients, especially those who failed to respond to therapy. Although increasing insulin sensitivity may be a rational option in chronic hepatitis C patients, especially in those with metabolic syndrome, the modalities of this intervention, however, have not been established. In addition, it is unclear whether one should start the antiviral treatment together with the insulin sensitizer or only once the HOMA-IR score has been decreased to a level predicting sufficient SVR rate[67].

However, specific inhibitors of SOCS family members and of the protein phosphatase 2A are either not suitable

for *in vivo* administration or are toxic. Alternatively, increasing insulin sensitivity may be achieved by modulating serum levels of specific cytokines, such as TNF-α, associated with insulin resistance[71,72], but the administration of anti-TNF-α antibodies to chronic hepatitis C patients may be risky[75]. Insulin sensitizers not only increase insulin sensitivity but may also inhibit HCV replication by decreasing serum free fatty acid flow to hepatocytes; saturated and monounsaturated free fatty acids have indeed been shown to stimulate HCV replication in an *in vitro* model[57]. It is not clear whether the best approach would be using a thiazolidindione, activating peroxisome proliferator-activated receptors (PPARs) (see below), or a biguanide such as metformin, whose mechanism of action is specifically directed to the hepatic AMP-activated protein kinase.

Recently, metformin-based triple therapy has been shown to be safe, improving insulin sensitivity and increasing SVR rate by 10% in patients with hepatitis C genotype 1 and insulin resistance (HOMA > 2). This therapy was especially effective in females in whom metformin significantly raised the SVR rate[76].

NUCLEAR RECEPTORS

PPAR receptor

The PPARs are nuclear factors (amongst others) involved in the regulation of glucose homeostasis. In addition to the direct effects on factors involved in lipid and glucose homeostasis[77-81], PPARs may have insulin sensitizing effects *via* their anti-inflammatory activity[82,83]. Thus, treatment with PPAR agonists results in improved insulin sensitivity *via* diverse mechanisms, both direct and indirect, and both at the level of the liver and at the level of extrahepatic tissues[77]. The relationship between HCV replication, protein expression and PPARs has been the focus of some recent studies. However, the data available so far are quite scanty and concern only the HCV genotype 3a[77].

In a recent randomized, double-blind, placebo-controlled study, adding concurrent (PPAR-γ agonist) pioglitazone 30 mg QD to the standard of care (i.e. without a preceding administration as monotherapy) markedly increased the on-treatment virological response, but failed to increase the SVR after the end of treatment[84]. In a related but smaller and shorter study, another research team reported that pioglitazone given as an adjuvant to pegylated interferon/ribavirin in HCV genotype one patients improved viral kinetic response during the first 4 wk of therapy[85].

Also, in a recent study, the level of PPARα mRNA was found to be profoundly suppressed in the liver of chronic hepatitis C patients (about 85% compared to control livers)[86]. The suppression of PPAR-α leads to the upregulation of nuclear factor (NF)-κB. NF-κB has been shown to accelerate virus replication[87], and it has been speculated that activation of PPAR-α with subsequent NF-κB suppression leads to decreased HCV replication in hepatocytes[88]. Given the availability of potent agonists, PPARs may represent a novel pharmacological target in the treatment of liver lesions observed in chronic hepatitis C.

Farnesoid X receptor (FXR)

The bile acid receptors were found to play a role in bile acid-mediated promotion of HCV replication[89]. Furthermore, it was discovered that bile acids compromised the anti-HCV effect of IFN in the cells. These findings suggest a mechanism for persistent infections of HCV in hepatocytes and for the failure of IFN-based treatment for certain HCV patients[89,90]. These data suggest a novel mechanism for bile acid-mediated gene regulation at virus and host levels. Importantly, these data may contribute to the finding of better regimens for the treatment of chronic HCV infections by including agents altering the bile acid-mediated FXR pathway[89].

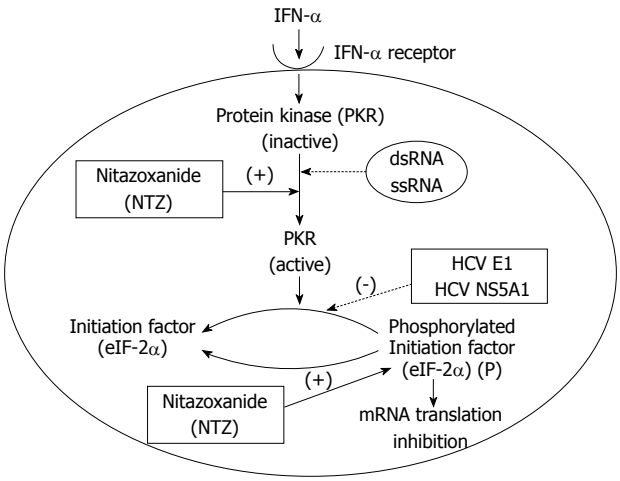

Figure 3 Antiviral mechanism of Nitazoxanide. Dotted lines: Represent inhibition; Continuous lines: Represent activation.

Estrogen receptor (ESR)

ESR belongs to the steroid hormone receptor family of the nuclear receptor super family. There are two different forms of the estrogen receptor, usually referred to as α and β, each encoded by a separate gene[91]. The novel role of ESR α in regulation of HCV replication has been recently reported[92]. Tamoxifen and other anti-estrogens suppress genome replication, as part of ESR resides on the endoplasmic reticulum and interacts with HCV RNA polymerase NS5B, so ESR is suggested to serve as a potential novel target for anti-HCV therapies[92].

OTHER PRINCIPLES

Nitazoxanide

Nitazoxanide is an oral prodrug of a thiazolide (tizoxanide), and was approved for the treatment of protozoal infections[93]. In addition to having antiprotozoal and antibacterial activity, nitazoxanide coincidently was discovered to inhibit HCV replication[94] through a recently identified host-mediated mechanism of action. The antiviral mechanism of action of nitazoxanide appears to be different from the mechanism of action of nitazoxanide in protozoa and anaerobic bacteria. Recent studies suggest that nitazoxanide and other thiazolides selectively induce PKR phosphorylation, which leads to increased cell concentration of phosphorylated eIF2, a naturally occurring antiviral intracellular protein (Figure 3)[95]. This mechanism of action is only triggered when a cell is infected with HCV

while nitazoxanide has no effect in uninfected cells, which provides a possible explanation for its very low rate of toxicity.

Furthermore, nitazoxanide does not appear to induce antiviral resistance, based on an attempt to produce a resistance to nitazoxanide and tizoxanide in HCV replicon-containing cell lines[96]. With serial exposure to nitazoxanide or tizoxanide, direct HCV viral resistance did not emerge, suggesting that the genetic barrier to the development of resistance to nitazoxanide is high. The drug has been recently studied in combination with the standard of care in 96 treatment-naive patients in Egypt infected with genotype 4 HCV infection. The combination of nitazoxanide, peginterferon α-2a, and ribavirin increased the percentage of patients with rapid and sustained virologic responses, compared with patients given peginterferon plus ribavirin, without an increase in adverse events[97].

Nitazoxanide, a novel protein kinase inducer, has the potential not only to increase the SVR rate but also potentially to shorten the duration of therapy.

In summary, the suboptimal response to the currently available standard therapy has led to an extensive search for novel therapies with new therapeutic approaches. Targeting host cofactors of the HCV life cycle by different strategies (inhibition of viral entry, targeting host metabolism, nuclear receptors and other principles) may be a novel rational option, especially because they impose higher genetic barriers for resistance than direct antiviral compounds. However, the principle drawback of these strategies is the greater potential for cellular toxicity.

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ORIGINAL ARTICLES

Characteristic pathological findings and effects of ecabet sodium in rat reflux esophagitis

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Abstract

AIM: To explore the pathological findings in the entire esophagus in rats with reflux esophagitis, and the effects of ecabet sodium (ES).

METHODS: A rat model of chronic acid reflux esophagitis was used. In the treatment group, ES was administered after surgery (n = 16). No drug was administered postoperatively to the esophagitis group (n = 9). Sham-operated rats were used as a control group (n = 5). Rats were sacrificed on day 7 after the operation. The epithelial thickness and leukocyte infiltration were examined in the upper, middle and lower areas of the esophagus. The survival rate, incidence of esophageal ulcer, and mean surface area and number of esophageal ulcers were determined in the esophagitis and ES groups. Esophageal histology was assessed in all three groups.

RESULTS: Leukocyte infiltration in the esophagitis group was 26.3 ± 22.0 in the middle esophagus and 8.2 ± 4.9 in the upper esophagus, which was significantly greater than that in the controls (1.3 ± 1.1 and 1.4 ± 1.0, respectively) (P < 0.05). The thickness of the epithelium in the esophagitis group was 210.8 ± 47.7 μm in the lower esophagus and 204.2 ± 60.1 μm in the middle esophagus, which was significantly greater than that in the controls (26.0 ± 5.5 and 21.0 ± 6.5 μm, respectively) (P < 0.05). The mean number of ulcers per animal in the ES group in the entire esophagus was 5.4 ± 2.5, which was significantly less than that in the esophagitis group (9.0 ± 3.5) (P < 0.05). The epithelial thickness in the ES group was 97.5 ± 32.2 μm in the lower esophagus, which was decreased compared with that in the esophagitis group (210.8 ± 47.7 μm) (P < 0.05).

CONCLUSION: Mucosal inflammation extended to the upper esophagus close to the hypopharynx. Our study suggested that ES may have a useful defensive role in reflux esophagitis.

Key words: Gastroesophageal reflux disease; Upper esophagus; Laryngopharyngeal reflux disease; Extraesophageal syndrome

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INTRODUCTION

Against the background of an aging society and changing dietary habits that now include western-style food, and despite a low rate of infection with *Helicobacter pylori* (*H pylori*), the number of patients with gastroesophageal reflux disease (GERD) has increased recently in Japan[1,2]. The symptoms of GERD decrease quality of life[3], and long-term reflux of gastric acid is known to increase the risk of Barrett's esophagus and Barrett's adenocarcinoma. As a result of the global ground swell

of GERD, a worldwide consensus definition of GERD has been developed recently[4]. Laryngopharyngeal reflux disease (LPRD) is a common condition in the primary care setting and is one of the extraesophageal syndromes regarded as secondary to GERD[5,6]. Exactly how reflux of gastric juice influences the esophagus and/or extraesophageal structures is unknown, and the causal association between gastric-juice reflux and pathogenesis of reflux esophagitis is a controversial subject.

There are limitations associated with the investigation of the pathophysiology of GERD in humans, thus, experiments using animal models are fundamental to this investigation. Proton pump inhibitors (PPIs) and histamine-2 receptor antagonists are likely to be used for reflux esophagitis, but there have been few reports about using mucosal-protective drugs. We previously induced chronic acid-reflux esophagitis in a rat model and investigated the underlying mechanism of reflux esophagitis, with a focus on the mechanism of esophageal mucosal resistance[7,8].

In the current study, we used a rat model of chronic acid-reflux esophagitis to explore the esophageal mucosal damage macroscopically and microscopically throughout the entire esophagus, including the upper esophagus close to the hypopharynx, and to investigate the protective effects of ecabet sodium (ES) on the esophageal mucosa.

MATERIALS AND METHODS

Chronic acid-reflux esophagitis model

Specific-pathogen-free male Wistar rats aged 9 wk were purchased from SLC (Tokyo, Japan). They were used after acclimatization for 1 wk in an animal room with a controlled temperature (23 ± 2℃). The rats were fed a standard diet but fasted for 12 h prior to the surgical induction of chronic acid-reflux esophagitis, which was induced by modifying the method of Omura *et al*[9]. Anesthesia was induced by inhalation of isoflurane. After laparotomy, duodenal stenosis was accomplished by wrapping the duodenum near the pylorus with a piece of 18 Fr Nelaton catheter (length: 3.0 mm, diameter: 4.0 mm; Terumo Inc., Tokyo, Japan). To prevent dislodgement, we sutured the edge of the catheter to the serosa of the pylorus using 4-0 nylon thread. The transitional zone between the fore-stomach and the glandular portion (i.e. the limiting ridge) was ligated with 2-0 silk thread. The animals were fasted for 48 h after the operation but were allowed free access to drinking water. In those allocated to the ES treatment group (n = 16), ES (65 mg/kg) was intragastrically administered only once immediately after surgery, and drinking water including ES (21.39 ± 2.74 mg/kg per day) was given from the day after surgery until day 7. ES was obtained from Tanabe Seiyaku Co. Ltd. (Osaka, Japan). No drug was administered after surgery to the animals in the esophagitis group (n = 9). Sham-operated rats were used as a control group (n = 5). The animals were sacrificed on day 7 after the operation. All the procedures performed on laboratory animals were approved by the Institutional Animal Care and Use Committee of Juntendo University School of Medicine (Tokyo, Japan), and all the animal experiments were carried out in compliance with the guidelines for animal experimentation of Juntendo University School of Medicine.

Measurement of gross esophageal lesions

In the chronic acid-reflux esophagitis and ES groups, the survival rate and incidence of esophageal ulcers were determined. The esophagus was resected up to the upper segment close to the hypopharynx. After taking photographs of esophageal lesions, the numbers of ulcers were measured in the upper, middle and lower segments of the esophagus. The surface areas of ulcers in these three segments of the esophagus were measured using a high-resolution computerized image analyzer (KS 400; Carl Zeiss Imaging Solutions GmbH, Hallbergmoos, Germany).

Histological assessment

After being fixed in 10% buffered formalin, the tissues were embedded in paraffin, and 3-μm sections were prepared and stained with HE. The epithelial thickness was assessed in the upper (approximately 5 mm below the cricopharyngeus), middle [midpoint between the cricopharyngeus and the esophagogastric (EG) junction] and lower (approximately 0.5 mm above the EG junction) segments of the esophagus, using light microscopy (high-power fields). The numbers of leukocytes that infiltrated each high-power field were counted in these three segments of the esophagus.

Statistical analysis

All data are presented as mean ± SD. Student's t test and χ^2 test were used, and $P < 0.05$ was regarded as statistically significant.

RESULTS

Modified chronic acid-reflux esophagitis model vs ES model

In the esophagitis group, the 7-d survival rate after the operation was 66.7% (6/9 rats), and the incidence of ulcers in the survivors was 100%. In the ES group, the 7-d survival rate after the operation was 62.5% (10/16), and the incidence of ulcers was 90.0% (9/10 rats). There were no statistically significant between-group differences in the survival rate and incidence of ulcers.

Macroscopic assessment

In both the esophagitis and ES groups, ulcers were noted in the mucosa, but none occurred in the control group. Also in the esophagitis and ES groups, macroscopic esophageal ulcers were found in the lower and middle parts of the esophagus in most cases (Figure 1). The mean number of ulcers per animal in the ES group in the

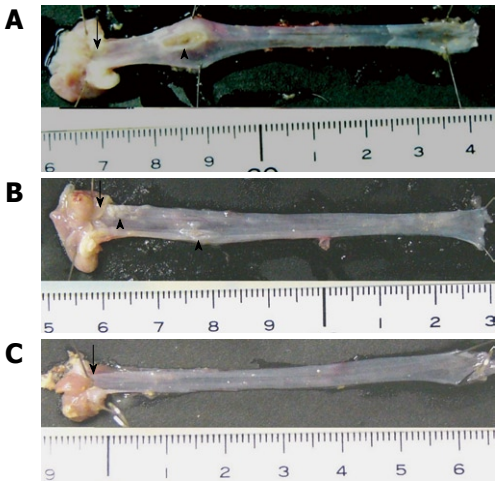

Figure 1 Macroscopic findings in the three groups. A: Esophagitis group on day 7 after operation; B: ES group on day 7 after operation; C: Control group on day 7 after operation. In the esophagitis and ES groups, ulcers were noted in the mucosa, but none occurred in the control group. The arrows indicate the EG junction. The arrowheads indicate ulcers.

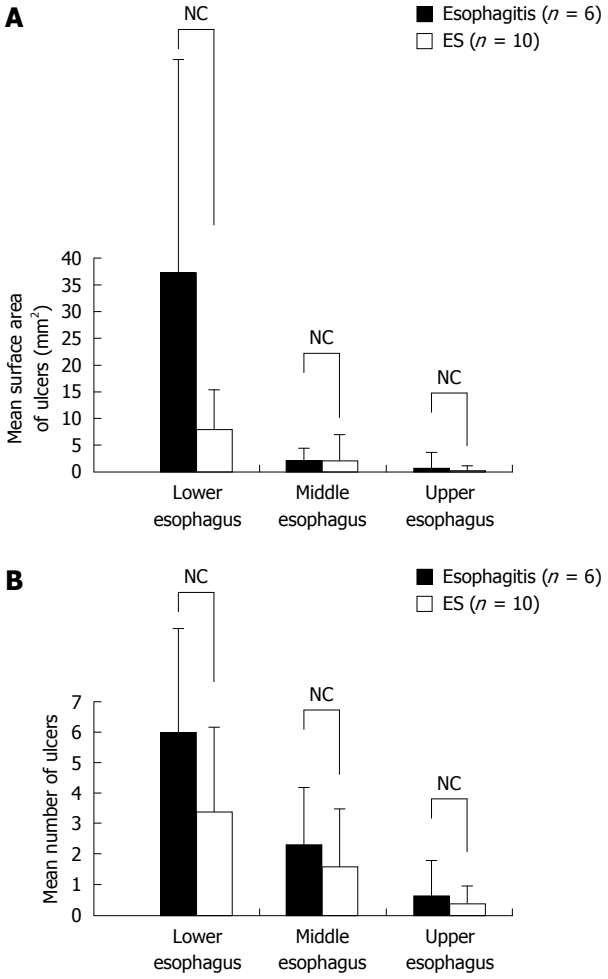

Figure 3 Mean surface area and number of ulcers in each of the three individual segments of the esophagus. A: Mean surface area of ulcers in each part of the esophagus in the esophagitis and ES groups; B: Mean number of ulcers in each part of the esophagus in the esophagitis and ES groups. There were no significant between-group differences in terms of the number and mean area of ulcers in each of the three individual segments of the esophagus.

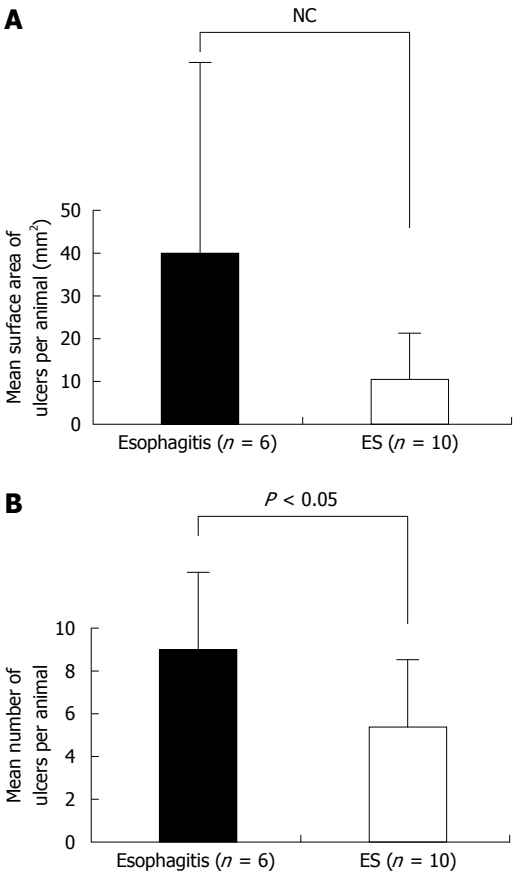

Figure 2 Mean surface area and number of ulcers per animal. A: Mean surface area of ulcers per animal in the esophagitis and ES groups. There were no significant differences in the mean area of ulcers between the two groups; B: Mean number of ulcers per animal in the esophagitis and ES groups. In the ES group, the number of ulcers was significantly less than in the esophagitis group (5.4 ± 2.5 *vs* 9.0 ± 3.5, $P < 0.05$).

entire esophagus was 5.4 ± 2.5, which was significantly less than the number in the esophagitis group (9.0 ± 3.5) ($P < 0.05$) (Figure 2). The mean surface area of ulcers per animal was 40.0 ± 56.8 and 10.5 ± 8.6 mm^2 in the esophagitis and ES groups, respectively ($P = 0.120$) (Figure 2). No significant between-group differences were found in terms of the number and area of ulcers in each of the three individual segments of the esophagus (Figure 3).

Histological assessment

Histologically, the esophagus showed a thin epithelial layer with squamous cells in the control group. In the esophagitis group, the epithelium was thickened markedly, and elongation of the lamina propria papillae into the epithelium was noted. Basal cell hyperplasia and inflammatory cell infiltration were marked in the lamina propria (Figure 4). The thickness of the epithelium in the lower esophagus was the most severely affected of the three areas. The thickness of the epithelium in the esophagitis group was 210.8 ± 47.7 μm in the lower esophagus and 204.2 ± 60.1 μm in the middle esophagus, which was significantly greater than that in the controls (26.0 ± 5.5 and 21.0 ± 6.5 μm, respectively) ($P < 0.05$)

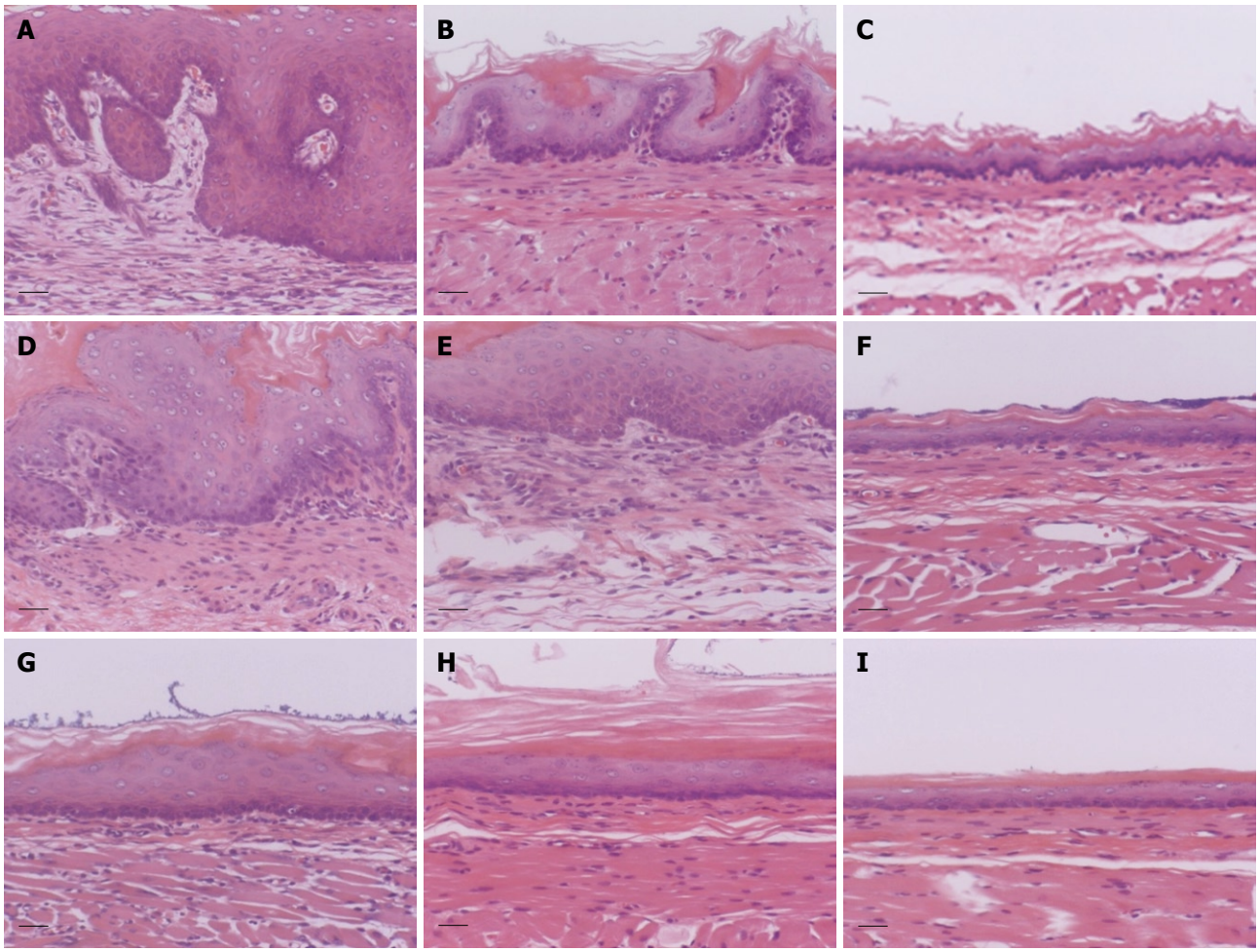

Figure 4 Histological findings in each part of the esophagus in the three groups (HE staining). Lower esophagus: esophagitis (A), ES (B) and control (C) groups. Middle esophagus: esophagitis (D), ES (E) and control (F) groups. Upper esophagus: esophagitis (G), ES (H) and control (I) groups. In the ES group, the thickness of the epithelium in the lower esophagus was significantly decreased compared with that in the esophagitis group. Scale bar, 30 μm.

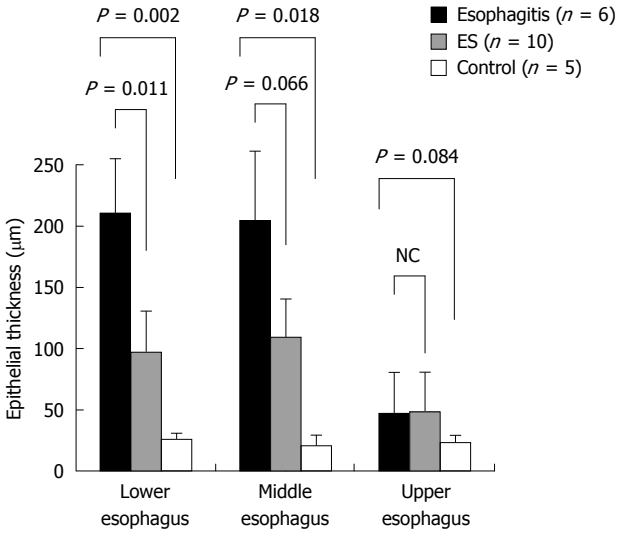

Figure 5 Thickness of the esophageal epithelium in the three groups. In the esophagitis group, the thickness of the epithelium in the lower and middle esophagus was significantly greater than that in the controls ($P < 0.05$). In the ES group, the thickness of the epithelium in the lower esophagus was significantly less than that in the esophagitis group ($P < 0.05$), and the thickness of the epithelium in the middle esophagus tended to be less than that in the esophagitis group ($P = 0.066$).

(Figure 5). Also, the epithelial thickness in the ES group was 97.5 ± 32.2 μm in the lower esophagus, which was decreased compared with that in the esophagitis group (210.8 ± 47.7 μm) ($P < 0.05$), and the epithelial thickness in the ES group in the middle esophagus also tended to be less than that in the esophagitis group.

Leukocyte infiltration in the esophagitis group was 26.3 ± 22.0 in the middle esophagus and 8.2 ± 4.9 in the upper esophagus, which was significantly greater than that in the controls (1.3 ± 1.1 and 1.4 ± 1.0, respectively) ($P < 0.05$) (Figure 6).

DISCUSSION

We revealed that epithelial thickening occurs at the same time as inflammatory cell infiltration in the middle to lower esophagus in chronic acid-reflux esophagitis. An imbalance between offensive factors (e.g. gastric acid and pepsin) and defensive factors (e.g. mucin, saliva and the esophageal epithelial lining) results in the development of reflux esophagitis[7,10,11]. However, there have been few reports about the pathological findings in the esophageal squamous epithelium, and there are differing opinions among pathologists about the findings considered

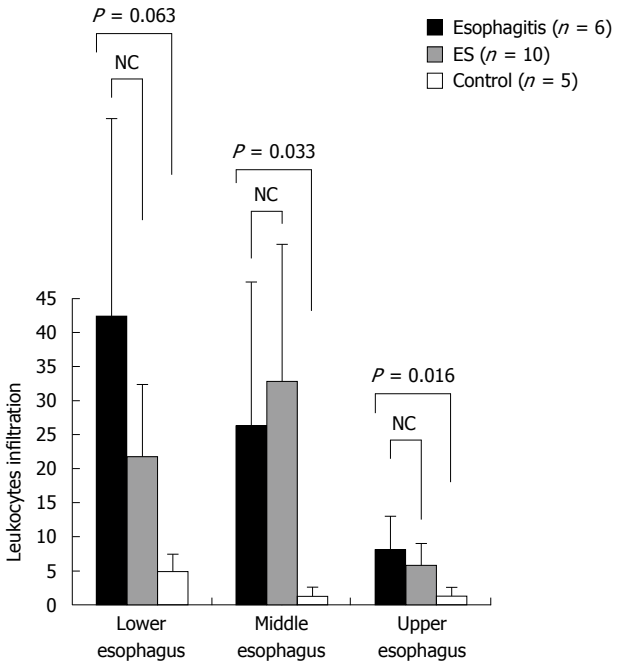

Figure 6 Leukocyte infiltration in the esophageal epithelium on day 7 after operation in the three groups. In the esophagitis group, leukocyte infiltration in the middle and upper esophagus was significantly greater than that in the controls ($P < 0.05$).

characteristic of chronic reflux esophagitis[12-14]. Some authors have reported the relationship between chronic inflammation and epithelial changes in other parts of the gastrointestinal tract. Yasunaga *et al*[15] have suggested that increased interleukin 1β and hepatocyte growth factor production caused by *H pylori* infection may contribute to fold thickening of the stomach by stimulating epithelial cell proliferation and foveolar hyperplasia in patients with enlarged fold gastritis[15]. In patients with erosive reflux disease, the thickness of the basal cell layer and length of the papillae were associated with the severity of esophagitis. After esomeprazole treatment, the basal layer thickness and length of papillae were reduced in both non-erosive and erosive reflux disease[16]. However, these studies were performed in human subjects, so detailed pathological investigation in chronic reflux esophagitis was not performed.

Recently, the mechanism by which acid may induce inflammation has been proposed by Tobey *et al*[17]. They clarified that the permeability of the esophageal epithelium to acid is increased by dilatation of the intracellular space, and PPIs may decrease inflammation of the esophageal epithelium[17,18]. These results suggest that acid may diffuse through the esophageal epithelium and induce infiltration of inflammatory cells. This increases the release of proinflammatory cytokines, which may induce thickening of the esophageal epithelium. Furthermore, we demonstrated that inflammatory cells infiltrated the epithelium of the upper esophagus close to the hypopharynx, where there was no evidence of ulcers. It has been reported previously that inflammation mainly occurs in the lower esophagus near the EG junction in cases of reflux esophagitis[8], but how the reflux of gastric juice influences esophageal and/or extraesophageal symptoms is unknown. Recently, GERD-related extraesophageal syndromes have attracted attention and it has been suggested that gastric-juice reflux can extend to the upper esophagus close to the hypopharynx. In a study of the pathogenesis of LPRD, which is one of the extraesophageal syndromes, Tokashiki *et al*[19] have found that patients with LPRD show a significantly longer acid reflux time in the upper esophagus than healthy volunteers do.

In our chronic acid-reflux esophagitis model, gastric juice passed through the EG junction and diffused directly into the esophagus. This model resembles the reflux esophagitis that is seen in the clinical setting. The direct acid injury to the hypopharynx appeared to be reflected in the microscopic pathological changes in the upper esophagus.

Secondarily, we demonstrated that ES inhibited the epithelial thickening of the lower esophagus, which was the most severely inflamed segment of the esophagus, and ES also tended to inhibit the epithelial thickening of the middle esophagus. ES, a dehydroabietic acid derivative from pine resin, has been used clinically in the treatment of gastritis and gastric ulcer, and is believed to exert its effects through various mechanisms[20-24]. ES binds directly to the gastric mucosa, thereby protecting the mucosa against ethanol binding, and it has been shown to inhibit pepsin activity in rat and human gastric juices.

In the present study, ES may decrease the number of ulcers in the entire esophagus by binding directly to the esophageal mucosa and inhibiting pepsin activity. ES was shown to be useful in preventing inflammation from the lower to the middle esophagus. ES may inhibit the increase in cytokines which are released as part of the inflammatory process and induce epithelial thickening. However, the relationship between the occurrence of ulcers and epithelial thickening is unknown, and further study about this relationship is necessary.

In conclusion, this study revealed that mucosal inflammation extended to the upper esophagus close to the hypopharynx, even where there was no evidence of ulcers. This finding of inflammation suggested that direct injury to the hypopharynx may occur as a result of the reflux of gastric juice. Our study also suggested that ES may play a useful defensive role in the prevention of reflux esophagitis. Further studies are necessary to explore the factors that are responsible for the protective effects of ES in reflux esophagitis.

COMMENTS

Background

Recently, the number of patients with gastroesophageal reflux disease (GERD) has increased in Japan. Although GERD-related extraesophageal syndromes have attracted attention, how gastric-juice reflux influences the esophagus and/or extraesophageal structures is unknown. In the present study, the authors explored the pathological findings in the entire esophagus and the effects of ecabet sodium (ES).

Innovations and breakthroughs

The authors revealed that epithelial thickening occurred at the same time as

inflammatory cell infiltration in the middle to lower esophagus in chronic acid-reflux esophagitis. Furthermore, they demonstrated that inflammatory cells infiltrated the epithelium of the upper esophagus close to the hypopharynx, where there was no evidence of ulcers. These findings suggested that the reflux of gastric juice can extend to the upper esophagus close to the hypopharynx.

Terminology

Laryngopharyngeal reflux disease is a common condition in the primary care setting and is one of the extraesophageal syndromes regarded as secondary to GERD.

Peer review

The authors evaluated the pathological findings in rat chronic acid-reflux esophagitis and the defensive effects of ES on esophageal mucosal injury. This an interesting, well-executed study.

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BRIEF ARTICLES

A survey of ampullectomy practices

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Abstract

AIM: To investigate the endoscopic ampullectomy practices of expert biliary endoscopists.

METHODS: An anonymous survey was mailed to 79 expert biliary endoscopists to assess ampullectomy practices.

RESULTS: Forty six (58%) biliary endoscopists returned the questionnaire. Of these, 63% were in academia and in practice for an average of 16.4 years (± 8.6). Endoscopists performed an average of 1.1 (± 0.8) ampullectomies per month. Prior to ampullectomy, endoscopic ultrasound was "always" utilized by 67% of respondents *vs* "sometimes" in 31% of respondents. Empiric biliary sphincterotomy was not utilized uniformly, only 26% "always" and 37% "sometimes" performed it prior to resection. Fifty three percent reported "never" performing empiric pancreatic sphincterotomy prior to ampullectomy. Practitioners with high endoscopic retrograde cholangiopancreatography volumes were the most likely to perform a pancreatic sphincterotomy (OR = 10.9; P = 0.09). Participants overwhelmingly favored "always" placing a prophylactic pancreatic stent, with 86% placing it after ampullectomy rather than prior to resection (23%). Argon plasma coagulation was the favored adjunct modality (83%) for removal of residual adenomatous tissue. Practitioners uniformly (100%) preferred follow-up examination to be within 6 mo post-ampullectomy.

CONCLUSION: Among biliary experts, there is less variation in ampullectomy practices than is reflected in the literature.

Key words: Endoscopic retrograde cholangiopancreatography; Ampullectomy; Papillectomy; Ampulla of Vater, Common bile duct neoplasms; Adenoma

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INTRODUCTION

Ampullary tumors account for approximately 5% of all gastrointestinal neoplasms[1]. In autopsy series, these tumors are seen in 0.04%-0.64% of the general population[2,3]. The most commonly affected patients are those with familial adenomatous polyposis with a 50%-100% lifetime incidence of peri-ampullary adenomas[4-6]. Given that the adenoma-carcinoma sequence for ampullary adenomas follows a similar progression to that of colorectal cancer, there is a need for prophylactic removal[7], However, the associated morbidity and mortality of surgical resection for ampullary adenomas have led clinicians to seek less invasive techniques. Endoscopic ampullectomy was first described in the 1980s[8-10]. Since then, numerous case and cohort series of ampullectomies, both retrospective and prospective, have been reported[11-19]. The first prospective, randomized, controlled trial of the use of prophylactic pancreatic duct stenting for endoscopic ampullectomy was published in 2005[20]. The trial was prematurely terminated because of an elevated incidence of pancreatitis in the unscented group (33% *vs* 0%) and suggested that pancreatic stent placement confers a protective effect.

Endoscopic ampullectomy guidelines have not been established. Desilets *et al*[14] performed endoscopic ampullectomy only in tumors less than 4.0 cm in size without induration/ulceration, and with the ability to be lifted by saline solution/epinephrine injection in the absence of

extension or stricturing into the pancreatic or biliary ducts. Similarly, Cheng *et al*[18] performed endoscopic ampullectomy in lesions less than 4.5 cm without endoscopic or pathologic evidence of malignancy and a soft consistency on palpation with any device. A recent editorial by Baillie *et al*[21] suggested a tumor size greater than 3 cm requires endoscopic ultrasound (EUS) assessment prior to ampullectomy. However, a recent literature review continues to reveal diverse endoscopic practices regarding the use of biliary/pancreatic sphincterotomy, use and timing of pancreatic stenting, thermal ablation therapy and follow-up in ampullectomy[22]. The majority of the literature guiding ampullectomy practice is comprised of case reports, retrospective and prospective clinical series, except for the aforementioned randomized, controlled trial by Harewood *et al*[20]. Subsequently, a consensus for endoscopic ampullectomy practices has not been established. In this respect, it is helpful to assess opinions on endoscopic ampullectomy practices in that it may set priorities for future research. Also, this type of data could be helpful for guideline development. Therefore, we surveyed expert biliary endoscopists on their endoscopic ampullectomy techniques to determine if a consensus exists in ampullectomy practice.

MATERIALS AND METHODS

Sample population

Seventy-nine expert biliary endoscopists were identified by the investigators, representing 55 medical centers in 33 States and Canada. Expert biliary endoscopists were identified by selecting the primary biliary endoscopists at tertiary care centers with a medium or large gastroenterology fellowship (2 or more fellows per year) (n = 52). Additional expert biliary endoscopists (n = 27) from private practice were selected based on the senior investigator's (G.E.) knowledge. No surveys were distributed at the sponsoring institution (University of Michigan and the Ann Arbor Veterans Affairs hospital). By utilizing biliary endoscopists from gastroenterology fellowship training institutions our study was likely to reflect routine gastroenterology practice since trainees tend to have similar practice patterns as their teachers.

Survey methods

An anonymous survey was sent to 79 potential respondents from May 2006 through October 2006. A self-addressed envelope was included with the survey to facilitate survey return. After 6 wk, a second survey was sent out to obtain results from endoscopists who did not respond initially with instructions to ignore the second mailing if they had already submitted the survey.

The survey instrument (Table 1) was composed of 16 questions based on an extensive literature review which identified diverse endoscopic ampullectomy practices. We performed an extensive literature search to identify endoscopic ampullectomy practices using the following search terms: ampullectomy, papillectomy, endoscopic ampullectomy/papillectomy, ampulla of Vater, major duodenal papilla and endoscopic retrograde cholangiopancreatography (ERCP). Based upon the results of this literature search, we determined diverse approaches to the following practices: use of EUS, timing of pancreatic stent placement, pre-ampullectomy biliary sphincterotomy and pancreatic sphincterotomy, type of electrosurgical currents used, type of adjunct modality for residual tumor removal and interval for follow-up. In order

Table 1 Ampullectomy survey

1 Please list your age.
2 Gender:
Male
Female
3 Please specify your type of practice?
Private practice
Multi-specialty group
Academic practice
Health maintenance organization (HMO)
Other
4 How many years have you been in practice?
5 On average, how many ERCPs do you perform in a month?
6 On average, how many ampullectomies for ampullary adenomas do you perform in a month?
7 How often do you perform an EUS or IDUS of the ampulla prior to ampullectomy?
Always
Sometimes-if there are concerning features known ahead of time
Never
8 How often do you perform an empiric biliary sphincterotomy prior to ampullectomy?
Always
Sometimes
Never
9 How often do you perform an empiric pancreatic sphincterotomy prior to ampullectomy?
Always
Sometimes
Never
10 How often do you place a prophylactic pancreatic stent prior to ampullectomy?
Always
Sometimes
Never
11 How often do you place a prophylactic stent after ampullectomy?
Always
Never
Only if there is delayed pancreatic duct drainage or a remnant lesion close to the pancreatic orifice that needs additional treatment
12 How often do you perform a submucosal injection of the ampullary adenoma prior to resection?
Always
Sometimes
Never
13 For endoscopic ampullectomy, what type of electrosurgical currents do you use most often?
Pure coagulation current
Blended current
Pure cutting current
ERBE-adjustable current
14 What is the largest ampullary adenoma that you have removed endoscopically?
15 What adjunct modality do you use most commonly to remove residual tissue after ampullectomy?
Cold forceps biopsy
Argon plasma coagulation
Monopolar/multipolar electrocoagulation probe
Nd: YAG laser photoablation
16 In general, after ampullectomy, at what interval do you recommend a follow-up endoscopic examination?
1 mo
3 mo
6 mo
12 mo

Table 2 Respondent characteristics (mean ± SD)

Characteristic	
Male, *n* (%)	46 (100)
Practice type, *n* (%)	
Academic	29 (63)
Private	14 (30)
Multi-specialty group	3 (7)
Years in practice	16.4 ± 8.6
ERCPs per month	36.78 ± 26.2
Ampullectomies per month	1.1 ± 0.8

ERCP: Endoscopic retrograde cholangiopancreatography.

to establish content validity, the results of this literature search were used to develop a draft questionnaire which was then reviewed by expert biliary endoscopists at the University of Michigan, followed by revision of the survey instrument. Since an adequate sample of expert biliary endoscopists were not available, test-retest reliability of the survey instrument could not be performed.

The study was approved by the Institutional Review Board (IRB) at the University of Michigan. In accordance to standard IRB guidelines, the need for documentation of informed consent was waived because of survey's anonymity.

Statistical analysis

All returned surveys were included in the analysis, regardless of the completeness of the survey. Percentage calculations were performed to determine if there were variations among expert biliary endoscopists in ampullectomy practices, including the use of biliary sphincterotomy, pancreatic sphincterotomy, timing of placement of pancreatic stents, use of submucosal injection, adjunctive ablative therapies and EUS. Multivariate models were used to determine if ampullectomy practices varied by academic or private practice and by volume of ERCPs. Since our ampullectomy practice data were collected as 3-level categories of "always", "sometimes" or "never", we first used multinomial logistic regression models for the 3-level outcomes and followed with logistic regression models for 2-level outcomes after collapsing the 3 levels into appropriate 2 levels. Given that the volume of ERCPs was highly skewed to the right, we considered this in various ways: as the number of ERCPs performed per month, as the number categorized into intervals ($\leqslant$ 20, 20-40, 40-60, > 60 per month), and as the number dichotomized to high (> 10 per month) versus low volume[23]. All statistical analyses were done using STATA 9.2 (StataCorp LP, College Station).

RESULTS

Demographic data

Forty-six respondents completed and returned the survey (58% response rate). Two-thirds of participants were from an academic medical practice. All respondents were male. Respondents had been in practice for a mean of 16.4 years (± 8.6 SD) at the time of the survey. There was a wide range in ERCP volume among respondents (ranging from 5 to 135 ERCPs per month) with an average of 36.7 (± 26.2, median = 30) ERCPs per month. One respondent stated that he no longer performed ERCPs. Thirty-nine (85%) reported a high volume (> 10) of ERCPs per month. Participants reported an average of 1.1 (± 0.79) ampullectomies per month (Table 2).

Table 3 Pre-ampullectomy practices

Practice	*n* (%)
EUS	
Always	30 (67)
Sometimes	14 (31)
Never	1 (2)
Biliary sphincterotomy	
Always	11 (26)
Sometimes	16 (37)
Never	16 (37)
Pancreatic sphincterotomy	
Always	10 (23)
Sometimes	10 (23)
Never	23 (53)
Pancreatic stent	
Always	10 (23)
Sometimes	15 (35)
Never	18 (42)
Submucosal injection	
Always	5 (12)
Sometimes	21 (49)
Never	17 (39)

EUS: Endoscopic ultrasound.

Practices performed prior to ampullectomy

Prior to ampullectomy, 67% of participants "always" used EUS to correctly assess tumor infiltration, 31% "sometimes" used EUS prior to ampullectomy, while only 2% "never" used EUS regularly. To maximally expose the affected ampullary epithelium, 26% of the respondents "always" performed biliary sphincterotomy and 23% "always" performed pancreatic sphincterotomy prior to resection. However, 53% of participants "never" performed pancreatic sphincterotomy and 37% "never" performed biliary sphincterotomy prior to ampullectomy. Only 12% of respondents "always" utilized submucosal injection of the ampullary adenoma to decrease the depth of thermal injury to the duodenal wall, while 49% and 39% of participants "sometimes" and "never" utilized this technique, respectively. For endoscopic ampullectomy, the most common type of electrosurgical current utilized was ERBE (67%) and blended current (17%) (Table 3).

Pancreatic stenting

For both pre- and post-ampullectomy, 98% of respondents reported placing a prophylactic pancreatic stent. A majority of participants (86%) favored "always" placing a pancreatic stent after resection. Some overlap in practice was identified with our 2 separate questions assessing the specific timing of pancreatic stent placement. Twenty-three percent of respondents always placed a pancreatic stent prior to resection, 35% "sometimes"

Table 4 Post-ampullectomy practices

Practice	*n* (%)
Pancreatic stent	
Always	37 (86)
Sometimes	4 (9)
Never	2 (4)
Adjunct therapy[1]	
APC	35 (83)
Multi/monopolar	3 (7)
Nd-Yag	1 (2)
Cold biopsy	3 (7)
Follow-up[2]	
One month	6 (16)
Three months	21 (55)
Six months	11 (29)
One year	0 (0)

[1]One respondent who checked both argon plasma coagulation (APC) and multipolar/monopolar electrocoagulation probe is not included here; [2]Four respondents who checked 2 follow-up intervals are not included here. They each checked 1 and 6 mo, 3 mo and 1 year, 1 and 3 mo, 3 and 6 mo.

placed a pancreatic stent prior to ampullectomy whereas 42% "never" placed a pancreatic stent prior to resection.

Practices performed after ampullectomy

The most frequently used adjunct modality to remove residual tissue post-ampullectomy was Argon Plasma Coagulation (83%). Follow-up examination at 3 mo was the most common time frame chosen (55%) by expert biliary endoscopists. Repeat examination at 6 mo (29%) and 1 mo (16%) were less frequently used. The largest reported adenoma removed by experts was 8.0 cm (Table 4).

Predictors of ampullectomy practices

Academic *vs* non-academic: Multinomial logistic regression showed that for empiric biliary sphincterotomy, the relationships between factors associated with a response of "sometimes" were similar to those associated with "never". On the other hand, for pancreatic sphincterotomy, the relationships between factors associated with "always" were similar to "sometimes". Thus for biliary sphincterotomy, we dichotomized the practice responses to "always" *vs* "sometimes or never", while for pancreatic sphincterotomy, we dichotomized the responses to "always or sometimes" *vs* "never". Logistic regression analysis showed that after controlling for years in practice and high ERCP volume, an odds of "always" doing empiric biliary sphincterotomy was 0.22 (95% CI = 0.05, 1.04; $P = 0.06$) for academic relative to private physicians, and an odds of "always or sometimes" doing empiric pancreatic sphincterotomy was 0.23 (95% CI = 0.05, 1.04; $P = 0.06$) for academic relative to private physicians. These indicated that academic practitioners tended to be less likely to do sphincterotomy than non-academic practitioners. Regarding prophylactic stenting, academic practitioners tended to be less likely to "always" do pre-ampullectomy prophylactic pancreatic stenting (OR = 0.42; $P = 0.28$), while they tended to be more likely to "always" do post-ampullectomy prophylactic pancreatic stenting (OR = 2.1; $P = 0.45$); however, these differences between academic *vs* private physicians were not statistically significant. Of the 18 practitioners who "never" placed a prophylactic pancreatic stent prior to ampullectomy, 17 "always" placed a prophylactic pancreatic stent after ampullectomy and only 1 "never" placed a prophylactic pancreatic stent after ampullectomy. These findings emphasize that regardless of the timing, almost all respondents utilized pancreatic stenting.

Volume of ERCPs

ERCP volumes were not associated with practice variation in empiric biliary sphincterotomy, but practitioners with high volumes of ERCPs (> 8 per month) tended to be more likely to "always or sometimes" do empiric pancreatic sphincterotomy (OR = 10.9; $P = 0.09$), controlling for academic status and years in practice. Practitioners with high volumes of ERCPs were also significantly less likely to "always" place prophylactic pancreatic stents prior to ampullectomy (OR = 0.08; $P = 0.04$), and more likely to "always" place prophylactic pancreatic stents after ampullectomy (OR = 12.8; $P = 0.06$).

DISCUSSION

This research describes the most commonly used endoscopic ampullectomy techniques by expert biliary endoscopists. This survey raises some interesting findings about current practices, showing some uniformity by expert endoscopists, which is important for future guideline development. For other practices, there is more variability. Therefore, these practices should be studied in prospective trials to help refine the best practice for our patients. In this regard, our survey has helped to identify key questions for future studies.

Universal agreement among participants regarding the use of prophylactic pancreatic stenting for ampullectomy was seen. This corresponds to findings by Brackbill *et al*[24] where 100% of respondents utilized prophylactic pancreatic stenting when performing ampullectomy. Previously, in some retrospective case series, prophylactic pancreatic stenting was performed only in the setting of delayed pancreatic duct drainage[11,15,16,19]. However, recent findings by Harewood *et al*[20] showing a markedly reduced rate of pancreatitis in those receiving prophylactic pancreatic stenting. Prophylactic pancreatic stenting was most commonly performed after ampullectomy by our expert biliary endoscopists. An argument against pre-ampullectomy pancreatic stenting is that it precludes *en bloc* removal of the adenomatous tissue by practitioners who favor complete transection of the polyp with a snare, rather than piecemeal resection. Only a minority of our respondents placed pancreatic stents prior to ampullectomy. For some practitioners, the possibility of not being able to find the pancreatic duct post-resection, and the increased risk of post-ampullectomy pancreatitis without a pancreatic stent may dictate their practice of pre-ampullectomy stent placement. To alleviate this concern, endoscopists may also consider wire placement in the pancreatic duct before ampullectomy, with snare

resection over the wire as an option.

EUS is frequently utilized by biliary experts prior to resection. EUS has the benefits of assessing the depth of tumoral infiltration with 70%-90% accuracy since endoscopic biopsies are not always reliable because of sampling error[25-31]. Size and characteristics of the ampullary tumor (evidence of ulceration, friability or spontaneous bleeding) should determine the need for EUS. Baillie suggested that EUS should be performed in large lesions to determine the need for surgery[32]. If concerning findings are noted, it obviates the need for endoscopic therapy. In the literature, there has been concern about overstaging the tumor with EUS. Desilets *et al*[14] felt that the suspicion for invasive disease is more accurately predicted by the behavior of the lesion with submucosal injection and careful evaluation of the cholangiogram and pancreatogram. Adding fine needle aspiration at the time of EUS is also a consideration. Defrain *et al* found adenocarcinoma in lesions ranging in size of 1.3-3.0 cm with sensitivity, specificity, positive and negative predictive values of 82.4%, 100%, 100% and 76.9%, respectively.

Endoscopic biliary and pancreatic sphincterotomy is utilized to assess ductal involvement prior to ampullectomy. In the literature, the use of biliary sphincterotomy prior to ampullectomy is not well defined. Ideally, biliary sphincterotomy maximally exposes the affected ampullary epithelium, aiding in future surveillance and preventing biliary stenosis. In the 2 largest series reporting ampullectomy outcomes, both reported using biliary sphincterotomy at the discretion of the endoscopists, although this was not well defined[18,33]. In other series, authors always performed biliary sphincterotomy in patients undergoing ampullectomy[13-16,19,20]. However, in a recent "Expert's Corner" on endoscopic ampullectomy, biliary sphincterotomy was not mentioned[32]. Auira *et al*[34] argued against biliary sphincterotomy since it carries the risk of bleeding, may interfere with *en bloc* resection and has the theoretical risk of seeding malignant cells present within the tumor. Unlike biliary sphincterotomy, the routine use of pancreatic sphincterotomy prior to ampullectomy has been advocated in the literature[14,19,32]. Our respondents utilized pancreatic sphincterotomy less frequently than biliary sphincterotomy prior to ampullectomy. However, Desilets *et al*[14], Kahaleh *et al*[19], and Baillie[32] all preferred pre-ampullectomy pancreatic sphincterotomy. The pancreatic sphincterotomy techniques that Desilets *et al*[14] described were wire-guided, involving sphincterotomies extending into normal duodenal tissue within the limits of safety. This was performed to further isolate the lesion, to remove the pancreatic orifice from the resection site and to ensure adequate drainage post-resection. Kahaleh and Baillie go on to further specify performing the pancreatic sphincterotomy solely with pure cutting current[19,32]. Our respondents with a high volume of ERCP were more likely to perform pre-resection sphincterotomy. However, Lee *et al*[35] questioned the use of pre-resection pancreatic sphincterotomy because of the higher risk of bleeding with papillary tumors, and the distortion of the resected specimen resulting from mechanical and thermal injury, making histopathologic interpretation of the lesion difficult.

Submucosal injection prior to ampullectomy has been recommended by some authors[14,19,36]. The technique is performed to separate the tumor from the muscularis propria. As a submucosal cushion, the fluid prevents deeper coagulation into the duodenal wall and theoretically reduces the risk of perforation and pancreatitis[37,38]. Epinephrine is added to prevent bleeding. Another benefit of submucosal injection is that it can serve as an indicator of malignancy. Lack of elevation with injection suggests invasive tumor growth. However, submucosal injection may actually impede optimal snare placement. This can be seen particularly in smaller tumors since the center of these lesions are tethered by the ducts and may not lift well. The surrounding normal tissue will lift and mushroom around the adenoma, thus partially burying it[36]. However, few respondents "always" performed this technique prior to ampullectomy. Factors determining when 48% of practitioners "sometimes" utilized submucosal injection were not defined.

Our study has several potential limitations. First, because of our study population, our findings may not apply to other practice settings. Unfortunately, a strict definition for "expert biliary endoscopists" does not exist. We therefore relied on the personal knowledge of leaders in the field to choose our sample population. This was the same method utilized by Brackbill *et al*[24]; however, we expanded the field to include more community gastroenterologists. Recall bias may also be present in these data since we are relying on self-reported data. There is also the possibility that our results may reflect what the respondents think they should do versus what they actually do in everyday practice.

In endoscopic ampullectomy, experts agreed (> 50%) on the use of EUS for large lesions, prophylactic pancreatic stenting, follow-up examination and adjunct modality for residual tissue removal. Few respondents used empiric pancreatic sphincterotomy. Practitioners with high volumes were more likely to "always" perform biliary and pancreatic sphincterotomy and place pancreatic stents after ampullectomy. Among biliary experts, there was less variation in ampullectomy practices than is reflected in the literature.

COMMENTS

Background

Ampullary tumors account for 5% of gastrointestinal neoplasms. Because of the morbidity and mortality associated with surgery, endoscopic retrograde cholangiopancreatography (ERCP) has been utilized as a less invasive procedure to perform an ampullectomy for removal of the ampullary tumor. Specific endoscopic guidelines for ampullectomy have not been established.

Research frontiers

Endoscopic ampullectomy has been reported widely in case reports and case series. Further study needs to be dedicated to the techniques involved in ampullectomy, in particular the use of biliary and pancreatic sphincterotomy, timing of pancreatic stenting and possible routine use of submucosal injection and endoscopic ultrasound (EUS).

Applications

This survey demonstrated the ampullectomy practices of expert endoscopists which is important for future guideline development. Experts agreed on the use of EUS for large lesions, prophylactic pancreatic stenting, follow-up examination and adjunct modality for residual tissue removal. Variability existed among experts regarding the use of biliary and pancreatic sphincterotomy prior to ampullectomy. Therefore, these practices should be studied in prospective trials to help refine the best practice for our patients.

Terminology

The ampulla is an orifice in the second portion of the duodenum where the biliary tree and pancreas drain. Ampullectomy describes a technique to remove a tumor at the ampulla. This can be performed by surgery or using a less invasive procedure named ERCP. ERCP is an endoscopic procedure used to access the biliary tree and pancreatic duct from the second portion of the duodenum. EUS is an endoscopic procedure where an endoscope with an ultrasound probe is used to obtain images of the internal organs.

Peer review

This article is very interesting and helpful to assess guidelines in endoscopic ampullectomy techniques for expert biliary endoscopists in North America.

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Cyclooxygenase-2 polymorphisms and the risk of esophageal adeno- or squamous cell carcinoma

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Abstract

AIM: To determine whether *-1195 A→G* and/or *-765 G→C* polymorphisms in *Cyclooxygenase-2* (*COX-2*) may have a risk modifying effect on the development of esophageal carcinoma in a Dutch Caucasian population.

METHODS: Two study groups were recruited, 252 patients with esophageal carcinoma and 240 healthy controls, matched for race, age, gender and recruiting area. DNA was isolated from whole blood and used for genotyping. PCR products were digested with restriction enzymes and products were analyzed by agarose gel electrophoresis. Odds ratios (OR) and 95% confidence intervals (CI) were estimated.

RESULTS: The distribution of the *-1195 A→G* polymorphism was significantly different in esophageal cancer patients compared to controls. The *-1195 GG* genotype resulted in a higher risk of developing esophageal adenocarcinoma (OR = 3.85, 95% CI: 1.45-10.3) compared with the *-1195 AA* genotype as a reference. The *-765 G→C* genotype distribution was not different between the two groups. The *GG/GG* haplotype was present more often in esophageal adenocarcinoma patients than in controls (OR = 3.45, 95% CI: 1.24-9.58; with *AG/AG* as a reference). The same trends were observed in patients with squamous cell carcinomas, however, the results did not reach statistical significance.

CONCLUSION: Presence of the *COX-2 -1195 GG* genotype and of the *GG/GG* haplotype may result in a higher risk of developing esophageal carcinoma.

Key words: Adenocarcinoma; Cyclooxygenase-2; Esophagus; Genetic polymorphism; Squamous cell carcinoma

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INTRODUCTION

During the last few decades, the incidence of esophageal carcinoma has sharply increased in Western-lifestyle countries. Two main types of esophageal carcinoma exist, adenocarcinoma and squamous cell carcinoma. The main difference between adenocarcinoma and squamous cell carcinoma is the cell type from which the tumor originates; glandular or squamous epithelial cells, respectively.

Adenocarcinoma of the esophagus predominantly occurs in Western societies. There is a strong and probably

causal relation between gastro-esophageal reflux and the development of esophageal adenocarcinoma[1]. Gastro-esophageal reflux may cause damage to the esophageal tissue due to the high concentrations of acid and bile salts, which may induce metaplasia and cell proliferation, thereby increasing the risk of mutations. This can lead to Barrett's esophagus with high grade dysplasia and ultimately to adenocarcinoma of the esophagus[1,2].

In contrast to adenocarcinoma, squamous cell carcinoma of the esophagus is thought to be caused predominantly by specific lifestyle or environmental factors such as heavy smoking in combination with alcohol use, chewing of tobacco or consumption of spicy foods and hot beverages[3]. In certain developing countries such as China, India or Iran, squamous cell carcinoma of the esophagus is very common, probably due to particular lifestyle habits[3]. As a result, damage to esophageal tissue may occur and tissue renewal may increase. This increased cell proliferation can lead to mutations, dysplasia and carcinoma.

Cell proliferation may play a key role in tumor genesis and cyclooxygenases (COXs) are important regulatory enzymes in this process. COXs are enzymes that catalyze the conversion of free arachidonic acid into prostaglandin H_2, which is the precursor of prostaglandins, prostacyclin and thromboxanes. These regulatory compounds play a role in many biological processes such as cell proliferation, angiogenesis, immune function and inflammation, which are all crucial in the development and progression of neoplasms[4]. The human COX family consists of three members, COX-1-3[4,5]. COX-1 is found in most tissues and plays a role in homeostasis of many physiologic processes. COX-3 is an alternative splice product of COX-1 and is believed to be involved in the regulation of pain and fever. COX-2 is probably very important in the development and progression of neoplasms. COX-2 is an inducible enzyme whose expression can be induced by pro-inflammatory and mitogenic stimuli like cytokines and growth factors. COX-2 plays an important role in the development of otherwise healthy tissue into metaplastic and dysplastic tissue, as well as in the development and progression of a tumor, by taking part in the regulation of cell proliferation, cell transformation, tumor growth, metastasis and invasion. COX-2 is often found over-expressed in gastrointestinal tumors, including those of the esophagus[6-10]. Tumors which exhibit a high level of COX-2 seem to be more aggressive[6] and patients bearing those tumors showed a significantly reduced survival[10]. In addition, when COX-2 expression in laboratory animals was suppressed with medication, fewer animals developed esophageal adenocarcinoma[11]. Therefore, the role of COX-2 in the development of normal or metaplastic tissue into neoplasms seems evident.

Recently, several functional Single Nucleotide Polymorphisms in the *COX-2* gene have been discovered which may contribute to the variance in inter-individual COX-2 expression. The *-1195 A→G* substitution in the COX-2 promotor was found to be associated with a lower expression of COX-2 in a Chinese population[12].

Another SNP, *-765 G→C* was first described in a UK population[13]. This polymorphism was shown to result in a lower promoter activity, which could subsequently lead to a lower expression of COX-2.

The purpose of this study is to determine the possible modulating effect of the *COX-2* polymorphisms *-1195 A→G* and *-765 G→C* on the risk for developing esophageal cancer in a Dutch Caucasian population.

MATERIALS AND METHODS

Patients and controls

A group of 252 patients with esophageal carcinoma was recruited during the period October 2002 to January 2008, in four hospitals all localized in the South-East area of The Netherlands. These hospitals were: (1) Radboud University Nijmegen Medical Center, (2) Canisius Wilhelmina Hospital, Nijmegen, (3) Hospital Gelderse Vallei, Ede and (4) Rijnstate Hospital, Arnhem. Only patients with a diagnosis of esophageal carcinoma as confirmed by a pathologist were included in the study.

Following an advertisement in local papers, a group of 240 healthy controls was recruited from the same geographical area of The Netherlands. Controls were matched with the esophageal carcinoma patients for age, ethnicity and gender.

The study was approved in 2002 by the Medical Ethical Review Committee, region Arnhem-Nijmegen (CMO 2002/114). EDTA blood was collected from patients and controls. The whole blood samples were stored at -22℃ until use. DNA was extracted from whole blood by using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, USA) according to the manufacturer's instructions. The extracted DNA was stored at 4℃ until use.

The extracted DNA was used for determination of the *-1195 A→G* and *-765 G→C* polymorphisms in the *COX-2* promoter by polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP), exactly as described by Zhang *et al*[12].

Statistical analysis

The differences between characteristics of patients with esophageal carcinoma and controls were analysed with the Student's *t*-test. All genotypes of controls and patients were tested to determine whether they were distributed according to the Hardy-Weinberg equilibrium. The chi-square test was used to test for differences in distribution of genotypes between the two groups, or to estimate differences in allele frequencies. Odds ratios (OR) with 95% confidence interval (95% CI) were calculated for genotypes associated with predicted normal versus predicted altered enzyme activities (variant genotypes). *COX-2* haplotypes were studied using the PL-EM software as described by Qin *et al*[14]. $P < 0.05$ was considered to be statistically significant. All data were processed using SPSS software for Windows version 16.0 (SPSS Inc, Chicago Illinois, USA).

RESULTS

Patients with esophageal carcinoma and controls were

Table 1 Characteristics of patients with oesophageal carcinoma and controls *n* (%)

Characteristics	Patients with oesophageal carcinoma				Controls
	Total	Adeno carcinoma	Squamous cell carcinoma	Mixed	
n	252	174 (69.0)	70 (27.8)	8 (3.2)	240
Age (yr; mean ± SD)	64.3 ± 10.8	64.7 ± 11.0	62.7 ± 10.2	69.9 ± 8.0	64.6 ± 10.9
Gender					
Female	51 (20.2)	24 (13.8)	26 (37.1)	1 (12.5)	51 (21.2)
Male	201 (79.8)	150 (86.2)	44 (62.9)	7 (87.5)	189 (78.8)

Table 2 Distribution of the *COX-2 -1195A→G* and *-765 G→C* genotypes and corresponding ORs in patients with oesophageal adenocarcinoma or squamous cell carcinoma *versus* controls

Genotype *COX-2*	Adenocarcinoma		Squamous cell carcinoma		Controls *n* (%)
	n (%)	OR (95% CI)	*n* (%)	OR (95% CI)	
-1195A→G					
-1195A/-1195A	100 (58)	Reference	39 (56)	Reference	154 (64)
-1195G/-1195A	59 (34)	1.13 (0.75-1.73)	26 (37)	1.28 (0.73-2.26)	80 (33)
-1195G/-1195G	15 (9)	3.85 (1.45-10.3)	5 (7)	3.29 (0.95-11.4)	6 (3)
Total	174		70		240
-765 G→C[1]					
-765G/-765G	112 (69)	Reference	41 (69)	Reference	157 (66)
-765G/-765C	46 (28)	0.88 (0.57-1.37)	16 (27)	0.84 (0.44-1.60)	73 (31)
-765C/-765C	5 (3)	1.17 (0.35-3.92)	2 (3)	1.28 (0.25-6.56)	6 (3)
Total	163		59		236

[1]In both the cases and control group, there are some missing data because of unsuccessful PCR; OR: Odds ratio; CI: Confidence interval.

Table 3 *COX-2* haplotype distribution and corresponding ORs in patients with oesophageal adenocarcinoma or squamous cell carcinoma carcinoma *versus* controls

Haplotype *COX-2*	Adenocarcinoma		Squamous cell carcinoma		Controls *n* = 236 (%)
	n = 163 (%)	OR (95% CI)	*n* = 59 (%)	OR (95% CI)	
AG/AG	59 (36.2)	Reference	18 (30.5)	Reference	94 (39.8)
AG/AC	29 (17.8)	0.87 (0.50-1.52)	12 (20.3)	1.18 (0.53-2.64)	53 (22.4)
AC/AC	5 (3.1)	1.33 (0.39-4.55)	2 (3.4)	1.74 (0.33-9.32)	6 (2.5)
GC/AC	0 (0)	-	0 (0)	-	0 (0)
GG/AC	17 (10.4)	1.29 (0.63-2.64)	4 (6.8)	0.99 (0.31-3.24)	21 (8.9)
GG/AG	40 (24.5)	1.14 (0.68-1.91)	19 (32.2)	1.77 (0.86-3.66)	56 (23.7)
GG/GG	13 (8.0)	3.45 (1.24-9.58)	4 (6.8)	3.48 (0.89-13.6)	6 (2.5)

matched for race, age, gender and recruiting area. Table 1 shows the characteristics of the patients and controls. The *COX-2* genotype distributions in patients and controls are summarized in Table 2. The polymorphisms tested here were distributed according to the Hardy-Weinberg criteria, *P*-values in patients and controls were 0.98 and 0.47 for the *-765 G→C* polymorphism and 0.21 and 0.24 for the *-1195 A→G* polymorphism, respectively.

No significant differences in the distribution of the *-765 G→C* polymorphism between patients with esophageal carcinoma and controls were observed ($P = 0.80$; χ^2 test). However, a significant difference in the distribution of the *-1195 A→G* polymorphism between patients and controls was observed ($P = 0.02$; chi square test). The *-1195 G/-1195 G* genotype was present more often in patients with esophageal carcinoma (whole group) as compared to the *-1195 A/-1195 A* genotype in controls (OR 3.57, 95% CI 1.39-9.13, $P = 0.005$). When analyzed according to the type of tumor, ORs were 3.85 (95% CI 1.45-10.3) for patients with adenocarcinoma and 3.29 (0.95-11.4) for patients with squamous cell carcinoma (Table 2). Allele frequencies in all patients with esophageal cancer (*-1195 A vs -1195 G*) also differed significantly from those in controls ($P = 0.02$).

When comparing the squamous cell carcinoma group ($n = 70$) with the adenocarcinoma group ($n = 174$), there were no significant differences with respect to the *-1195* genotype distribution: *-1195 AA*, 55.7% *vs* 57.5%; *-1195 AG*, 37.1% *vs* 33.9% and *-1195 GG*, 7.2% *vs* 8.6% ($P = 0.97$). For the *-765* genotypes no differences in distribution between the squamous cell carcinoma and adenocarcinoma groups were found: *-765 GG*, 69.5% *vs* 68.7%; *-765 GC*, 27.1% *vs* 28.2% and *-765 CC*, 3.4% *vs* 3.1% ($P = 0.95$).

Table 3 shows the results of a comparison of the distribution of the *COX-2 -765* and *-1195* haplotypes, according to the type of tumor. Only one significant difference was found, the *GG/GG* haplotype was present more often in the esophageal adenocarcinoma group than in the control group (OR = 3.45, 95% CI = 1.24-9.58). However, the number of individuals

present in these subgroups was very small (n = 13 *vs* n = 6, respectively). The same trend was observed in the squamous cell carcinoma group, however, statistical significance was not reached.

DISCUSSION

The *-1195 GG* genotype was present more often in patients with esophageal carcinoma than in controls. This is in contrast to the findings of Zhang *et al*[12] who identified the *-1195 AA* genotype as a risk factor for esophageal carcinoma. It is commonly reported that COX-2 expression is higher in cancerous tissue, because high COX-2 expression contributes to and sustains inflammatory and pre-cancerous processes[4,6]. Zhang *et al*[12] also concluded that COX-2 mRNA expression in *-1195 AA* genotypes was much higher than the mRNA expression in tissues of patients with the *-1195 GG* genotype. Our findings now suggest that the *COX-2 -1195* polymorphism has the opposite effect on esophageal carcinoma risk in Caucasians, as compared to Chinese patients. However, two limitations must be noted: firstly, we did not measure whether the COX-2 mRNA expression in *-1195 AA* genotypes was highest in our group of Caucasian patients, similar to the findings of Zhang *et al*[12] in Chinese patients. Secondly, there is a difference between our study population and that of Zhang *et al*[12]; the majority of our patients had adenocarcinoma (69%) and the minority suffered from squamous cell carcinoma (28%), whereas the Chinese patients in the study by Zhang *et al*[12] all had squamous cell carcinoma. In China, esophageal squamous cell carcinoma is significantly more common than adenocarcinoma, as it is mainly caused by lifestyle factors such as drinking hot beverages and eating spicy foods, whereas adenocarcinoma is associated with acid reflux as a result of the Western lifestyle[1]. In our patient group, we found no differences in the distribution of both *COX-2* polymorphisms between patients with adenocarcinoma and squamous cell carcinoma, which suggests that the differences found when compared to the results of Zhang *et al*[12] could be assigned merely to racial differences rather than to differences in the type of tumor.

Another indication that racial differences in the study populations may explain the apparent contradictory results is obtained by comparing the distribution of the *COX-2* polymorphisms in the Chinese and Dutch control populations. The genotype frequencies found in our Dutch controls for the *-765 G→C* and *-1195 A →G* polymorphisms were: 66.5% *GG*, 30.9% *GC*, 2.9% *CC* and 64.2% *AA*, 33.3% *GA*, 2.5% *GG*, respectively. Zhang *et al*[12] in a Chinese population reported genotype frequencies of 95.7% *GG*, 4.3% *GC*, 0% *CC* and 24.1% *AA*, 53.4% *GA* and 22.5% *GG*, respectively. Tan *et al* in Chinese controls more recently reported approximately the same genotype frequencies as Zhang *et al*: 95.2% *GG*, 4.8% *GC*, 0% *CC* and 23.7% *AA*, 53.2% *GA* and 23.1% *GG*, respectively[15].

On the other hand, our control group data on the *COX-2 -765* genotype were in good agreement with other European control data recently reported from Denmark, being 73.2%, 24.8% and 2.0% for -765 *GG*, *GC* and *CC* genotypes, respectively[16]. In addition, the *COX-2* polymorphism data in our patients are very similar to the recently reported *COX-2 -765* and *-1195* genotype distributions in Dutch esophageal adenocarcinoma patients by Moons *et al*[17], except for the *-1195 GG* genotype, which was present in 8.0% of our patients *vs* only 2.0% in the patients in the study by Moons *et al*[17].

The distribution of the *-765* genotypes in the control group was not found to be significantly different when compared to the esophageal carcinoma group, whereas Moons *et al*[17] reported a significantly different *-765 CC* genotype distribution between a Dutch esophageal carcinoma group (n = 140) and a Barrett's esophagus (n = 255) or reflux esophagitis (n = 240) patient group. It should be noted, however, that the number of *-765 CC* genotype individuals in these patient groups was very low, being seven, four and zero individuals, respectively[17]. Two main reasons for the difference in results between the two Dutch studies are as follows: firstly, our study was performed on a larger patient population than the study by Moons *et al*[17] (252 *vs* 140 patients), and secondly in our study, similar to the study by Zhang *et al*[12], a comparison between patients with esophageal cancer and healthy controls was made, in contrast to the study by Moons *et al*[17] where patients with Barrett's esophagus or reflux esophagitis, both of which are at risk for esophagus carcinoma, were used for comparison.

Analyzing the *COX-2* haplotypes showed that the *GG/GG* haplotype was present more often in the esophageal carcinoma group, which again is not in accordance with the results of Zhang *et al*[12] and Moons *et al*[17], who both found that the *CA* containing haplotypes carried the highest risk. Since the results of Zhang *et al*[12] and Moons *et al*[17] on different types of tumors (squamous cell carcinoma *vs* adenocarcinoma, respectively) are very similar, and more or less contradict our results, it was of interest to compare the haplotype distribution between our patients with squamous cell carcinoma *vs* adenocarcinoma. However, no significant differences were found.

In conclusion, the presence of the *COX-2 -1195 GG* genotype and of the *GG/GG* haplotype may result in a higher risk of developing esophageal adenocarcinoma and possibly also squamous cell carcinoma.

COMMENTS

Background

Cyclooxygenase-2 (COX-2) is claimed to be a key enzyme in the development and progression of neoplasms. COX-2 is often found over-expressed in gastrointestinal tumors, including those of the esophagus. The corresponding *COX-2* gene is polymorphic and two single nucleotide polymorphisms; *-1195 A →G* and *-765 G→C* were demonstrated to influence the expression of COX-2. Therefore, these polymorphisms might modulate the risk for gastrointestinal cancers, including cancer of the esophagus.

Research frontiers

In this study, the *COX-2 -1195 GG* genotype was found to be present more often in Caucasian patients with esophageal carcinoma than in controls. This is in contrast to earlier findings in a Chinese population, where the *-1195 AA* genotype was revealed as a risk factor for esophageal carcinoma.

Innovations and breakthroughs

Presence of the *COX-2 -1195 GG* genotype and of the *GG/GG* haplotype may result in a higher risk of developing esophageal carcinoma.

Applications

Screening for the *COX-2 -1195 GG* genotype in a population at risk for esophageal cancer may be valuable in the future in order to select high risk patients. Information and prevention programs can than be focused on these patients.

Terminology

COX-2 is an enzyme that catalyzes the conversion of arachidonic acid in prostaglandin H_2, the precursor of other prostaglandins, prostacyclin and thromboxanes. These regulatory compounds play a role in many biological processes such as cell proliferation, angiogenesis, immune function and inflammation, which are all crucial in the development and progression of neoplasms.

Peer review

This study offered a controversial view of COX-2 polymorphisms in the esophageal carcinomas, compared with existing studies in Europe and China. The authors thoroughly discussed various possibilities that may lead to the different findings among studies. This manuscript is well written. Although the finding is controversial, the authors discussed this issue very well.

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BRIEF ARTICLES

Gallbladder emptying in patients with primary sclerosing cholangitis

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Abstract

AIM: To assess gallbladder emptying and its association with cholecystitis and abdominal pain in patients with primary sclerosing cholangitis (PSC).

METHODS: Twenty patients with PSC and ten healthy subjects were investigated. Gallbladder fasting volume, ejection fraction and residual volume after ingestion of a test meal were compared in patients with PSC and healthy controls using magnetic resonance imaging. Symptoms, thickness and contrast enhancement of the gallbladder wall and the presence of cystic duct strictures were also assessed.

RESULTS: Median fasting gallbladder volume in patients with PSC [67 (19-348) mL] was twice that in healthy controls [32 (16-55) mL] ($P < 0.05$). The median postprandial gallbladder volume in patients with PSC was significantly larger than that in healthy controls ($P < 0.05$). There was no difference in ejection fraction, gallbladder emptying volume or mean thickness of the gallbladder wall between PSC patients and controls. Contrast enhancement of the gallbladder wall in PSC patients was higher than that in controls; (69% ± 32%) and (42% ± 21%) ($P < 0.05$). No significant association was found between the gallbladder volumes and occurrence of abdominal pain in patients and controls.

CONCLUSION: Patients with PSC have increased fasting gallbladder volume. Gallbladder Mucosal dysfunction secondary to chronic cholecystitis, may be a possible mechanism for increased gallbladder.

Key words: Cholecystitis; Ejection fraction; Gallbladder volume; Magnetic resonance imaging

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INTRODUCTION

Primary sclerosing cholangitis (PSC) is an idiopathic chronic cholestatic inflammatory liver disease characterized by diffuse fibrosing inflammation of intra- and/or extrahepatic bile ducts, resulting in bile duct obliteration, biliary cirrhosis, and eventually hepatic failure[1,2]. Gallbladder abnormalities, including gallstones, cholecystitis and gallbladder masses are common in patients with PSC[3,4], and seems to be part of the spectrum of the disease. Inflammation of the bile ducts in PSC is similar to that found in the gallbladder epithelium in these patients.

Functional impairment of the gallbladder in PSC is rarely studied. Van de Meeberg *et al*[5] showed enlarged fasting gallbladder volumes and increased postprandial volumes in patients with PSC compared with patients with primary biliary cirrhosis and healthy controls, although the ejection fraction of bile was normal. Other studies in patients with PSC have not found gallbladder enlargement[6,7]. Increased gallbladder volume or gallbladder retention are known to occur in conditions other than PSC such as truncal vagotomy[8,9], chronic

pancreatitis[10], octreotide therapy[11], obesity[12], diabetes mellitus[13], pregnancy[14] and distal biliary obstruction[15]. PSC associated inflammation of the gallbladder epithelium and cholangiographic abnormalities of the cystic duct have been reported in patients with PSC[3,7,16].

One of the most common symptoms at the time of presentation of PSC is mild to severe abdominal pain localized in the right upper quadrant[17-20]. The cause of abdominal pain is unclear, it seems, however, unrelated to the grade of bile duct strictures. In addition, cholecystectomy seldom improves abdominal pain in these patients. A possible association between enlarged fasting gallbladder volume, ejection fraction and abdominal pain has never been investigated in patients with PSC.

The primary aim of the present study, using magnetic resonance imaging (MRI), was to evaluate the fasting and postprandial gallbladder volumes and to assess whether or not gallbladder emptying is associated with abdominal pain in patients with PSC. Secondly, we studied if the presence of imaging signs of chronic cholecystitis is correlated to gallbladder volume, the emptying process or abdominal pain.

MATERIALS AND METHODS

Ethics

The study was approved by the Ethics committee at Karolinska University Hospital, Huddinge and written informed consent was received from all patients and controls.

Subjects

Twenty patients, (14 men and 6 women) who ranged in age from 24 to 59 years (mean age 39 ± 10 years), with well-defined PSC[21] treated at the Liver Unit, Karolinska University Hospital, Huddinge were included in the study between January 2005 and July 2006. Clinical data were obtained by review of the complete medical history collected from patient files. Patients with hepatobiliary malignancy, diabetes mellitus, chronic pancreatitis or distal biliary obstruction (dominant extrahepatic strictures) were excluded. Ten healthy subjects (5 men and 5 women), who ranged in age from 31-79 years (mean age 47 ± 13.5 years), without any history of gastrointestinal disease or previous abdominal surgery served as controls. Informed consent for study participation was received from all patients and controls.

Procedure

After overnight fasting, two intravenous cannulas were inserted into the antecubital veins: one for blood sampling and one for intravenous injection of a contrast agent for MRI. The fasting gallbladder volume was analysed by MRI, prior to injection of the contrast agent, time = 0 min. One hour later (time = 1 h) a test meal consisting of 200 g "Swedish hash" (fried diced meat, onions and potatoes served with beetroot), 250 mL milk (3% fat) and an apple, totalling 2064 kJ including 21 g fat was ingested. Postprandial gallbladder volume and ejection fraction were obtained at 2.5 h (time = 2.5 h), that is an hour and a half after ingestion of the fat-meal at which point gallbladder contraction is supposed to be maximal[5,22-24].

Laboratory data

Biochemical variables including alkaline phosphatase, serum transaminases, total bilirubin, International normalized ratio (INR), serum albumin and CRP were obtained at the beginning of the procedure and analysed using standard procedures at the Karolinska University Hospital.

Questionnaire

Every subject filled in a questionnaire for the assessment of abdominal pain localized in the right upper quadrant, abdominal discomfort and nausea, before the first MRI, just before, and one and three hours after meal ingestion. The questionnaire consisted of visual analogue scales (VAS) where the patient marked the degree of symptoms including abdominal pain, nausea and abdominal discomfort.

Magnetic resonance imaging (MRI)

Examinations including the gallbladder and the hepatobiliary system were performed (after overnight fasting) using a 1.5 T magnetic resonance system [Magnetom Symphony (n = 1 PSC), Vision (n = 7 PSC) or Avanto (n = 12 PSC and 10 controls]; Siemens, Erlangen, Germany]. Each patient was examined using the same unit before and after the meal combining the spine and the flexible body array coil. The use of different units was therefore not considered to influence the results. Gd-BOPTA (MultiHance® 0.5 mmol/mL, Bracco, Milan, Italy) at a dosage of 0.1 mmol/kg of was injected. Axial breath-hold 3D-T1-weighted scans (VIBE, slice thickness 1.7-2.5 mm) were performed natively and dynamically in arterial, portal-venous and delayed 5 min phase for clinical diagnosis. Postprandially, in the hepatobiliary phase, the hepatobiliary system was rescanned (VIBE).

Gallbladder wall thickness, cystic duct and gall stones

The thickness of the gallbladder wall was measured on the axial T2 Haste slices at three different areas of the gallbladder. The mean values of the measurements were calculated for each patient. The presence of biliary stones and perivesical fluid was noted and the cystic duct was evaluated for the presence of strictures. Cystic duct abnormalities were defined as mural irregularities of the cystic duct on magnetic resonance cholangiography (MRC).

Gallbladder wall contrast enhancement

Contrast enhancement of the gallbladder wall was analyzed in % using the formula: Contrast enhancement = [SI (portalvenousphase) - SI (native)]/SI (native) × 100.

In each patient the signal intensity (SI) of the wall was measured, in a single voxel, in three different areas, trying to avoid vessels and adjacent intestinal loops or the liver parenchyma. The same areas were measured natively

and in the portal venous phase and the enhancement was calculated for each part. The mean of the measurements was calculated for each patient.

Gallbladder volume measurements

The 3D-T1-weighted scans were analysed using a Voxar® 3D workstation (Barco NV, Kortrijk, Belgium) using 3D segmentation and volume measurements. The volume of the gallbladder was measured fasting (delayed 5 min phase) and in the postprandial phase. In the latter hepatobiliary phase, contrast filling of the gallbladder was also noted. The analyses were made in consensus by two radiologists (NE and NA).

The ejection volume was measured in microliter using the formula: Ejection volume = volume (fasting) - volume (postprandial).

The ejection fraction or gallbladder emptying was measured in % using the formula: Ejection fraction = [volume (fasting) - volume (postprandial)]/volume (fasting) × 100.

Gallbladder fasting volume, ejection fraction and postprandial gallbladder volume of patients with PSC were compared with healthy controls. Postprandial gallbladder refilling of bile (that is, of contrast excreted to the common bile duct) was noted.

Statistical analysis

Data were analyzed using statistical software (v 7.0, Stat Soft Inc.). Values are expressed as mean and standard deviation or as median (range). For comparison of categorical data, the Chi-square test was used, or Fisher's exact test when appropriate. Spearman's correlation test was used to determine the association between contrast enhancement and gallbladder wall thickness. $P < 0.05$ was considered significant.

RESULTS

The clinical characteristics of the 20 PSC patients and the healthy controls are shown in Table 1. There was no significant difference between the two groups regarding age and body mass index (BMI). The mean levels of plasma alkaline phosphatase (ALP) and plasma bilirubin were significantly higher in patients with PSC than in the healthy controls.

The median fasting gallbladder volume in patients with PSC was twice that of the healthy controls; 67 (range 19-348) mL and 32 (range 16-55) mL, respectively ($P < 0.05$) (Figure 1A). The mean fasting gallbladder volume (mean ± SD) in patients with PSC was 91 ± 78 mL compared with 35 ± 11 mL in healthy controls. The median postprandial gallbladder volume in patients with PSC was significantly higher than that in healthy controls; 40 (9-345) mL and 16 (9-26) mL, respectively ($P < 0.05$) (Figure 1B). Median ejection volume was 16 (1-100) mL in PSC patients and 16 (8-38) mL in healthy controls (n.s.) (Figure 1C).

There was no significant difference in ejection fraction between PSC patients and controls as shown in Figure 1D. Ninety percent (18/20) of patients had inflammatory bowel disease (IBD) and two of these patients had active disease. We found no significant difference in gallbladder volumes between the PSC patients with active IBD and patients who were in remission. None of the women in the PSC group were pregnant at the time of the study.

Table 1 Clinical characteristics of 20 patients with PSC and 10 healthy controls

Clinical characteristic	PSC patients (n = 20)	Healthy controls (n = 10)	P value
Gender			
Male	70% (14/20)	50% (5/10)	NS
Female	30% (6/20)	50% (5/10)	NS
Age in years (± SD)	39 ± 10	47 ± 13	NS
Associated IBD	90% (18/20)	0	
UC	85% (17/20)		
CD	5% (1/20)		
Liver cirrhosis	10% (2/20)	0	
Treatment with UDCA	85% (17/20)	0	
Cholangiographic distribution of PSC		-	
Extra-and intrahepatic involvement	75% (15/20)		
Intrahepatic changes	25% (5/20)		
Duration of PSC in years	9.7 ± 5	-	
Mean P-ALP (< 1.9 μkat/L)	7 ± 6	1 ± 0.3	$P < 0.05$
Mean P-ALT (< 0.76 μkat/L)	1.4 ± 1.2	0.4 ± 0.1	$P < 0.05$
Mean P-Bilirubin (< 26 μmol/L)	20 ± 14	10 ± 5	NS
Mean P-Albumin (36-45 g/L)	40 ± 6	39 ± 5	NS
Mean P-Cholesterol (3.9-7.8 mmol/L)	5 ± 01	5 ± 0.1	NS
Mean BMI			
Male	25 ± 4	25 ± 2	NS
Female	25.5 ± 2.5	25 ± 5	NS

PSC: Primary sclerosing cholangitis; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; IBD: Inflammatory bowel diseases; UDCA: Ursodeoxycholic acid; UC: Ulcerative colitis; CD: Crohn's disease; NS: Not significant; BMI: Body mass index.

Two of the patients with PSC had liver cirrhosis. One of them, a 42-year-old man had the largest fasting gallbladder volume (348 mL) with an ejection fraction of only 1% (Figure 2). The other cirrhotic patient was a 37-year-old man with a fasting gallbladder volume of 20 mL and an ejection fraction of 55%.

Gallstones were found in the gallbladder in 20% (4/20) of the PSC patients but not in the controls. The median gallstone size was 2.5 mm (2-6 mm), these gallstones had no impact in the cystic duct in preprandial images. None of the patients had visible stones in the common bile duct. Perivesical fluid was seen in small amounts in 20% (4/20) of the PSC patients. None of the patients had a previous history of pancreatitis and we found no significant difference in gallbladder size between patients with and without abdominal pain.

The thickness of the gallbladder wall (mean ± SD) did not differ significantly between PSC patients and controls; 2.2 ± 0.5 mm and 1.9 ± 0.3 mm, respectively. However, there was a significant increase in contrast enhancement of the gallbladder wall (mean ± SD) in PSC patients compared to controls; 69% ± 32% and

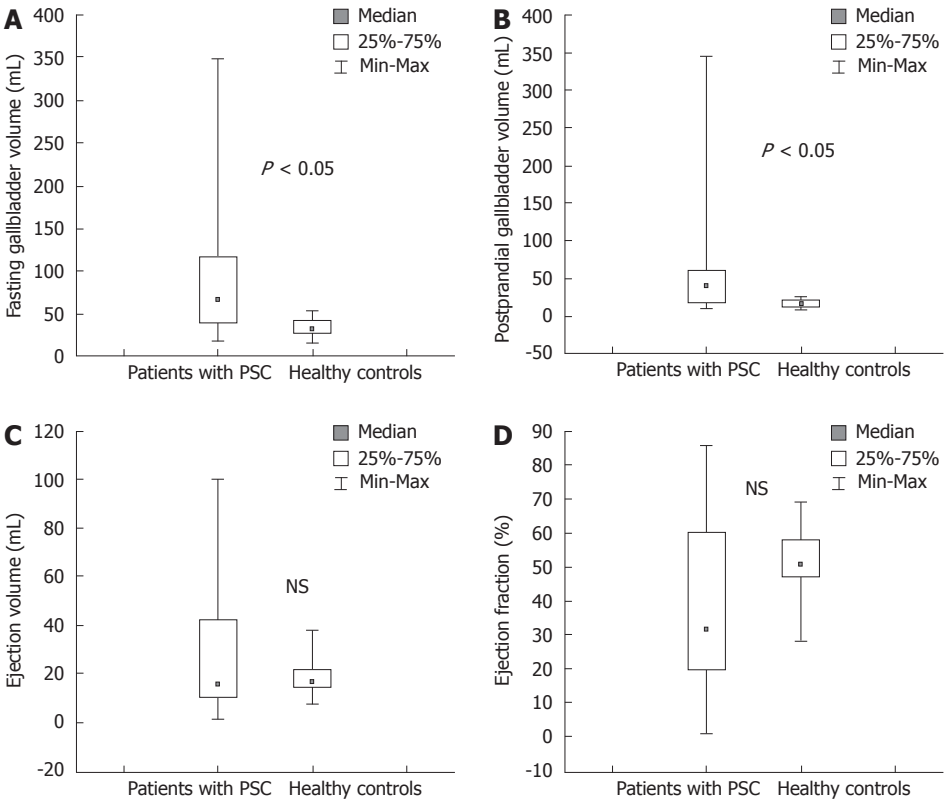

Figure 1 Fasting gallbladder volume (A), postprandial gallbladder volume (B), ejection fraction (C, ejection volume as percentage of fasting gallbladdervolume) and Ejection volume (D) measured with magnetic resonance imaging (MRI) in patients with primary sclerosing cholangitis (PSC) (*n* = 20) and in healthy controls (*n* = 10).

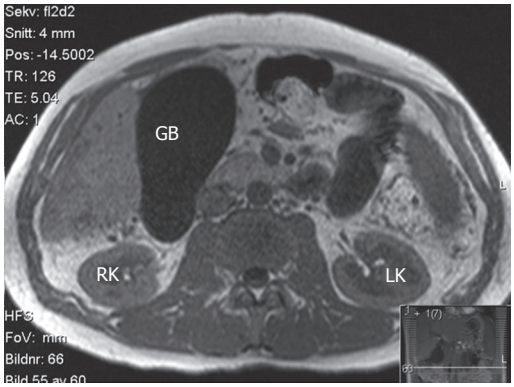

Figure 2 MRI showing a large gallbladder volume of 348 mL in a patient with PSC.

43% ± 21%, respectively ($P < 0.05$). There were no other signs of acute cholecystitis.

In all subjects there was a significant correlation between high contrast enhancement of the gallbladder wall and large gallbladder volume at fasting ($P < 0.05$) ($r = 0.39$). Postprandial gallbladder volume and ejection fraction were not significantly correlated to contrast enhancement of the gallbladder wall.

We found no correlation between patients with increased gallbladder volume, increased postprandial gallbladder volume and decreased ejection fraction and levels of P-Bilirubin, P-Cholesterol, P-Albumin, P-ALP, duration and distribution of PSC, treatment with ursodeoxycholic acid (UDCA), presence of IBD or age. In all subjects there was no significant correlation between BMI and fasting gallbladder volume or ejection fraction of the gallbladder. Seventeen of 20 patients with PSC were taking 10-15 mg/kg per day of UDCA. The fasting gallbladder volume and ejection fraction was similar in patients who were on UDCA treatment (96 ± 83 mL and 38% ± 27%) and patients who were not treated with UDCA (57 ± 19 mL and 36% ± 15%) (n.s.). Seventy five percent (15/20) of the PSC patients and 100% (10/10) of the healthy controls showed contrast in the gallbladder in the hepatobiliary phase (n.s.). Abnormalities of the cystic ducts were visualized in 13 (60%) of the PSC patients and in none in the control group. No significant correlation was found between increased gallbladder fasting volume, decreased ejection fraction or lack of postprandial gallbladder refilling and presence of abnormalities in the cystic ducts.

Symptoms

No significant association was found between gallbladder volumes or contrast enhancement and occurrence of abdominal pain, abdominal discomfort and nausea in PSC patients and controls. Before the fatty meal, 25% of PSC patients experienced abdominal pain, the visual analogue scale (VAS) ranging from 1 to 4. Twenty five percent of PSC patients experienced nausea with the VAS ranging from 1 to 2. Twenty percent of PSC patients experienced abdominal discomfort with the VAS ranging from 1 to 4. There was no significant increase in symptoms in the PSC group at one or three hours after meal ingestion. None of the healthy controls experienced symptoms pre- and postprandially.

DISCUSSION

In the present study, we showed that patients with PSC have a significant increase in gallbladder volume both pre- and postprandially compared with healthy control

subjects. This is in agreement with a previous sonographic study by van de Meeberg *et al*[5], who reported a fasting gallbladder volume of 73 ± 13.7 mL compared with 91 ± 77.9 mL in our study. The reason for the increased fasting gallbladder volume in patients with PSC is unclear. Several mechanisms may cause gallbladder enlargement, for example obstruction of the cystic duct or the common bile duct distal to the cystic duct, gallbladder dysmotility or gallbladder mucosal dysfunction may all contribute to increased fasting gallbladder volumes. In our study we excluded patients with significant extrahepatic strictures. Seventy-five percent of the PSC patients showed contrast in the gallbladder indicating the absence of a dominant stricture in the cystic duct. We also found that the gallbladder ejection fraction in PSC patients was similar to that of the healthy controls. Taken together, these findings do not suggest mechanical obstruction and/or gallbladder dysmotility as reasons for the enlarged gallbladder fasting volume in these patients.

Gallbladder abnormalities are common in PSC, and include cholecystitis which is found in 25% of all PSC patients[4]. In experimental cholecystitis the process of fluid absorption in the gallbladder epithelium changes to fluid secretion[25,26]. The secretory function of the gallbladder in PSC has been described previously in a case report of one PSC patient with concomitant cholecystitis. This patient produced between 39 mL and 52 mL of fluid daily from the gallbladder epithelium[27]. We found a similar gallbladder wall thickness in cases and controls. This finding may represent an underestimation of the wall thickness in PSC patients since the gallbladder is larger and more distended. The increased enhancement of the gallbladder wall may indicate the presence of inflammation of the gallbladder epithelium and wall in PSC patients. This sign of cholecystitis, in combination with normal ejection fraction of the gallbladder in PSC patients, support the notion that gallbladder mucosal dysfunction is a possible cause of the increased fasting gallbladder volume and residual volume in patients with PSC. The presence of inflammatory changes in the gallbladder epithelium and its effect on absorption/secretory functions is difficult to evaluate in a clinical setting. There are obvious problems in obtaining daily gallbladder volume measurements in patients, and biopsies are needed for the proper evaluation of inflammation. Measurement of bile concentration in the gallbladder could be a surrogate marker for absorption/secretory dysfunction and such measurements may be possible in the future using MR spectroscopy techniques.

The effect of UDCA treatment on gallbladder motility is unclear. Several studies have shown that UDCA treatment results in increased fasting and postprandial gallbladder volume, whereas gallbladder emptying has not been shown to be reduced or modified[28-31]. PSC patients in the study conducted by Van de Meeberg *et al*[5], which showed similar results to ours, discontinued their UDCA medication for four weeks before commencement of the study. We decided not to discontinue therapy with UDCA based on the above studies in order to study the patients' symptoms in a true clinical setting. Eighty-five percent of our PSC patients were treated with UDCA. We did not ascertain any significant difference in fasting gallbladder volume and gallbladder emptying between UDCA treated and untreated patients. However, this should be interpreted with caution due to the small number of patients involved.

Up to a third of all PSC patients experience pain in the upper right quadrant of the abdomen[19,20]. This abdominal pain is most often intermittent, but may occasionally be of a more continuous nature[32]. Abdominal pain has been hypothesized to result from constriction of the bile ducts. One third of patients with small duct PSC, which is characterized by the absence of strictures in the large ducts also suffer from abdominal pain[33] indicating that biliary strictures do not play a role in the development of abdominal pain. In our study, we did not find any significant difference among PSC patients regarding gallbladder dysfunction and the occurrence of abdominal pain, abdominal discomfort and nausea pre- or postprandially. Gallbladder motility dysfunction as a pathophysiological factor in the development of abdominal pain in patients with PSC is therefore unlikely.

In conclusion, patients with PSC have increased fasting and residual gallbladder volumes, whereas gallbladder emptying is normal. The reason for the increased fasting gallbladder volume is unclear. However, gallbladder mucosal dysfunction secondary to chronic inflammation of the gallbladder is a possible mechanism. Gallbladder size or emptying does not seem to be involved in the development of abdominal pain in patients with PSC.

COMMENTS

Background

The mechanisms responsible for the abdominal pain in primary sclerosing cholangitis (PSC) are not fully understood. The aim of the present study was to assess gallbladder emptying and its association with cholecystitis and abdominal pain in patients with PSC.

Research frontiers

The authors compared gallbladder volumes at fasting and after ingestion of a test meal in patients with PSC and healthy controls using magnetic resonance imaging (MRI). Symptoms, thickness and contrast enhancement of the gallbladder wall and the presence of cystic duct strictures were also assessed.

Innovations and breakthroughs

The increased enhancement of the gallbladder wall on MRI may indicate the presence of inflammation of the gallbladder epithelium and wall in PSC patients. Gallbladder size or emptying does not seem to be involved in the development of abdominal pain in patients with PSC.

Applications

The present study provides information on the possible mechanisms of increased fasting gallbladder volume in patients with PSC.

Peer review

This is an interesting report addressing a clinically important question: whether gallbladder emptying is associated with cholecystitis and the abdominal pain in patients with PSC. The authors analyzed 20 patients with PSC and compared with ten healthy subjects. Results indicate that patients with PSC have increased fasting and residual gallbladder volumes, probably resulting from gallbladder mucosal dysfunction secondary to chronic cholecystitis. However, gallbladder size or the emptying process does not seem to cause abdominal pain in patients with PSC.

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BRIEF ARTICLES

Perianal disease, small bowel disease, smoking, prior steroid or early azathioprine/biological therapy are predictors of disease behavior change in patients with Crohn's disease

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Abstract

AIM: To assess the combined effect of disease phenotype, smoking and medical therapy [steroid, azathioprine (AZA), AZA/biological therapy] on the probability of disease behavior change in a Caucasian cohort of patients with Crohn's disease (CD).

METHODS: Three hundred and forty well-characterized, unrelated, consecutive CD patients were analyzed (M/F: 155/185, duration: 9.4 ± 7.5 years) with a complete clinical follow-up. Medical records including disease phenotype according to the Montreal classification, extraintestinal manifestations, use of medications and surgical events were analyzed retrospectively. Patients were interviewed on their smoking habits at the time of diagnosis and during the regular follow-up visits.

RESULTS: A change in disease behavior was observed in 30.8% of patients with an initially non-stricturing, non-penetrating disease behavior after a mean disease duration of 9.0 ± 7.2 years. In a logistic regression analysis corrected for disease duration, perianal disease, smoking, steroid use, early AZA or AZA/biological therapy use were independent predictors of disease behavior change. In a subsequent Kaplan-Meier survival analysis and a proportional Cox regression analysis, disease location (P = 0.001), presence of perianal disease (P < 0.001), prior steroid use (P = 0.006), early AZA (P = 0.005) or AZA/biological therapy (P = 0.002), or smoking (P = 0.032) were independent predictors of disease behavior change.

CONCLUSION: Our data suggest that perianal disease, small bowel disease, smoking, prior steroid use, early AZA or AZA/biological therapy are all predictors of disease behavior change in CD patients.

Key words: Crohn's disease; Smoking; Azathioprine; Infliximab; Monoclonal antibodies; Colectomy; Risk; Reoperation

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INTRODUCTION

Inflammatory bowel disease (IBD) is a multifactorial disease with probable genetic heterogeneity[1]. In addition, several environmental risk factors (e.g. diet, smoking, measles or appendectomy) may contribute to its pathogenesis. During the past decades, the incidence pattern of both forms has significantly changed[2], showing some common but also quite distinct characteristics for the two disorders.

Phenotypic classification of Crohn's disease (CD) plays an important role in determining treatment and may assist in predicting the likely clinical course of disease[3]. In 2005, the Montreal revision of the Vienna classification system was introduced[4]. Although the broad categories for CD classification remained the same, changes were made within each category. Upper gastrointestinal disease can now exist independently of, or together with, disease present at more distal locations. Finally, perianal disease, which occurs independently of small bowel fistulae, is no longer classified as penetrating disease. Instead, a perianal modifier has been introduced, which may coexist with any disease behavior.

Using the Vienna classification system, it has been shown in clinic-based cohorts that there can be a significant change in disease behavior over time, whereas disease location remains relatively stable[3,5]. An association between the presence of perianal disease and internal fistulation (OR: 2.6-4.6) was reported earlier by Sachar *et al*[6] in patients with colonic disease. Most recently, Australian authors[3] have shown that although > 70% of CD patients had inflammatory disease at diagnosis, the proportion of patients with complicated disease increased over time. Progression to complicated disease was more rapid in those with small bowel than colonic disease location ($P < 0.001$), with perianal disease being also a significant predictor of change in CD behavior (HR: 1.62, $P < 0.001$). Similarly, small bowel location and stricturing disease were predictors for surgery in a long-term follow-up study[7]. Finally, perianal lesions, the need for steroids to treat the first flare-up and ileocolonic location, but not an age below 40 years were confirmed as predictive markers for developing disabling disease (according to the predefined criteria) at 5 years[8]. In the same study, stricturing behavior (HR: 2.11, 95% CI: 1.39-3.20) and weight loss (> 5 kg) (HR: 1.67, 95% CI: 1.14-2.45) at diagnosis were independently associated with the time to development of severe disease.

A further environmental factor which may be of importance in determining change in disease behavior is smoking. In CD, smoking was reported to be associated with disease location: most, but not all, studies report a higher prevalence of ileal disease and a lower prevalence of colonic involvement in smokers[9,10]. A recent review[10] and previous data have demonstrated that smoking, when measured up to the time-point of disease behavior classification, was more frequently associated with complicated disease and penetrating intestinal complications[9,11,12], a greater likelihood of progression to complicated disease, as defined by the development of strictures or fistulae[10], and a higher relapse rate[13]. In addition, the risk of surgery as well as the risk for further resections during disease course were also noted to be higher in smokers in some studies[9,14] and a recent meta-analysis[15]. The need for steroids and immunosuppressants was found to be higher in smokers compared to non-smokers[16]. Noteworthy, in one study by Cosnes *et al*[17], immunosuppressive therapy was found to neutralize the effect of smoking on the need for surgery. In a recent paper by Aldhous *et al*[18], using the Montreal classification, the harmful effect of smoking was only partially confirmed. Although current smoking was associated with a lower rate of colonic disease, the smoking habits at diagnosis were not associated with time to development of stricturing disease, internal penetrating disease, perianal penetrating disease, or time until first surgery.

Finally, early postoperative use of azathioprine (AZA, at a dose of 2-2.5 mg/kg per day) appeared to delay postoperative recurrence in comparison to a historical series or placebo groups in randomized, controlled trials[19]. Furthermore, in a recent withdrawal study by the GETAID group[20], the authors provide evidence for the benefit of long-term AZA therapy beyond 5 years in patients with prolonged clinical remission. In contrast, initial requirement for steroid use [OR: 3.1 (95% CI: 2.2-4.4)], an age below 40 years (OR: 2.1, 95% CI: 1.3-3.6), and the presence of perianal disease (OR: 1.8, 95% CI: 1.2-2.8) were associated with the development of disabling disease in the study by Beaugerie *et al*[21]. The positive predictive value of disabling disease in patients with two and three predictive factors for disabling disease was 0.91 and 0.93, respectively.

In this study, the authors aimed to assess the combined effect of disease phenotype, smoking, and medical therapy (steroid, AZA, AZA/biological) on the probability of disease behavior change in a cohort of Hungarian CD patients.

MATERIALS AND METHODS

Patients

Three hundred and forty well-characterized, unrelated, consecutive CD patients (age: 38.1 ± 13.2 years, M/F: 155/185, duration: 9.4 ± 7.5 years) with a complete clinical follow-up were included. A detailed clinical phenotypic description of these patients is presented in Table 1.

The diagnosis was based on the Lennard-Jones Criteria[22]; age, age at onset, presence of familial IBD, presence of extraintestinal manifestations; arthritis: peripheral and axial; ocular manifestations: conjunctivitis, uveitis, iridocyclitis; skin lesions: erythema nodosum, pyoderma gangrenosum; and hepatic manifestations: primary sclerosing cholangitis, frequency of flare-ups (frequent flare-up: > 1 per year). The disease phenotype (age at onset, duration, location, and behavior) was determined according to the Montreal Classification[4] (non-inflammatory behavior: either stricturing or penetrating disease). Perianal disease and behavior change during follow-up were also registered. Medical therapy was registered in detail [e.g. steroid and/or immunosuppressive/biological therapy use, AZA intolerance as defined by the ECCO (European Crohn's and Colitis Organisation) Consensus Report[23]], need for surgery/reoperation (resections) in CD, and time-point of surgery/reoperation and smoking habits, were investigated by reviewing the medical charts and completing a questionnaire. Only patients with a confirmed diagnosis for more than 1 year were enrolled.

Definitions of AZA/biological therapy use and smoking: patients were regarded as AZA users if they took a dose of ⩾ 1.5 mg/kg body weight for at least 6 mo. Early use was considered if the use of immuno-

Table 1 Clinical characteristics of patients with Crohn's disease *n* (%)

	CD (*n* = 340)
Male/female	155/185
Age (yr)	38.1 ± 13.2
Age at presentation (yr)	28.7 ± 12.4
Duration (yr)	9.4 ± 7.5
Familial IBD	39 (11.4)
Location	
L1	75
L2	99
L3	161
All L4	22
L4 only	5
Behavior at diagnosis	
B1	198
B2	65
B3	77
Behavior change from B1 to B2/B3	61 (30.8)
Perianal disease	117 (34.4)
Frequent relapse	126 (37.1)
Arthritis	127 (37.5)
PSC	10 (2.9)
Ocular	17 (5.0)
Cutaneous	43 (12.6)
Steroid use	264 (77.6)
Azathioprine use	216 (63.5)
Azathioprine intolerance	36 (14.3)
Biological use	71 (20.9)
Surgery/reoperation	158 (46.5)/52 (32.8)
Smoking habits	
No	151
Ex	36
Yes	153

L1: Ileal; L2: Colonic; L3: Ileocolonic; L4: Upper gastrointestinal disease; B1: Inflammatory; B2: Stenosing; B3: Penetrating disease behavior. IBD: Inflammatory bowel disease.

modulatory therapy preceded the behavior change by at least 6 mo. According to the Center's policy, if AZA was started, its use was not halted even in patients with long-term clinical remission. The rate of AZA intolerance was 14.3% (including 36 additional patients), and these patients were classified as AZA-non-users. Common intolerance reactions were leukopenia, abdominal pain, and in three patients, pancreatitis. Biological therapy use was considered if the patient received at least a full, anti-tumor necrosis factor induction therapy at an appropriate dose. The definition of smoking consisted of smoking $\geqslant$ 7 cigarettes/wk for at least 6 mo[15-18] at the time of diagnosis and/or during follow-up, within 1 year of diagnosis or behavior change. Patients were interviewed on their smoking habits at the time of diagnosis and during the regular follow-up visits. Moreover, due to Hungarian health authority regulations, a follow-up visit is obligatory for IBD patients at a specialized gastroenterology centre every 6 mo. Otherwise, the conditions of the health insurance policy change and they forfeit their ongoing subsidized therapy. Consequently, the relationship between the IBD patients and their specialists is a close one. The referred patients were retrospectively interviewed about their smoking habits at the time of referral and thereafter. Smoking cessation was defined as complete abstinence of at least 1 year's duration. Only 16 (4.7%) CD patients stopped smoking during the course of the disease, while 2 additional CD patients started smoking after the diagnosis. In patients with change in disease behavior, all CD patients stopped smoking following the change. Since macroscopic lesions on the ileal side of the anastomosis observed 1 year following surgery were not different between smokers and non-smokers and there was no significant difference reported between ex-smokers and non-smokers in reoperation rates in a recent meta-analysis [15,24], ex-smokers at the time of diagnosis were included in the non-smoker group.

Detailed clinical phenotypes were determined by thoroughly reviewing the patients' medical charts, which had been collected in a uniform format. The central coordination of sample and database management was completed at the 1st Department of Medicine, Semmelweis University (by PLL, TS). Data capture was prospective except for referred patients in whom disease course until referral was registered at the date of the referral and prospectively thereafter. Data analysis was done retrospectively. The study was approved by the Semmelweis University Regional and Institutional Committee of Science and Research Ethics.

Statistical analysis

Variables were tested for normality using Shapiro Wilk's W test. The *t*-test with separate variance estimates, ANOVA with *post hoc* Scheffe test, χ^2-test, and χ^2-test with Yates correction were used to evaluate differences within subgroups of IBD patients. Kaplan-Meier survival curves were plotted for analysis with the LogRank and Breslow tests. Additionally, forward stepwise Cox regression analysis was used to assess the association between categorical clinical variables and surgical requirements. $P < 0.05$ was considered as significant. For the statistical analysis, SPSS 15.0 (SPSS Inc, Chicago, IL) was used with the assistance of a statistician (Dr. Peter Vargha).

RESULTS

Association between clinical markers and disease behavior change in CD patients

In a univariate analysis, behavior change from B1 to B2/B3 during follow-up was associated with disease duration, location, presence of perianal disease, smoking at diagnosis, frequency of relapses, steroid use, early AZA use, AZA/biological therapy use and need for resective surgery (Table 2). Although ocular manifestations were also associated with behavior change (3.6% *vs* 11.5%, $P = 0.033$), this became non-significant after Bonferroni correction. Patients with a change in disease behavior had significantly longer disease duration (12.3 ± 7.6 years *vs* 7.4 ± 6.5 years, $P < 0.001$).

In a logistic regression model, disease duration, presence of perianal disease, smoking, steroid use, and early AZA use prior to behavior change were independent predictors for change in disease behavior (Table 3). If early AZA use was changed to early AZA and/or biological therapy use (Coefficient: -1.221, $P = 0.002$, OR: 0.29, 95% CI: 1.34-0.64) in the same logistic regression model, the associations remained unchanged.

Table 2 Clinical factors associated with behavior change from inflammatory to complicated disease behavior *n* (%)

Factor	Prevalence without behavior change	Prevalence with behavior change	*P*-value	OR	95% CI
Disease location					
L1	19 (13.9)	17 (27.9)			
L2	55 (40.1)	18 (29.5)	0.04	-	-
L3	63 (46)	25 (41)			
L4	0	1 (1.6)			
Perianal disease	30 (22.2)	30 (49.2)	< 0.001	3.4	1.78-6.46
Frequent relapses	18 (13.1)	19 (31.1)	0.003	3.0	1.43-6.23
Disease duration (> 10 yr)	28 (20.4)	35 (57.4)	< 0.001	5.3	2.7-10.1
Smoking	51 (37.2)	32 (52.5)	0.04	1.9	1.02-3.45
Steroid use	106 (77.4)	59 (96.7)	0.001	8.6	2.00-37.3
Early azathioprine use	79 (57.7)	24 (39.3)	0.017	0.48	0.26-0.88
Early azathioprine/biological use	83 (60.6)	25 (41)	0.01	0.45	0.24-0.84
Need for operation	19 (13.9)	37 (60.7)	< 0.001	9.6	4.72-19.4

Table 3 Logistic regression: predictive factors for behavior change from non-stricturing, non-penetrating to complicated disease behavior in Crohn's disease

Factor	Coefficient	*P*-value	OR	95% CI
Disease location	-0.385	0.13	-	-
Longer disease duration (≤ 10 yr *vs* > 10 yr)	1.476	< 0.001	4.37	2.04-9.38
Perianal disease	1.351	0.001	3.86	1.72-8.67
Frequent relapses	0.388	0.404	-	-
Smoking	1.015	0.009	2.76	1.29-5.89
Steroid use	2.089	0.01	8.07	1.64-39.7
Early azathioprine use	-1.055	0.006	0.35	0.16-074

The coefficient is equivalent to the natural log of the OR.

Association between clinical markers and time to disease behavior change in CD patients

Disease location, perianal disease, early AZA or AZA/biological therapy, steroid use (LogRank $P = 0.004$ and Breslow $P = 0.005$) and smoking were significant determinants for time to behavior change surgery in a Kaplan-Meier analysis using LogRank and Breslow tests (Figure 1).

To further evaluate the effect of the above variables on the probability of behavior change, we performed a forward stepwise proportional Cox regression analysis. Each of the above variables was independently associated with the probability of disease behavior change (Table 4). The result was the same if early AZA/biological therapy use ($P = 0.002$, HR: 0.43, 95% CI: 0.25-0.73) was incorporated in the same analysis.

Table 4 Summary of Cox regression model: factors affecting time to disease behavior change

	P-value	Hazard ratio	95% CI
Disease location	0.001		
L1	0.023	2.13	1.11-4.08
L2	0.05	0.54	0.27-1.001
L3	Reference		
Perianal disease			
Yes	< 0.001	3.26	1.90-5.59
No	Reference		
Steroid use			
Yes	0.006	7.48	1.79-31.2
No	Reference		
Early azathioprine use			
Yes	0.005	0.46	0.27-0.79
No	Reference		
Smoking status			
Yes	0.032	1.79	1.05-3.05
No	Reference		

DISCUSSION

In the present study, we assessed, for the first time, the combined effect of disease phenotype and medical therapy on the probability of disease behavior change in a well-characterized CD cohort with a strict clinical follow-up. This study has shown that perianal disease, disease location, prior steroid, early AZA or AZA/biological therapy were significant independent predictors of disease behavior change during follow-up in patients with CD, in a stepwise proportional Cox regression and Kaplan-Meier analysis.

In accordance with the findings by Tarrant *et al*[3], the presence of perianal disease was associated with a 3-4-fold increased risk of developing complicated disease behavior in patients with non-stricturing, non-penetrating disease at the time of diagnosis. Interestingly, in both studies, approximately 50%-55% of patients with perianal disease and only 15%-20% of patients without perianal disease developed a complicated disease behavior after 10 years of follow-up. In the present study, however, only 60% of the patients presented with B1 behavior, representing the more severe disease population in referral centers. In contrast, the Australian study reports the results of a population-based cohort follow-up. Therefore, our results are not only confirmatory, but enable us to extrapolate on the finding that perianal disease is a poor prognostic factor in referral center IBD populations.

In addition, similar to the Australian study, the present study found that progression to complicated disease was more rapid in those with small bowel than colonic disease location. The HR was significantly lower (0.54, $P = 0.05$) for colonic disease and significantly higher for ileal disease (2.13, $P = 0.023$) compared to an ileocolonic location in the Cox regression analysis. The same conclusions were reached when data were analyzed

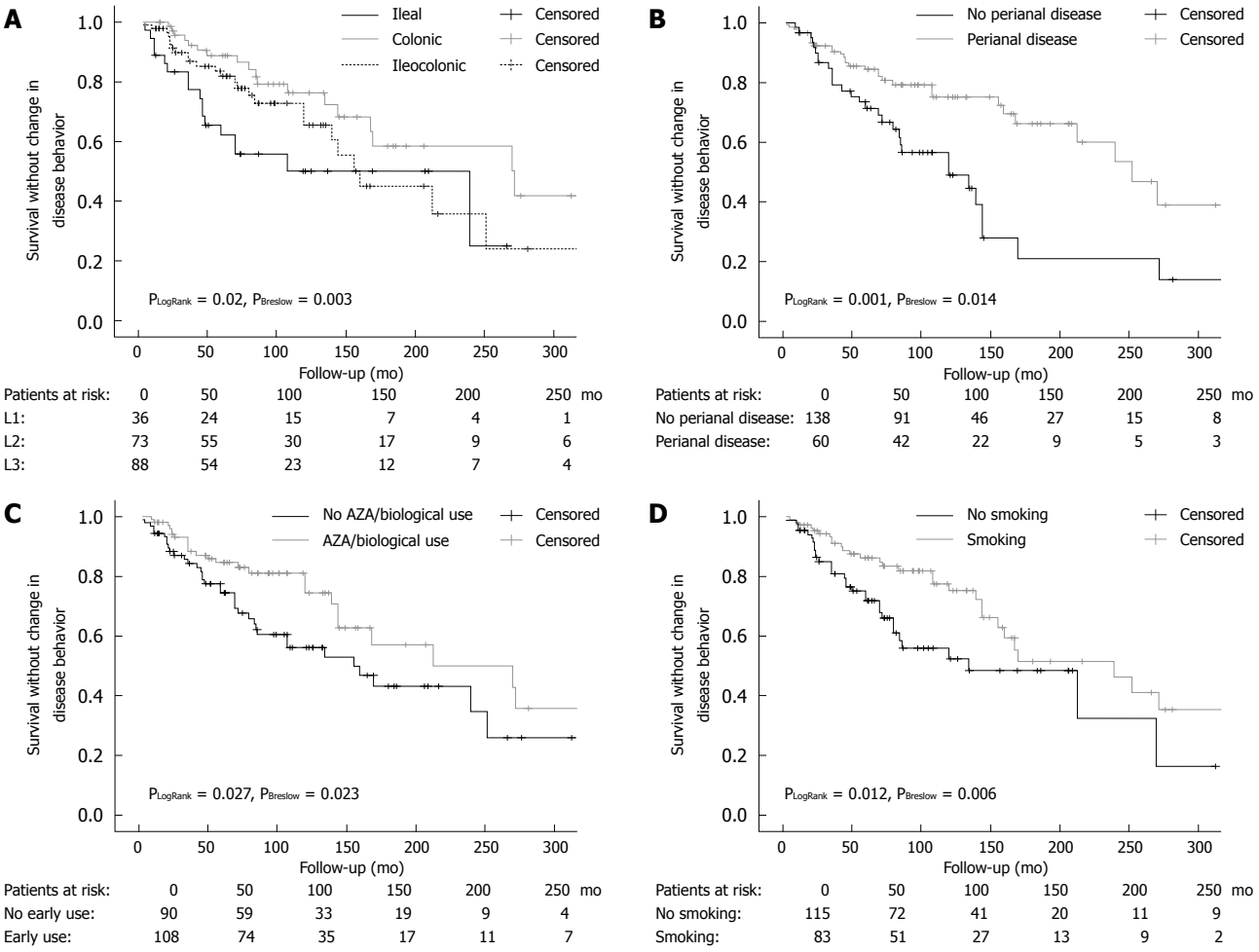

Figure 1 Association between disease location (A), perianal disease (B), early azathioprine/biological therapy use (C) or smoking (D) and disease behavior change from non-stricturing, non-penetrating to complicated disease behavior in Crohn's disease.

by the Kaplan-Meier method using the LogRank and Breslow tests for comparison. Of note, while the L1 and L3 curves ran parallel after 150 mo, the relatively small number of patients in the L1 group may have introduced a bias in the analysis. These results were only partially confirmed by Aldhous *et al*[18]. In 275 CD patients, colonic disease was negatively associated with the time required for development of stricturing complications ($P < 0.001$), while any upper gastrointestinal disease (and tendentially ileocolonic disease, $P = 0.066$) was the only factor significantly increasing the risk of development of fistulizing complications. Finally, small bowel involvement, stricturing disease, and a young age at diagnosis were associated with disease recurrence in another Dutch population-based study[7].

In the present study, we could not confirm frequency of relapses as an independent prognostic factor for disease behavior change. This is somewhat in contrast with the findings by Munkholm *et al*[25], where the relapse rate within a year of diagnosis and in the following 2 years, was positively correlated ($P = 0.00001$) with the relapse rate in the following 5 years in patients with CD.

The findings were slightly different if the authors assessed the clinical factors associated with the development of irreversible structural damage. After a multivariate analysis, only stricturing behaviour at diagnosis (HR: 2.11, $P = 0.0004$) and weight loss (> 5 kg) at diagnosis (HR: 1.67, $P = 0.0089$) were independently associated with time to the development of severe disease in the study by Loly *et al*[8]. The definition of severe, non-reversible damage was, however, much more rigorous. It was defined by the presence of at least one of the following criteria: the development of complex perianal disease, any colonic resection, two or more small-bowel resections (or a single small-bowel resection measuring more than 50 cm in length) or the construction of a definite stoma. Nonetheless, medical therapy was not included in either of the previous studies.

Similarly, although the effect of smoking was extensively investigated in IBD, most studies failed to investigate the complex associations between smoking, disease phenotype, and medical therapy. A recent review[10] and previous data have demonstrated that current smoking was more frequently associated with complicated disease, penetrating intestinal complications[9,10], and greater likelihood to progress to complicated disease, as defined by the development of strictures or fistulae[11]. In accordance, in the present study, smoking at the time of diagnosis was independently associated with time to behavior change from a non-stricturing, non-penetrating phenotype to complicated disease behavior in CD in a Cox regression model.

However, this was not a universal finding. In a recent paper by Aldhous *et al*[18], using the Montreal classification, the harmful effect of smoking was only partially confirmed. Although current smoking was associated with less colonic disease, the smoking habits at diagnosis were not associated with time to development of stricturing disease, internal penetrating disease, perianal penetrating disease, or time to first surgery. In contrast, disease location was associated with the need for surgery.

A more solid end-point was also deleteriously affected by smoking in the present study; the need for intestinal resection but not reoperation was also increased in smokers (HR: 3.19, $P < 0.001$) not treated with immunosuppressive therapy, especially in females, in accordance with data from Cosnes *et al*[26]. Much emphasis was also placed, by some authors, on investigating the association between the amount of smoking and the above variables in both CD and ulcerative colitis. In a recent publication, the authors[18] did not find a significant association between pack-years of smoking and disease behavior or need for surgery. In addition, in a recent French publication[27], light smokers had higher resection rates compared to non-smokers in CD, suggesting that complete smoking cessation should be advised for all smokers with CD.

The key to explaining these conflicting results lies partly in the study by Cosnes *et al*[17], where the authors have demonstrated that immunosuppressive therapy neutralizes the effect of smoking on the need for surgery. Therefore, we aimed to analyze the effect of smoking in a more complex setting. After obtaining the results of the univariate and Kaplan-Meier analyses, we performed both a logistic regression analysis adjusted to disease duration as an independent variable and a stepwise proportional Cox regression analysis to investigate the relative weight of the risk factors. In this analysis, perianal disease, smoking, steroid use, and AZA or AZA/biological therapy use before the behavior change were independently associated with time to disease behavior change. However, a partial recall bias, especially in the referral patients, where smoking habits were analyzed in a partially retrospective manner, cannot be excluded.

Finally, the initial requirement for steroid use (OR: 3.1, 95% CI: 2.2-4.4), an age below 40 years at diagnosis (OR: 2.1, 95% CI: 1.3-3.6), and the presence of perianal disease (OR: 1.8, 95% CI: 1.2-2.8) were associated with the development of disabling disease in the study by Beaugerie *et al*[21] The positive predictive values of disabling disease in patients with two and three predictive factors for disabling disease were 0.91 and 0.93, respectively. Nonetheless, the prevalence of disabling disease was approximately 80.5% at 5 years in the entire patient group, which makes these criteria less valuable in clinical practice. Moreover, the authors classified the need for immunosuppressive therapy as one potential disabling factor, which in light of the present study, is rather controversial. In the present study, prior steroid use was an independent predictor of time to change in disease behavior. However, again, because of the high prevalence of overall steroid use, the confidence interval is wide and, consequently, the clinical usefulness of this marker is relatively low.

In adults, early postoperative use of AZA at a dose of 2-2.5 mg/kg per day seemed to delay postoperative recurrence in comparison to a historical series or placebo groups in randomized, controlled trials[19]. Furthermore, in a recent withdrawal study by the GETAID group[20], the authors provided evidence for the benefit behind long-term AZA therapy beyond 5 years in patients with prolonged clinical remission. The most convincing data to support the benefit from early use of AZA, however, comes from the pediatric literature[28], where in a randomized, controlled trial in 55 children, early 6-mercaptopurine use was associated with a significantly lower relapse rate (only 9%) compared with 47% of controls ($P = 0.007$). Moreover, the duration of steroid use was shorter ($P < 0.001$) and the cumulative steroid dose lower at 6, 12 and 18 mo ($P < 0.01$). More recently, also in a pediatric setting, this strategy was found to be associated with a lower hospitalization rate[29]. Similarly, in the present study, early AZA or AZA/biological therapy was an independent preventive factor associated with decreased probability of developing complicated disease behavior during the course of the disease (HR: 0.46 and 0.43).

In conclusion, in the present study, we have shown that the complex analysis of disease phenotype, medication history, and smoking habits is needed, in order to study the factors associated with change in disease behavior in patients with IBD. Our data suggest that perianal disease, current smoking, prior steroid use, early AZA or AZA/biological therapy are predictors of disease behavior change in patients with CD.

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COMMENTS

Background

Using the Vienna classification system, it has been shown in clinic-based cohorts that there can be a significant change in inflammatory bowel disease (IBD) disease behavior over time, whereas disease location remains relatively stable. Early age at diagnosis, disease location, perianal disease and, in some studies, smoking were associated with the presence of complicated disease and surgery in previous studies.

Research frontiers

The combined effect of markers of disease phenotype (e.g. age, gender, location, perianal disease) and medical therapy (steroid use, early immunosuppression) on the probability of disease behavior change have not, however, been studied in detail thus far in the published literature.

Innovations and breakthroughs

In the present study, the authors have shown in a well-characterized Crohn's disease (CD) cohort with strict clinical follow-up, that the complex analysis of disease phenotype, medication history, and smoking habits is needed in order to study the factors associated with change in disease behavior in patients with IBD. Their data suggest that perianal disease, current smoking, prior steroid use, early azathioprine (AZA) or AZA/biological therapy are predictors of disease behavior change in patients with CD.

Applications

New data with easily applicable clinical information may assist clinicians in everyday, practical decision-making, when choosing a treatment strategy for their CD patients.

Terminology

Vienna-Montreal classification: classification systems of CD disease phenotypes. The Vienna classification assesses the age at presentation, disease location and disease behavior. In the Montreal classification, the broad categories for CD classification remain the same; however, changes were made within each category. First, a new category was introduced for those aged 16 years or younger at the time of diagnosis, to separate pediatric from adult-onset IBD. Second, upper gastrointestinal disease and perianal disease became disease modifiers, which may coexist with any disease behavior or location.

Peer review

The present article provides valuable information regarding clinical prognostic factors of phenotype changes of CD over time. The study was performed in a large cohort of patients.

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Barrett's esophagus: Prevalence and risk factors in patients with chronic GERD in Upper Egypt

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Abstract

AIM: To determine the prevalence and possible risk factors of Barrett's esophagus (BE) in patients with chronic gastroesophageal reflux disease (GERD) in El Minya and Assuit, Upper Egypt.

METHODS: One thousand consecutive patients with chronic GERD symptoms were included in the study over 2 years. They were subjected to history taking including a questionnaire for GERD symptoms, clinical examination and upper digestive tract endoscopy. Endoscopic signs suggestive of columnar-lined esophagus (CLE) were defined as mucosal tongues or an upward shift of the squamocolumnar junction. BE was diagnosed by pathological examination when specialized intestinal metaplasia was detected histologically in suspected CLE. pH was monitored in 40 patients.

RESULTS: BE was present in 7.3% of patients with chronic GERD symptoms, with a mean age of 48.3 ± 8.2 years, which was significantly higher than patients with GERD without BE (37.4 ± 13.6 years). Adenocarcinoma was detected in eight cases (0.8%), six of them in BE patients. There was no significant difference between patients with BE and GERD regarding sex, smoking, alcohol consumption or symptoms of GERD. Patients with BE had significantly longer esophageal acid exposure time in the supine position, measured by pH monitoring.

CONCLUSION: The prevalence of BE in patients with GERD who were referred for endoscopy was 7.3%. BE seems to be associated with older age and more in patients with nocturnal gastroesophageal reflux.

Key words: Barrett's esophagus; Gastrointestinal; Endoscopy; Gastroesophageal reflux; Risk factors

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INTRODUCTION

Barrett's esophagus (BE) was first identified by N.R. Barrett in 1950, who described the replacement of the normal squamous mucosa of the distal esophagus by a columnar epithelium of both gastric and intestinal types[1,2]. The definition of BE has been modified over subsequent years to include only intestinal metaplasia within the tubular esophagus.

The exact cause of BE remains unclear. A popular explanation for the occurrence of BE is that it results from mucosal damage caused by gastroesophageal reflux[3,4]. When visible upon upper gastrointestinal endoscopy, this mucosal damage is termed erosive esophagitis. However, not all patients with gastroesophageal reflux and erosive

esophagitis go on to develop BE, and not all patients with BE have a history of gastroesophageal reflux[5]. In most patients with reflux esophagitis, the epithelium heals through regeneration of the normal squamous lining[6]. Other patients, however, will develop BE with the risk of ultimately progressing to esophageal adenocarcinoma (EAC)[7].

The prevalence of BE varies in different geographic areas worldwide. Multiple risk factors for the development of BE besides reflux have been studied, including being Caucasian and/or male, a history of smoking, and hiatus hernia[8].

The aim of this study was to determine the prevalence and possible risk factors of BE in patients with chronic gastroesophageal reflux disease (GERD) symptoms in Upper Egypt.

MATERIALS AND METHODS

Ethics

This study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. All patients provided informed written consent.

Location

The study was performed at two clinical centers in Upper Egypt (Southern part of Egypt): the endoscopy units of the Department of Tropical Medicine at El Minya University, and the Department of Internal Medicine at Assuit University, from January 2006 to January 2008. Both centers are referral centers for a large number of patients in Upper Egypt.

Patients

In a prospective manner, 1000 consecutive patients with chronic GERD symptoms were recruited. All patients had a history of longstanding heartburn and or regurgitation for at least three times weekly for the last year. A questionnaire was completed by every patient, including age, sex, occupation, smoking and alcohol consumption. The symptom questionnaire also included the following criteria: primary referral symptom; frequency of GERD symptoms such as heartburn, regurgitation, and acid taste; extra esophageal symptoms; and history of systemic diseases such as scleroderma and diabetes.

Endoscopic examination

Endoscopic examination was performed using an Olympus Evis CLV-U 200 Videoscope (Olympus, Japan). All patients were examined in our units by a well-trained endoscopist. BE was diagnosed by the presence of columnar-lined esophagus at endoscopy and the confirmed presence of intestinal metaplasia upon biopsy. In addition, information on the presence of intestinal metaplasia, evidence of dysplasia (a premalignant condition characterized by increased cell growth, cellular atypia, and altered cell differentiation) and its severity, and the presence of coexistent EAC was obtained from histopathology records. We defined short-segment BE by the presence of less than 3 cm of columnar-lined esophagus at endoscopy. The distinction between long- and short-segment BE was made. We also recorded the presence of esophagitis or any esophageal lesions. Repeat endoscopy was done in patients with erosive esophagitis after complete healing, to confirm the presence of BE. Then the patients were classified into two groups according to presence or absence of BE.

Histopathological examination

Fresh endoscopic biopsy samples were obtained from the operating theatre and fixed in 10% formalin within 13 h at room temperature. Tissues were subjected to a series of processing steps, which included fixation, dehydration with ethanol, clearing with xylene, and wax impregnation with paraffin, and then stained with hematoxylin and eosin (HE).

Ambulatory pH monitoring was done for 40 patients (20 patients with BE and 20 without). All patients were studied with ambulatory pH monitoring using an antimony pH electrode placed 5 cm above the proximal border of the manometrically located lower esophageal sphincter, and another electrode placed 10 cm above this point in the proximal esophagus, connected to a portable Digitrapper (Synectics, San Antonio, TX, USA) data storage unit. Intraesophageal pH was recorded continuously, with sampling obtained every 4 s. All pH tracings were analyzed for the percentage time that the distal and proximal esophageal pH was < 4, determined for the upright and recumbent time periods in each study. Average esophageal acid clearance (EAC) time was calculated for each patient by dividing the total time (in minutes) that distal esophageal pH remained < 4 by the total number of GERD episodes. This was calculated for both the upright and supine periods.

Statistical analysis

Statistical analyses were performed using Stats Direct version 2.2.5 statistical software (Stats Direct Ltd., Sale, Cheshire, UK) and SPSS for Windows version 11.5 (SPSS, Inc., Chicago, IL, USA). All data are presented as means ± SD. χ^2 and Fisher's tests were used to compare BE and GERD groups according to sex, smoking and alcohol consumption. Student's *t* test was used for some factors. ANOVA was used for pH measurement analysis. Significance was accepted at $P \leqslant 0.05$.

RESULTS

This study included 1000 patients with chronic GERD symptoms (764 male and 236 female) with a mean age of 38.81 ± 14.52 years. Seventy-three of these patients (7.3%) had BE suspected endoscopically and detected histopathologically. The remaining 927 patients (92.7%) were negative for BE. Accordingly, we classified the patients into two groups: group A included patients with

Table 1 Age, sex, symptoms and endoscopic findings *n* (%)

	Group A	Group B	*P* value
Age (yr, mean ± SD)	48.3 ± 8.2	37.6 ± 13.4	< 0.05
Sex			NS
Male	68 (93)	785 (85)	
Female	5 (7)	142 (15)	
Total	73	927	
Smoking (508 patients)			
Smokers	45 (61.6)	463 (49.9)	NS
Non-smokers	28 (38.4)	464 (50.1)	
Alcohol consumption (60 patients)			
Alcoholic	6 (8)	54 (6)	NS
Non-alcoholic	67 (92)	871 (94)	
Main symptoms			
Heartburn	71 (97)	908 (98.1)	NS
Regurgitation	61 (83.6)	769 (82.9)	NS
Dyspepsia	58 (80)	742 (80.1)	NS
Epigastric pain	58 (80)	797 (86.2)	NS
Dysphagia	23 (31.5)	222 (24)	NS
Endoscopic findings			
Hiatus hernia	29 (39.7)	389 (42.1)	0.8 (NS)
Gastritis	53 (72.7)	667 (71.6)	0.8 (NS)
Duodenitis	21 (28.7)	297 (31.8)	0.7 (NS)

NS: Not significant; χ^2 and Student's *t* test used for statistical analysis. The patients with BE (group A) were significantly older than those with GERD (group B) ($P < 0.05$).

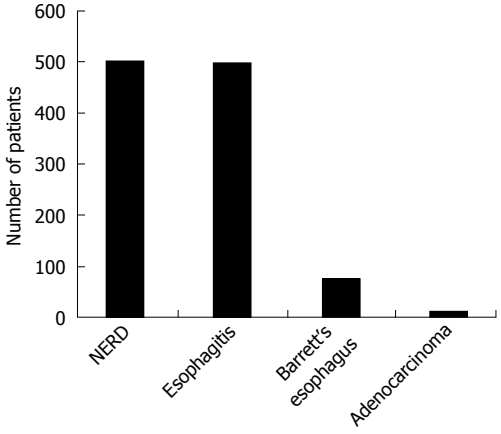

Figure 1 Endoscopic findings. Esophagitis was detected in 498 patients (49.8%) and NERD was seen in 502 patients. BE was present in 73 patients. EAC was detected in eight patients.

BE and group B included those with chronic GERD without BE. The mean length of BE was 5.3 ± 2.6 cm. Short-segment BE was present in 61 patients (84%), while long-segment BE was present in 12 (16%). Four cases with BE were detected in endoscopy-negative patients, while the remainder was detected in patients with esophagitis. EAC was detected in eight patients (six in group A and two in group B).

Regarding age and sex, the mean age of patients in group A (48.3 ± 8.2 years) was significantly older than that in group B (37.6 ± 13.4 years) ($P < 0.05$), and there was no significant difference regarding sex between the groups (Table 1). Although smoking and alcohol consumption were more frequent in the BE group, there was no significant difference from those without BE.

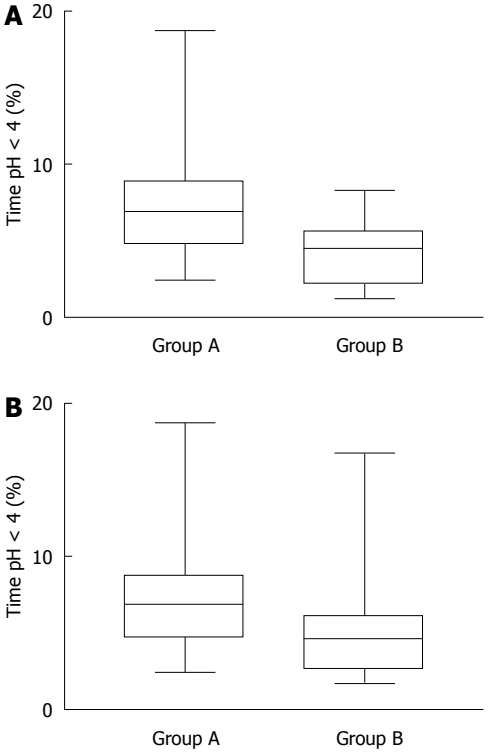

Figure 2 Distal esophageal acid exposure in both groups. A: In the supine position. The median time at pH < 4 in the distal esophagus in the supine position was significantly longer in patients with BE (group A) than in those with GERD without BE (group B) ($P < 0.03$); B: In the upright position. The median time at pH < 4 in the distal esophagus in the upright position did not differ significantly between patients with BE (group A) and those with GERD without BE (group B).

Also, there was no significant difference detected between the groups regarding GERD symptoms (Table 1).

Endoscopic examination detected esophagitis in 498 patients (49.8%) and non-erosive reflux disease (NERD) was seen in 502 patients (Figure 1). Only four cases with BE were seen among patients with NERD.

The percentage of patients with abnormal esophageal acid exposure was higher in the supine position in group A (16 patients, 80%) than in group B (nine patients, 45%). The median time at pH < 4 in the distal esophagus in the supine position was significantly longer in group A than group B, while no significant difference was detected in the upright position (daytime) between the groups (Figure 2A and B).

The histopathology of BE and EAC is shown in Figures 3 and 4.

DISCUSSION

To the best of our knowledge, this is the first study reporting the prevalence of BE in patients with chronic GERD symptoms in Upper Egypt. We recruited patients with GERD symptoms. We could not perform the study on the general population as it is difficult to convince asymptomatic people to undergo endoscopic procedures. One thousand consecutive patients were referred to endoscopy units and evaluated for chronic GERD

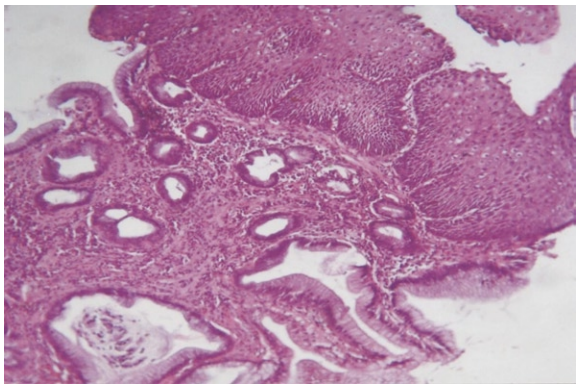

Figure 3 BE without dysplasia, showing true barrel-shaped goblet cells and intervening columnar cells, with incomplete brush-border, intestinal type metaplasia. The squamous epithelium of the normal esophagus was transformed into columnar epithelium (HE, × 200).

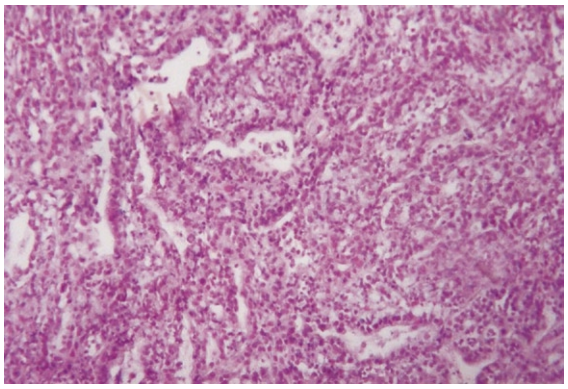

Figure 4 Well-differentiated adenocarcinoma showing gland formation, and low levels of nuclear pleomorphism and atypia (HE, × 200).

symptoms by a well-trained endoscopist in a prospective manner.

The prevalence of BE was 7.3% in patients with GERD symptoms. Taking into considerations the type of subjects included, the prevalence could have been much lower in the general population because of the known association of BE and GERD. In Northern Egypt, Hak *et al*[9] have found a prevalence rate of 9.9% of BE in patients with GERD, which agrees with the results of our study in the Southern part of Egypt (Upper Egypt). Hak *et al*[9] recruited symptomatic patients with GERD, but with an emphasis on the effect of acid and bile reflux on the esophageal mucosa.

The prevalence of BE varies around the world and it seems to be higher in western than eastern countries. Focusing on patients who presented for their initial endoscopy in the setting of suspected GERD, Westhoff *et al*[8] studied 378 consecutive patients who had biopsies taken from areas suspicious for BE. The overall prevalence of BE was found to be 13.2%. The majority of patients diagnosed had short-segment BE, which agreed with previous data that showed the prevalence of endoscopically recognizable short-segment BE at 5%-7% *vs* 1%-3.4% for long-segment BE[9,10].

Ronkainen and colleagues have used a population-based study to estimate the prevalence of BE in Sweden. Of 19 000 subjects within a target age range of 20-80 years, a random sample of 3000 was surveyed by questionnaire. A random sub-sample of 1000 subjects then underwent upper digestive system endoscopy, in which an overall BE prevalence of 1.6% was observed. However, when reflux symptoms were present, the prevalence rose to 2.3%[11]. In another study in Korea, Kim *et al*[12] have found that, in the general population, the prevalence of BE was < 1%, and remained less common in Korea than in western countries.

In our study, the prevalence of BE did not differ significantly between men and women, although the number of men recruited was much higher than women. Probably, men have more reflux symptoms or seek medical advice and endoscopic evaluation more than women do. Lin *et al*[13] have studied 543 patients with GERD symptoms, and have shown that while male and female patients demonstrated an equal severity of erosive esophagitis, only 14% of female patients had BE, compared to 23% of male patients ($P < 0.05$). However, Banki *et al*[14] have shown that there was an equal prevalence of BE in men and women diagnosed with severe reflux by 24-h pH monitoring. A chart review of almost 22 000 first endoscopies identified 492 patients with BE, and suggested that there was a 20-year age shift between men and women in prevalence patterns, which resulted in a male to female OR of 4.15 (95% CI: 2.99-5.77)[15].

In our study, the mean age in patients with BE was significantly older than in those without BE. Other studies have demonstrated that increased age is a risk factor for developing BE, as well EAC[16,17].

We found no significant difference between the groups regarding smoking and alcohol consumption, but it seemed that the number of smokers was high in both groups. While Ronkainen *et al*[11] and Kim *et al*[12] have found that alcohol consumption and smoking are significant risk factors, others have shown no significant importance of alcohol consumption and smoking in patients with BE[18-20].

Trying to explore the pattern of acid reflux in patients with BE, we found a significant difference between patients with BE and those with GERD for night-time acid reflux, which was more evident in patients with BE. The nocturnal gastroesophageal reflux that occurs in the recumbent position causes more injury to esophageal mucosa and may contribute to more severe chronic esophageal mucosal changes. Hak *et al*[9] have found more prolonged reflux periods in patients with BE than in those with GERD or NERD without BE. Gutschow *et al*[21] have reported that patients with BE have significantly more acid reflux events and a higher percentage of reflux time during the supine and upright phase than patients with NERD and GERD without BE. Also, Koek *et al*[22] in a multivariate analysis have found that BE is associated with male sex and exposure to both acid and duodenogastroesophageal reflux.

We conclude that BE is present in about 7.3% of patients with chronic GERD symptoms in our area.

It may be associated with older age and nocturnal gastroesophageal reflux.

COMMENTS

Background

Barrett's esophagus (BE) is gaining importance because of its association with esophageal adenocarcinoma (EAC). Its prevalence varies around the world. However, there are no data about its prevalence in upper Egypt.

Innovations and breakthroughs

This is the first study to report prevalence of BE in Upper Egypt.

Applications

This study may represent a future strategy for screening for BE and AEC in patients with chronic gastroesophageal reflux disease.

Peer review

This was a well-conducted and interesting study that investigated the prevalence of BE in Upper Egypt.

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BRIEF ARTICLES

Bone mineral density and disorders of mineral metabolism in chronic liver disease

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Abstract

AIM: To estimate the prevalence and identify the risk factors for metabolic bone disease in patients with cirrhosis.

METHODS: The study was performed on 72 Indian patients with cirrhosis (63 male, 9 female; aged < 50 years). Etiology of cirrhosis was alcoholism (n = 37), hepatitis B (n = 25) and hepatitis C (n = 10). Twenty-three patients belonged to Child class A, while 39 were in class B and 10 in class C. Secondary causes for metabolic bone disease and osteoporosis were ruled out. Sunlight exposure, physical activity and dietary constituents were calculated. Complete metabolic profiles were derived, and bone mineral density (BMD) was measured using dual energy X ray absorptiometry. Low BMD was defined as a Z score below -2.

RESULTS: Low BMD was found in 68% of patients. Lumbar spine was the most frequently and severely affected site. Risk factors for low BMD included low physical activity, decreased sunlight exposure, and low lean body mass. Calcium intake was adequate, with unfavorable calcium: protein ratio and calcium: phosphorus ratio. Vitamin D deficiency was highly prevalent (92%). There was a high incidence of hypogonadism (41%). Serum estradiol level was elevated significantly in patients with normal BMD. Insulin-like growth factor (IGF) 1 and IGF binding protein 3 levels were below the age-related normal range in both groups. IGF-1 was significantly lower in patients with low BMD. Serum osteocalcin level was low (68%) and urinary deoxypyridinoline to creatinine ratio was high (79%), which demonstrated low bone formation with high resorption.

CONCLUSION: Patients with cirrhosis have low BMD. Contributory factors are reduced physical activity, low lean body mass, vitamin D deficiency and hypogonadism and low IGF-1 level.

Key words: Bone mineral density; Liver disease; Chronic disease; Cirrhosis; Bone mineral metabolism; Hepatic osteodystrophy

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INTRODUCTION

Metabolic bone disease is a common complication of long-standing liver disease, ranging from cholestatic disorders to alcoholic, autoimmune and post-viral cirrhosis[1]. Often known as hepatic osteodystrophy (HO),

it is well-recognized among individuals with chronic liver disease (CLD). Its etiology is poorly understood and is thought to vary according to the type, severity and progression of the liver disease, along with a multitude of other contributing factors including the ethnicity of the population studied. It can result in spontaneous low-trauma fractures that significantly impact on the morbidity, quality of life, and even survival, through pain, deformity and immobility. With liver transplantation steadily taking the center stage in treatment of end-stage cirrhosis of varying etiology and offering long-term survival, bone disease has snowballed into one of the major determinants of survival and quality of life in this cohort[1].

Keeping in view the numerous therapeutic options for bone disease[2] already available and those under development, it is prudent to characterize this condition in order to give these patients a better chance of survival. The medical fraternity around the world has recognized this and has started characterizing the disorder. In various international studies, the overall incidence has varied from 11% to 48%[3], with a fracture rate of 3%-44%[3]. This has not been studied extensively in the Indian population[4].

MATERIALS AND METHODS

Patients

The study was performed on 72 Indian patients with cirrhosis. The group consisted of 63 men and nine women with a median age of 45 years (43.1 ± 7.4, range 22-50 years). Twenty-five patients had hepatitis B (22 men and 3 women), 10 had hepatitis C (5 men and 5 women), and 37 had alcoholic cirrhosis (36 men and 1 woman). A diagnosis of cirrhosis was confirmed histologically or clinically if biopsy was not available. A clinical diagnosis was established in patients who demonstrated a Child-Pugh index > 6 or ultrasound findings suggestive of cirrhosis (the presence of at least two of the findings of nodular irregular surface, distorted vascular pattern, or ascites). Signs of portal hypertension (endoscopically proven esophageal varices or dilated portal venous system with ultrasonography) were taken as additional corroborative evidence. The etiology of post-viral cirrhosis was proven if any of the serological markers were positive [hepatitis B surface antigen by ELISA, anti-hepatitis C virus (HCV) by third generation ELISA, or HCV RNA]. Diagnosis of alcoholic cirrhosis was made with a positive answer to more than one question in the CAGE questionnaire and a previous history of alcohol intake of > 80 g/d in men and > 40 g/d in women for > 10 years. An aspartate aminotransferase to alanine aminotransferase (AST/ALT) ratio of > 1.5 was taken as corroborative evidence. We selected only patients who had abstained from alcohol for > 3 mo prior to the study.

All patients with acute exacerbation or flair of disease (suggested by a bilirubin concentration > 5 mg/dL, AST > 2.5 times the upper limit of normal, leukocytosis > 10 000/mm^3, or diagnostic lesions of hepatitis on biopsy) and those with recent gastrointestinal bleeding were excluded. Patients with serum creatinine levels > 1.4 mg/dL were excluded, as were those with any form of acute illness. None of the patients had a previous history of chronic disorders associated with changes in mineral metabolism (thyroid disorders, parathyroid disorders, Cushing's syndrome, diabetes, immobilization in the past, or renal failure). None had a family history of osteoporosis, nor did they receive calcium, vitamin D or any medication which may have influenced bone metabolism (corticosteroids, hormone replacement therapy, calcitonin, bisphosphonates, cytotoxics, antimetabolites, anticoagulants, anticonvulsants, thyroxine, interferon or lamivudine). Nineteen patients were receiving spironolactone and 14 were receiving spironolactone and furosemide. Patients with major sclerosis of the aorta, osteophytes, or scoliosis on X-ray, which precluded accurate measurements of lumbar bone mineral density (BMD) by dual energy X ray absorptiometry (DXA), and those who had the criteria for more than one etiology of chronic liver disease were also excluded. All patients signed informed consent and the protocol was approved by the institutional ethics committee.

Methods

Demographic and disease-related data including anthropometry at the time of enrollment were captured. Each subject was interviewed to characterize sunlight exposure, physical activity and dietary intake.

Sunlight exposure was calculated in terms of length of usual weekly outdoor activity, sunscreen use, and usual outdoor attire. The "rule of nine" was adapted to estimate the fraction of body surface area (BSA) exposed to sunlight by each subject's usual outdoor attire[5]. With this, sun index was calculated as the product of hours of sun exposure per week and fraction of BSA exposed to sunlight. Mumbai is at latitude 18° 56' North and all of the study population were from areas below 37° latitude. Only sunlight exposure between 8 am to 5 pm in summer and 9 am to 3 pm in winter was measured. All our patients belonged to the same ethnicity and were of skin type 5. Physical activity was assessed using the Global Physical Activity Questionnaire (GPAQ) developed by WHO (www.who.int/chp/steps). Nutritional intake was calculated using a questionnaire with specific reference to calorie, protein, calcium (dairy and non-dairy), phosphorus and salt intake. These parameters were calculated in two different periods of life; prior to illness (5 years prior to patient perceived onset) and present state of illness.

Biochemical and hormonal determinations

Blood samples were drawn in the morning after an overnight fast. In addition to standard liver function tests, serum levels of calcium, phosphate, magnesium, alkaline phosphatase, and creatinine were measured on the same day with an auto analyzer (Biosystems S.A.,

Table 1 Demographic data of patients with normal and low BMD

Parameter	Low BMD	Normal BMD	*P* value
Age (yr)	44.4 ± 6.1 (47)	42 ± 8.7 (45.5)	0.220
BMI (kg/m^2)	21.14 ± 3.55 (20.3)	23.16 ± 5.46 (22.4)	0.275
Child score	7.4 ± 1.8 (7)	7.85 ± 1.9 (8)	0.350
Lean body mass (kg)	43.3 ± 7.3 (43)	46.7 ± 8.75 (44.7)	0.290
Fat mass (kg)	11.6 ± 6.5 (11.2)	14.4 ± 7.1 (12.43)	0.150

BMD: Bone mineral density. Parameters are expressed as mean ± SD. Median value is given in parentheses.

Barcelona, Spain). The rest of the sample was centrifuged immediately and stored at -70℃ for measurement of hormonal parameters, which were analyzed in a single batch. Serum was assayed using commercially available kits for 25 hydroxy vitamin D [25 (OH)D; radioimmunoassay (RIA); DiaSorin Inc., Stillwater, MN, USA], 1,25, dihydroxy vitamin D [1,25 $(OH)_2D$; enzyme immunoassay; Immunodiagnostic Systems Inc, Fountain Hills, AZ, USA], parathyroid hormone (PTH), osteocalcin, follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), testosterone (RIA; Diagnostic Products Corp., Los Angeles, CA, USA), sex hormone binding globulin (SHBG), free T4 (FT4), thyroid stimulating hormone (TSH), insulin like growth factor 1 (IGF-1) and IGF binding protein 3 (IGFBP3). Free testosterone was calculated from total testosterone, SHBG and albumin concentration (www.issam.ch). All hormonal investigations except testosterone, 25 (OH)D and 1,25 $(OH)_2D$ were done by chemiluminescent immunometric assay with Immulite 1000 systems (Diagnostic Products Corporation). Serum testosterone, estradiol, FSH and LH were estimated from pooled sera collected three times at 20-min intervals. The morning second void urinary sample was used for urinary parameters. Urine was analyzed for calcium, creatinine and free deoxypyridinoline (UDPD). UDPD was expressed as the ratio to creatinine.

BMD and X-ray measurements

BMD of the lumbar spine (L1-L4) and proximal femur (femoral neck and trochanter) was measured by DXA (Delphi W 70460; Hologic Inc., USA). All scans were carried out on the same machine by the same operator and were analyzed with the same software. BMD was expressed as g/cm^2 as well as Z score, compared to reference data for Caucasian populations. As there are no normative data available for the Indian population, no such comparison could be made. Low BMD was considered to be a Z score of -2 or less obtained at any site. X-ray analysis of lumbosacral spine (lateral view) and pelvis (antero-posterior view) was done to rule out any fracture. Lean body mass was also assessed by DXA.

Statistical analysis

Statistical analysis was done using SPSS version 14 software (Chicago, IL, USA). All results are expressed as means ± SD and median. The statistical significance between means was calculated by Student's *t* test, analysis of variance (ANOVA), or Mann-Whitney *U* test when appropriate. Differences between proportions were assessed by the χ^2 test. $P < 0.05$ was considered significant.

RESULTS

BMD

Among the 72 patients, 49 (68%) had low BMD. There were no significant differences in demography between the patients with normal and low BMD (Table 1). When patients were classified according to etiology of liver disease, the incidence was 56.7% alcoholic, 72% hepatitis B, and 100% hepatitis C. Incidence of low BMD was the same across all Child classes. Lumbar spine was the most frequently and severely affected site. It was involved in all patients with low BMD. Mean BMD at each site was: spine, -2.28 ± 1.1; hip, -1.27 ± 0.74; trochanter, -1.3 ± 0.8; and femoral neck, 0.75 ± 0.86. Bone mass loss in trabecular bone (lumbar spine) was more severe than that in cortical bone (femoral neck). The percentage of patients with low BMD of the hip was 14%, trochanter was 18%, and femoral neck was 7%.

Risk factors for low BMD

Patients were evaluated further for the possible predisposing factors for low BMD. The following data pertain to the 63 men in the study. As there were only nine women, they were analyzed separately. Patients were subdivided into low BMD (Z score ≤ -2, 43 patients) and normal BMD (Z score > -2, 20 patients) groups and further analyzed.

Relationship of BMD with physical activity, sunlight exposure and diet

Both groups showed considerable reduction in sunlight exposure and physical activity after the onset of illness (Table 2). Past and present sunlight exposure was lower in the group with low BMD, although it reached significance ($P < 0.05$) only with present exposure. Low physical activity (defined as < 600 MET.min/wk) was seen in 15 patients (23%) prior to disease onset but in 76% after developing chronic liver disease. It can be seen that the median activity level and sunlight exposure in the affected population was zero, compared to some amount of activity (120 MET.min/wk) and sunlight exposure (sun index of 0.15) in patients who were able to maintain their bone strength.

Dietary intake was comparable between the two groups. Calorie and protein intake were adequate. Calcium intake was also adequate according to the Indian Council of Medical Research guidelines (ICMR)[6]. The calcium:protein ratio (8.5-11.5) was much below the advocated range of 16-20[6]. The calcium:phosphorus ratio (0.45) was also not in the recommended range of 1:1[6].

Table 2 Sunlight exposure, physical activity and dietary parameters in men with normal and low BMD

Parameter		Low BMD	Normal BMD	*P* value
Sun index	Past	2.49 ± 3.3 (1.3)	3.54 ± 5.3(2.5)	0.13
	Present	0.21 ± 0.41 (0)	0.83 ± 1.4 (0.15)	0.035[a]
Physical activity (MET.min/wk)	Past	2188 ± 2340 (1920)	2378 ± 1855(2450)	0.7
	Present	468 ± 1260 (0)	351 ± 659.7 (120)	0.47
Total Calorie (kcal/d)	Past	2111 ± 768 (2078)	1867 ± 524 (1878.0)	0.18
	Present	2020 ± 662 (2071)	1997 ± 422 (2078)	0.65
Total Proteins (g/d)	Past	59.9 ± 22.6 (59.7)	54.9 ± 20 (56)	0.3
	Present	59.3 ± 20.7 (50.6)	60.2 ± 18.6 (61.2)	0.56
Calcium (mg/d)	Past	550 ± 160 (426.5)	582.6 ± 197 (557.8)	0.45
	Present	528 ± 180.7 (391.3)	620.3 ± 259.7(639.8)	0.3
Phosphorus (mg/d)		1436 ± 652 (1478)	1214.8 ± 535.7(1202)	0.1
Salt intake (g/d)		6.9 ± 2.9 (7)	7.2 ± 2.8(7.4)	0.53

[a]Statistically significant, $P < 0.05$.

Table 3 Biochemical parameters and markers of bone turnover in men with normal and low BMD

Parameter	Low BMD	Normal BMD	*P* value
Serum calcium (mg/dL[1])	9.17 ± 0.52 (9.22)	9.29 ± 0.47 (9.33)	0.29
Serum phosphorus (mg/dL)	3.63 ± .9 (3.59)	3.74 ± 1 (3.8)	0.69
Serum alkaline phosphatase (IU/L)	138 ± 61 (132.7)	140.2 ± 65.6(128.5)	0.96
Serum magnesium (mg/dL)	2.0 ± 1.9 (1.58)	1.42 ± 0.3 (1.48)	0.08
Urine calcium/creatinine ratio	0.06 ± 0.1 (.03)	0.053 ± 0.053 (0.035)	0.72
25 (OH) vit D (ng/mL)	11.2 ± 7.6 (9.4)	10.5 ± 5.7 (8)	0.90
1,25 $(OH)_2$ vitD (pg/mL)	46.54 ± 26.4 (41)	36.6 ± 21.6 (30)	0.19
Serum PTH (pg/mL)	45.5 ± 28.1 (43.9)	42.2 ± 18.9 (42.1)	0.95
Serum osteocalcin (ng/mL)	2.99 ± 2.5 (1.8)	2.14 ± 1.8 (1.14)	0.37
UDPD/creatinine ratio[2]	11.5 ± 4.5 (12.1)	12.5 ± 6.2 (11.6)	0.84

[1]Corrected calcium is used; [2]Unit - nM DPD/mmol/L creatinine.

Table 4 Hormone parameters in men with normal and low BMD

Parameter	Low BMD	Normal BMD	*P* value
FSH (IU/L)	8.2 ± 9.4 (4.62)	19.8 ± 40.2 (7.53)	0.34
LH (IU/L)	6.8 ± 6.2 (4.78)	11.1 ± 13.9 (6.47)	0.31
Estradiol (pg/mL)	76.1 ± 61.5 (63.8)	100.6 ± 61.8 (79.2)	0.02[1]
SHBG (nmol/L)	76.7 ± 31.1 (75.4)	72.3 ± 25.4 (72.6)	0.60
Free testosterone[2] (ng/dL)	7.6 ± 4.7 (7.27)	7.2 ± 4.0 (7.06)	0.85
IGF-1 (pmol/L)	44.8 ± 24.7 (35.2)	73.7 ± 58.54 (38.4)	0.049[1]
IGFBP3 (μg/mL)	1.21 ± 0.6 (1.03)	1.8 ± 1.2 (1.51)	0.071

[1]Statistically significant, $P < 0.05$; [2]Calculated from total testosterone.

Relationship of BMD with biochemical parameters and markers of bone turnover

Biochemical parameters were comparable between both groups (Table 3). Serum calcium, magnesium, phosphorus and alkaline phosphatase levels were normal. Prevalence of vitamin D deficiency was high among patients with CLD. Among the 63 patients, vitamin D values were < 10 ng/mL in 38 patients (60%), 10-20 ng/mL in 20 patients (32%), and > 20 ng/mL in 5 patients (8%). Despite having a low vitamin D level in 92%, PTH was within the physiological range in 87% of patients.

Markers of bone turnover indicated high resorption with low formation. Serum osteocalcin was low in 43 patients (68%) and UDPD: creatinine ratio was high in 50 patients (79%). This suggests uncoupling of bone remodeling as the cause for low BMD in CLD. The levels of bone turnover markers were comparable between the two groups.

Relationship of BMD to hormone parameters

There was a high incidence of hypogonadism in patients with cirrhosis. Twenty-six patients (41%) had low calculated free testosterone. This was distributed equally between the low and normal BMD groups. Among the hypogonadal patients, 18 (69%) had central hypogonadism and eight (31%) had primary testicular failure. An FSH value of > 10 IU/L with normal free testosterone was seen in an additional nine (14%) patients. Serum estradiol level was significantly elevated ($P < 0.05$) in patients with normal BMD as compared to those with low BMD (Table 4). Forty-six patients (73%) had a high estradiol level, which was distributed unequally within the groups, with 90% of patients with normal BMD and 65% in the group with low BMD having values above the physiological upper range of 50 pg/mL.

IGF-1 levels were below the age-related normal range in both groups, but significantly lower ($P < 0.05$) in patients with low BMD (Table 4). Forty-one patients (95%) of patients in the low BMD group and 15 (75%) in the normal BMD group had IGF-1 level below normal, which accounted for 89% of patients with CLD.

Low IGFBP3 was almost a universal finding in patients with CLD (61 of 63 patients; 97%), although it did not differ significantly between the groups ($P = 0.071$).

DISCUSSION

The purpose of the current study was to determine the prevalence of low BMD, to estimate the bone turnover and hormonal status, and to identify the factors associated with bone disease in patients with CLD. The only available Indian data on this subject are those of Sachdev *et al*[4] from 1976. The current study shows that patients with CLD have a high prevalence of decreased BMD, with the lumbar spine being the most frequently and intensely affected site. Furthermore, there was no relation between severity of hepatic dysfunction (Child class) and incidence of low BMD. These factors point to the need for early evaluation for HO in patients with CLD.

In the present cohort, low BMD was found in 68% of patients. This is comparable to the only available Indian data of Sachdev *et al*[4], in which 64% of cirrhotic patients had low BMD. The method of evaluation and diagnosis differed greatly in that era. In the earlier study of 25 patients with cirrhosis (all aged < 40 years), diagnosis of cirrhosis was made from liver biopsy and osteoporosis from iliac crest biopsy. Scanning through western studies has indicated marked heterogeneity in BMD findings in CLD, ranging from no effect to a large BMD deficit. Leslie *et al*[7] have pooled the results from uncontrolled and controlled studies of bone mineral content in CLD. They have shown a Z score less than -2 in 21% of patients. Studies on patients with end-stage liver disease of varying etiology confirm a high but variable incidence of osteoporosis (11%-48%) and osteopenia (18%-35%)[3]. The incidence of 68% in the present study is much higher than that in any previous study. This may be because Indians have a lower BMD as compared to Caucasians[8,9]. Thus, the use of Z scores based on a Caucasian database might have resulted in overestimation of osteoporosis. However there are no published data for BMD in healthy Indian populations.

The major influences on bone metabolism are genetic, but also essential are mechanical stress (exercise and muscle activity), good nutrition, adequate calcium and vitamin D, and a normal hormonal environment. The patient with CLD could have any of these factors acting alone or in concert, which potentially predispose him/her to thin bones. Each of the above factors were assessed and compared between patients with low and normal BMD. It was found that patients with CLD had all the above and known risk factors: low sunlight exposure, reduced physical activity, low lean body mass, vitamin D deficiency and hypogonadism. The presence of risk factors in the low and normal BMD groups was probably the reason for the absence of a statistically significant difference in risk factors between the normal and low BMD groups. This indicates that all patients with cirrhosis, unless prevented, will develop the disease. In addition, although the calcium intake was adequate by ICMR guidelines, it was well below the internationally accepted daily allowance. This added to an unfavorable calcium:protein ratio of 8.5-11.5 mg/g, and calcium:phosphorus ratio of 0.45 may have resulted in inadequate recommended daily allowance of calcium in these patients.

Vertebrae consist of 50% trabecular bone, while other bones (hip, neck and trochanter) consist mainly of cortical bone. Sites with a high proportion of trabecular bone are affected earliest in diseases that produce increased bone turnover[10]. In the present study, serum osteocalcin was low in 68% and UDPD: creatinine ratio was high in 79% of patients, which indicated a high resorptive state added to low formation. This suggests uncoupling of bone remodeling as the cause of low BMD in CLD. This can explain the predominant involvement of the spine in HO. This is also compatible with other similar studies[11,12].

In the present study, 41% of patients were hypogonadal, although this was not correlated with the severity of bone loss. Diamond *et al*[13] and Monegal *et al*[11] have shown that hypogonadism is common in men with cirrhosis but it is not correlated with osteoporosis. A particularly interesting finding in the present study was the significantly elevated estradiol level ($P < 0.05$) in patients with normal compared with low BMD. Estrogen is known to have a positive influence on the male skeleton[14]. It is also known to be increased in men with cirrhosis. Probably the anabolic and antiresorptive qualities of estrogens in bone act as protective factors in preventing bone loss in these patients with cirrhosis.

More than 90% of circulating IGF-1 is synthesized in the liver. It is a proven early marker of hepatocellular functional capacity[15,16], and shows a marked decline in the early stages of cirrhosis (Child-Pugh class A). It starts decreasing before other liver-function parameters such as albumin, bilirubin and prothrombin become involved[15]. GH levels are increased correspondingly, which creates a state of IGF resistance[17]. IGF-1 is also a major determinant of BMD in healthy men[18]. In the present study, IGF-1 levels were below the age-related normal range in both groups, and were significantly lower ($P < 0.05$) in patients with low BMD. IGF-1 values were low in 89% of patients with CLD. Previous studies have shown IGF-1 levels to correlate directly with BMD and inversely with disease severity[12,19,20]. Studies have described a role for decreased serum IGF-1, even in idiopathic osteoporosis[21]. IGF-1 expression is increased during early osteoblast recruitment, but declines as these cells undergo differentiation. It is known to stimulate osteoblast proliferation[22] and play a key role in bone remodeling and maintenance of bone mass. Simonet *et al*[23] have shown that low levels of IGF-1 may lead to increased bone resorption. Thus, the link between cirrhosis and bone loss also involves low levels of IGF-1. A significant difference in IGF-1 level between normal and low BMD patients may be a pointer to why some patients deteriorate faster, despite sharing equally all the risk factors.

IGFBP3 play a very important role in bioavailability of circulating IGF-1. It forms a stable ternary complex with an acid-labile subunit and IGF-1, and binds > 95% of circulating IGF-1. In the present cohort, low IGFBP3 was seen in 97% of patients with CLD, although this did not differ significantly between the normal and low BMD groups ($P = 0.071$). This is plausible because hepatocytes are the major site of IGFBP3 synthesis. This may have further decreased the tissue bioavailability of IGF-1[24,25].

In conclusion, the present study confirms the high incidence of low BMD in patients with CLD. Disease onset is early in the course of cirrhosis. Decreased bone formation with increased bone resorption imply that uncoupling of bone remodeling is the mechanism involved. Contributing factors are inadequate sunlight exposure, reduced physical activity, low lean body mass, vitamin D deficiency and hypogonadism. The presence of most risk factors in low and normal BMD groups indicates that all patients with cirrhosis are vulnerable, and unless prevented, will develop the disease. Our results provide evidence of the key roles played by IGF-1 and estrogen in this condition. Although risk factors are prevalent in all patients, the severity of bone loss may be accelerated in patients with low IGF-1 level. The present study also suggests a possible protective role for the high estrogen level seen in cirrhosis.

COMMENTS

Background

Long-standing liver disease has long been recognized to result in fragile bones with increased risk of fractures. In various international studies, the overall incidence has varied from 11% to 48%, with a fracture rate of 3%-44%. The reason for this is poorly understood. With liver transplantation becoming a viable option in liver disease and offering complete cure and long-term survival, bone disease is becoming the major determinant of survival and quality of life in these patients. The present study tried to characterize the problem and identify contributing risk factors.

Research frontiers

Much work has been done and is still going on in the field of hepatic osteodystrophy (HO). It is a hot topic of research, as liver transplantation is improving survival of patients with end-stage liver disease, and bone disease and fracture are limiting the survival and quality of life of these patients. The medical fraternity has recognized that bone health has to be taken care of to fully translate the benefits of modern treatment into patient survival.

Innovations and breakthroughs

Most of the data obtained in this study conform to those in the literature. Two significant findings of the study (that low levels of IGF-1 is a risk factor for decreased BMD, and increased estrogen is protective) are relatively new.

Applications

This article provide an entirely new frontier in research, namely, to look forward to the therapeutic benefit of IGF-1 therapy in these patients. Synthetic IGF-1 is available under the name mecasermin and is used currently for the long-term treatment of growth failure in children with severe primary IGF-1 deficiency.

Peer review

This work represents an original contribution regarding HO in patients with advanced liver disease in India. The study was well-conducted. The authors identified in cirrhotic patients that low levels of IGF-1 are a risk factor for decreased BMD, while increased estrogens protect against osteopenia.

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Clinical outcomes of self-expandable metal stents in palliation of malignant anastomotic strictures caused by recurrent gastric cancer

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Abstract

AIM: To examine the technical feasibility and clinical outcomes of the endoscopic insertion of a self-expandable metal stent (SEMS) for the palliation of a malignant anastomotic stricture caused by recurrent gastric cancer.

METHODS: The medical records of patients, who had obstructive symptoms caused by a malignant anastomotic stricture after gastric surgery and underwent endoscopic insertion of a SEMS from January 2001 to December 2007 at Kangnam St Mary's Hospital, were reviewed retrospectively.

RESULTS: Twenty patients (15 male, mean age 63 years) were included. The operations were a total gastrectomy with esophagojejunostomy (n = 12), subtotal gastrectomy with Billroth-Ⅰ reconstruction (n = 2) and subtotal gastrectomy with Billroth-Ⅱ reconstruction (n = 8). The technical and clinical success rates were 100% and 70%, respectively. A small bowel or colon stricture was the reason for a lack of improvement in symptoms in 4 patients. Two of these patients showed improvement in symptoms after another stent was placed. Stent reobstruction caused by tumor ingrowth or overgrowth occurred in 3 patients (15%) within 1 mo after stenting. Stent migration occurred with a covered stent in 3 patients who underwent a subtotal gastrectomy with Billroth-Ⅱ reconstruction. Two cases of partial stent migration were easily treated with a second stent or stent repositioning. The median stent patency was 56 d (range, 5-439 d). The median survival was 83 d (range, 12-439 d).

CONCLUSION: Endoscopic insertion of a SEMS provides safe and effective palliation of a recurrent anastomotic stricture caused by gastric cancer. A meticulous evaluation of the presence of other strictures before inserting the stent is essential for symptom improvement.

Key words: Stents; Surgical anastomosis; Stricture; Endoscopic gastrointestinal surgery; Stomach neoplasms

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INTRODUCTION

Local recurrence causing dysphagia occurs in approximately 20% of stomach cancer patients treated with a gastrectomy[1]. These patients are usually poor surgical candidates because of advanced malignancy, poor performance status or malnutrition. Palliative surgery carries a high risk of mortality and morbidity.

A self-expandable metal stent (SEMS) is currently the main palliative nonsurgical treatment for malignant gastric outlet obstructions[2]. Metal stents are also used to treat malignant anastomotic obstructions after esophagojejunostomy, gastrojejunostomy and gastroduodenostomy. However, there are only a few

reports on the clinical outcome of SEMS for the palliation of a recurrent anastomotic obstruction after gastric surgery[3-11]. In particular, there is one study on endoscopic insertion of a SEMS in a recurrent anastomotic stricture[11] instead of a fluoroscopically-guided method. The clinical outcomes and complications might differ according to the surgical technique because of the different anastomotic angle or different anatomical alterations during surgery.

This study evaluated the technical feasibility and clinical effectiveness of endoscopic SEMS placement in the palliation of patients with a recurrent anastomotic obstruction after gastric surgery.

MATERIALS AND METHODS

Patients

Twenty consecutive patients (M:F = 15:5, mean age 63.1 ± 10.3 years), who had a documented postoperative anastomotic stricture caused by recurrent gastric cancer and had undergone endoscopic SEMS insertion from January 2001 to December 2007, were enrolled in this study. All patients had a symptomatic obstruction characterized by nausea, vomiting, reduced oral intake and weight loss. The recurrent gastric cancer, which was the underlying cause of the obstruction, was confirmed by pathological diagnosis in all patients. None of the patients were surgical candidates based on the presence of advanced, metastatic disease or medical comorbidity.

The exclusion criteria were patients who were mildly symptomatic or patients in whom an adult endoscope could be passed through the malignant anastomotic stricture or patients showing evidence of peritonitis. An abdominal computed tomography (CT) scan or contrast media radiographic study to document multiple strictures was not performed routinely.

The surgical technique was a total gastrectomy with esophagojejunostomy in 10 patients, subtotal gastrectomy with Billroth- I reconstruction in 2 patients and subtotal gastrectomy with Billroth-Ⅱ reconstruction in 8 patients. The type of reconstruction after total gastrectomy was loop esophagojejunostomy except for 2 patients with a Roux-en-Y esophagojejunostomy. Gastrojejunostomy without jejunojejunostomy was used for Billroth-Ⅱ reconstruction after subtotal gastrectomy. Strictures occurring in the efferent loop were included in this study. One patient had a stricture in both afferent and efferent loops. Therefore 2 stents were inserted in both sites. Patients with recurrent cancer only in the afferent loop were excluded. Table 1 lists the patients' characteristics.

Table 1 Patients' characteristics

Age ± SD (yr)	63.1 ± 10.3
Male:Female	15:5
Prior surgery (*n*)	
Total gastrectomy with	Covered stent (6)
esophagojejunostomy (10)	Uncovered stent (4)
Subtotal gastrectomy with Billroth- Ⅰ	Covered stent (1)
reconstruction (2)	Uncovered stent (1)
Subtotal gastrectomy with Billroth-Ⅱ	Covered stent (6)
reconstruction (8)	Uncovered stent (2)
Chemotherapy after stent insertion (number of patients)	10
Follow-up loss (*n*, %)	3 (15%)
30-d mortality	3/17 (18%)
Survival [median (range)]	83 (12-439) d
Stent patency duration [median (range)]	56 (5-439) d

Methods

NITI-S® stents (Taewoong, Seoul, Korea, *n* = 10), Choo stent (M.I. Tech, Seoul, Korea, *n* = 10) were used. These stents are commonly used commercial pyloric stents. The degree, length and site of the stenosis were evaluated using an endoscopic procedure or barium meal prior to stent insertion. Thirteen covered stents and 7 uncovered stents were inserted. The covered stent was coated with polyurethane around the body and contained the proximal flare portion. The diameter of the body and flare portions were 18 and 26 mm, respectively. The length of these SEMS ranged from 8 to 22 cm. The outer diameter of the delivery system was 10F to 11F with an overall length of 180 cm. The stent delivery system was advanced over the guidewire. Under direct guidance of endoscopic and fluoroscopic vision, a guidewire was passed through the malignant stricture. The stent was then released and the position and location of the stent were assessed by both endoscopy and fluoroscopy. Compensatory hydrostatic dilatation of the stent was not required in any of the patients. The patients usually resumed a water or a liquid diet 24 h after stent placement. The patients started a soft or solid diet after the follow up X-ray showed full extension. There was one patient whose stent was not sufficiently expanded. He could not restart a soft diet.

After inserting the stent, a combination of 5-fluorouracil, cisplatin, and epirubicin or paclitaxel-based or docetaxel-based chemotherapy was administered when the oral intake improved and the Eastern Cooperative Oncology Group performance status was ≤ 2 (graded as follows: 0 = normal activity, 1 = symptoms but ambulatory, 2 = in bed less than 50% of time, 3 = in bed more than 50% of time, and 4 = totally bedridden). Palliative chemotherapy after stent insertion was performed in 10 patients (50%).

Definitions

The outcome of the stent was evaluated using the following parameters: (1) technical success and clinical success; (2) complications; (3) stent patency.

Technical success was defined as the successful insertion of a stent in the proper position and the confirmation of patency using a combination of endoscopy and fluoroscopy with oral contrast opacification.

Clinical success was defined as an improvement in the obstructive symptoms and oral intake 1 to 3 d after placing the stent. The degree of oral intake was assessed using the Gastric Outlet Obstruction Scoring System as follows: 0 = no oral intake; 1 = exclusively liquid diet; 2 = exclusively soft solids diet; 3 = full diet possible. The improvement in oral intake was evaluated as the best degree at least 3 d after stent insertion. A primary stent dysfunction was defined as a failure to resume an oral intake after stent insertion.

Table 2 Improvement in the oral intake status compared to before stent insertion (n = 20)

Oral intake status (by GOOSS)	Number of cases	
	Pre-stenting	Post-stenting
No oral intake (0)	15	3
Liquids only (1)	3	6
Soft solids (2)	2	8
Low-residual or full diet (3)	0	3
Mean score[b]	0.35 ± 0.61	1.55 ± 0.94

[b]P < 0.01 by Wilcoxon signed rank test; GOOSS: Gastric Outlet Obstruction Scoring System.

The stent patency time was defined as the duration between the initial stent placement and the recurrence of obstructive symptoms caused by a stent occlusion. It was considered to be equal to the survival time if there were no obstruction symptoms or stent occlusion.

Follow-up

The patients were followed up to determine their clinical outcomes until they died or the stent malfunctioned, such as by migration or occlusion by tumor ingrowth or overgrowth. The data were obtained from the hospital records, radiology or endoscopic records, the patients themselves during a clinical visit and their relatives by a telephone survey. The status of oral food intake was monitored at 1 mo intervals on an outpatient basis. A follow-up barium study or endoscopy was performed only if obstructive symptoms recurred in order to evaluate stent occlusion or migration.

Statistical analysis

The values for the patients' characteristics are expressed as the median (range). The categorical data were examined using Fisher's exact test. The degree of oral intake before and after stent insertion was compared by a Wilcoxon signed rank test. The overall survival and stent patency were estimated by Kaplan-Meier life table analysis. A P-value < 0.05 was considered significant. All analyses were carried out using SPSS version 10.0 (SPSS Inc, USA).

RESULTS

Technical and clinical success

Endoscopic stent placement was technically successful in all patients. Clinical success was achieved in 14 out of the 20 cases (70%). The reasons for the lack of improvement in obstructive symptoms were small bowel or colon stricture (n = 4), ileus induced by peritoneal dissemination (n = 1) and primary stent dysfunction caused by stent expansion failure (n = 1). The symptoms in 2 of the 4 patients with single small bowel or colon stricture improved after placing a second stent. Table 2 summarizes the improvement in the dietary status.

Complications

There was no procedure-related mortality. In one patient who underwent a distal gastrectomy with Billroth- I reconstruction, the uncovered stent did not expand fully and was compressed by the tumor mass until 5 d after stent placement. The symptoms were not improved. However, he refused further treatment and was lost to follow-up 7 d after stent placement.

Recurrent symptoms of an obstruction were observed in 3 patients (15%) as a result of tumor overgrowth (n = 2) or tumor ingrowth (n = 1) within 1 mo after stenting. The reobstruction rate (1/13 *vs* 2/7, P = 0.55) of a covered stent and uncovered stent, and stent patency duration [56 d (range, 7-439) for the covered stent *vs* 37 d (range, 15-141) for the uncovered stent, P = 0.7] were similar. Tumor overgrowth occurred in patients who underwent a total gastrectomy with esophagojejunostomy. Tumor ingrowth occurred in a patient who underwent a subtotal gastrectomy with Billroth-Ⅱ reconstruction, in whom an uncovered stent was inserted. Two patients were treated successfully with an overlapping second covered stent. Stent migration occurred in 3 patients (15%) who underwent a subtotal gastrectomy with Billroth-Ⅱ reconstruction, in whom covered stents was inserted. Complete stent migration occurred at 64 d in one patient who received palliative chemotherapy. The migrated stent was not detected until the endoscopy or radiologic study revealed no stent remaining at the previous stricture site. Therefore, the stent was believed to have migrated downward and pass out of the anus without the patient's awareness. She was asymptomatic even though there was stent migration. The reobstructive symptoms appeared 319 d after stent migration. She was treated with the placement of 3 stents at the efferent loop, the afferent loop and distal colon stricture.

Partial stent migration to the more distal side of the efferent loop occurred in a patient 2 d after stent placement. The patient was treated by overlapping a second stent into the first stent. The proximal half of one stent slipped upward to the body of the stomach in one patient, which was repositioned by grasping with the forceps. The symptoms improved. Table 3 gives a summary of the complications.

Survival

Three patients were lost during the follow-up period and the remaining 17 patients died. The median survival period was 83 d (range, 12-439 d) and the median stent patency was 56 d (range, 5-439 d, Figure 1). There were no differences in median survival or stent patency between the patients who received palliative chemotherapy and those who did not (P = 0.66).

DISCUSSION

A SEMS is a simple, safe and effective palliation treatment for patients with a malignant obstruction of the gastrointestinal tract[12,13]. A SEMS has clinical advantages, compared with surgical gastrojejunostomy, such as rapid resumption of oral intake, shorter hospital stay and rapid improvement in the quality of life in malignant gastric obstruction[14,15].

Patients with an anastomotic stricture caused

Table 3 Complications associated with stent placement

Patient	Complication	Type of operation	Type of stent	Days after stenting	Treatment
1	Expansion failure	Billroth-Ⅰ subtotal gastrectomy	Uncovered	5	Refusal of treatment
2	Tumor overgrowth	Total gastrectomy	Covered	7	Second stent
3	Tumor overgrowth	Total gastrectomy	Uncovered	28	TPN
4	Tumor ingrowth	Billroth-Ⅱ subtotal gastrectomy	Uncovered	15	Second stent
5	Stent migration (complete)	Billroth-Ⅱ subtotal gastrectomy	Covered	64	Not needed
6	Stent migration (partial)	Billroth-Ⅱ subtotal gastrectomy	Covered	20	Reposition
7	Stent migration (partial)	Billroth-Ⅱ subtotal gastrectomy	Covered	2	Second stent

TPN: Total parenteral nutrition.

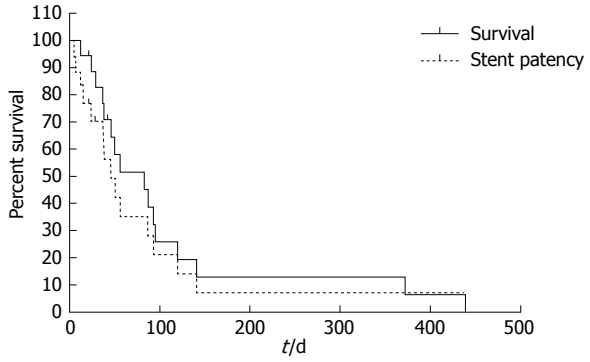

Figure 1 Cumulative survival and stent patency of 20 patients obtained using the Kaplan-Meier method.

by recurrent gastric cancer are likely to be severely debilitated. These patients generally have a relatively short life expectancy. Bypass or resective operations are usually impossible because of the extensive tumor invasion and metastasis[5]. Therefore, a less invasive procedure is preferred. This study evaluated the clinical effectiveness and the technical feasibility of SEMS insertion in the palliation of patients with a recurrent anastomotic stricture after various gastric surgical procedures.

The surgical techniques used were total gastrectomy with esophagojejunostomy (n = 10), subtotal gastrectomy with Billroth-Ⅰ reconstruction (n = 2), subtotal gastrectomy with Billroth-Ⅱ reconstruction (n = 8). All procedures were performed using endoscopic guidance. The technical success rate was 100%, which is comparable to those with a primary malignant gastric outlet obstruction (83%-100%)[15]. There is one report on endoscopically-guided stent insertion in a recurrent anastomotic stricture[11]. The advantages of endoscopically-guided stent insertion are the ease of accessing the stricture site and the avoidance of looping the delivery system through the dilated gastric lumen because endoscopy offers sufficient stiffness, so that the delivery system can easily pass through the dilated gastric lumen. There was no erroneous stent placement in the incorrect loop. The efferent loop was differentiated by identifying the ampulla of Vater in the afferent loop by endoscopy, which was confirmed by fluoroscopy during stent insertion. Before stenting, knowledge of the anatomy is important because it can be altered by the surgical procedures or recurrent tumor mass occluding the efferent loop[7].

The dietary intake improved in 14 out of the 20 patients (70%) after stent placement, which is comparable to the clinical success rate of SEMS insertion in a malignant gastric outlet obstruction (75%-85%)[13]. The improvement in symptoms after SEMS insertion in the anastomotic stricture caused by recurrent gastric cancer was reported to be 80%-90%[3-8]. The average score of the dietary status improved from 0.35 ± 0.61 to 1.55 ± 0.94 (P < 0.01). Five patients whose symptoms did not improve had another single stricture at the small intestine or colon, or ileus by peritoneal dissemination. The dietary state in 2 of them improved after inserting an additional stent. This suggests that a precise study of the distal bowel loop using a CT scan or barium study before stent insertion is essential in order to exclude a concealed obstruction. A single stent may not be helpful if there are multiple strictures. Moreover, the insertion of 2 stents at one time may be necessary if the patients have another single stricture.

Stent reobstruction caused by tumor ingrowth or overgrowth occurred in 3 patients (15%) within 1 mo after stent placement. A recent study reported that early restenosis within 1 mo tended to occur more frequently in postoperative anastomosis than a gastric outlet obstruction caused by primary cancer (4/6 *vs* 2/6, P < 0.01)[16]. The covered stents had the merit of less frequent reobstruction by tumor ingrowth[17]. However, in this study, the reobstruction rate and stent patency duration of covered stents and uncovered stents were similar. The incidence of stent reobstruction in recurrent anastomotic stricture after gastric surgery was reported to be 0%-17%[3-8]. Most studies used covered stents. In 2 studies using uncovered stents, Lee *et al*[6] reported that one out of 4 patients had tumor ingrowths, and Song *et al*[7] reported a 50% stent reocclusion rate within 2 wk of stent placement. A recent retrospective study suggested that a double coaxial stent had a longer patency and lower migration rate than an uncovered stent in postoperative anastomotic obstructions[11]. A prospective, randomized, comparative study to determine which stent is favorable in this situation will be needed.

Three cases of stent migration (15%, 3/20) were encountered in patients who underwent a subtotal gastrectomy with Billroth-Ⅱ reconstruction and had a covered stent inserted. Complete stent migration occurred in one patient who received palliative chemotherapy after approximately 64 d. Because chemotherapy might stabilize or reduce the tumor burden, it could influence stent migration. Two cases of partial stent migration were

easily treated by repositioning the stent and overlapping a second stent. The incidence of stent migration was reported to be 0%-16% in studies using a covered stent in an anastomotic stricture in various types of gastric cancer surgery[3-8]. The surgical technique can influence the rate of migration. The relatively acute angle between anastomosis and the efferent loop in gastrojejunostomy compared with the relatively obtuse angle in esophagojejunostomy or gastroduodenostomy, the radial force of the stent in the angulated loop, or the use of a covered stent may influence stent migration.

In this study, the 30-d mortality was 18%. The median survival was 83 d (range, 12-432 d). The median stent patency was 56 d (range, 5-439 d). Because the median survival in an anastomotic obstruction is comparable to that in a malignant gastric outlet obstruction, strategies to prolong stent patency and avoid the need for additional intervention are important in patients with recurrent cancer, particularly those with a relatively good performance status or who are expected to have a longer survival.

In summary, endoscopic insertion of a SEMS is a safe, technically feasible, and effective treatment for the palliation of anastomotic strictures caused by recurrent gastric cancer. A meticulous evaluation of the presence of another stricture before inserting the stent is essential for symptom improvement.

COMMENTS

Background

A self-expandable metal stent (SEMS) was used to treat malignant anastomotic obstruction during esophagojejunostomy, gastrojejunostomy and gastroduodenostomy. There are only a few reports on the clinical outcome of SEMS insertion for the palliation of a recurrent anastomotic obstruction after gastric surgery.

Research frontiers

The authors aimed to evaluate the technical feasibility and clinical effectiveness of an endoscopic SEMS placement in the palliation of patients with a recurrent anastomotic obstruction after gastric surgery.

Innovations and breakthroughs

This retrospective study has shown that the technical and clinical success of SEMS insertion for anastomotic strictures caused by recurrent gastric cancer were 100% and 70%, respectively. The main reasons for the clinical failure were small bowel or colon stricture in addition to anastomotic stricture. Stent migration (15%) was encountered in patients who underwent subtotal gastrectomy with Billroth-Ⅱ reconstruction and had a covered stent inserted.

Applications

A meticulous evaluation of the presence of other strictures is essential before inserting the stent for anastomotic strictures from recurrent gastric cancer. The possibility of stent migration is a consideration in anastomotic strictures after gastrojejunostomy.

Peer review

This paper is a good retrospective report on the usage of a SEMS for palliation of malignant anastomotic stricture caused by recurrent gastric cancer, showing that it is a safe and effective palliation treatment.

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BRIEF ARTICLES

Osteoporosis in adult Sri Lankan inflammatory bowel disease patients

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Abstract

AIM: To determine if inflammatory bowel disease (IBD) is a risk factor for osteoporosis in adult Sri Lankans.

METHODS: We identified eligible subjects from among consecutive patients diagnosed with IBD who attended our outpatient clinic. We included only patients aged between 20 and 70 years. Patients who were pregnant, had significant comorbidity, or were on calcium supplements or treatment for osteoporosis within the past 6 mo, were excluded. Healthy, age- and sex-matched controls were also recruited, in a control to patient ratio of 3:1. Both groups were screened for osteoporosis using peripheral dual energy X-ray absorptiometry scanning.

RESULTS: The study population consisted of 111 IBD patients (male:female = 43:68; mean age 42.5 years) and 333 controls (male:female = 129:204; mean age 43.8 years). The occurrence of osteoporosis among IBD patients (13.5%) was significantly higher than among controls (4.5%) (P = 0.001). The frequency of osteoporosis was not significantly different between ulcerative colitis (14.45%) and Crohn's disease (10.7%). However, on multivariate analysis, only age (P = 0.001), menopause (P = 0.024) and use of systemic steroids (P < 0.001) were found to be associated independently with the occurrence of osteoporosis, while IBD, severity of disease, number of relapses, duration of illness or treatment other than systemic steroids were not.

CONCLUSION: IBD does not appear to be an independent risk factor for the occurrence of osteoporosis in this population. However, the use of systemic steroids was a risk factor.

Key words: Osteoporosis; Inflammatory bowel disease; Asians; Crohn's disease; Ulcerative colitis

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INTRODUCTION

The incidence of inflammatory bowel disease (IBD)

is rising in Asian populations[1]. IBD, both ulcerative colitis (UC) and Crohn's disease (CD), is a recognized risk factor for development of osteoporosis among Caucasians[2-4] but this association does not seem to have been investigated adequately in Asian populations[5].

Osteoporosis is usually diagnosed by dual energy X-ray absorptiometry (DEXA) scanning[6]. However, peripheral DEXA (pDEXA), quantitative computed tomography (QCT), radiographic absorptiometry, and ultrasound have become useful in community screening[7-9]. In the literature, the reported prevalence of osteoporosis/osteopenia in IBD varies from 7% to 56%[10,11]. A retrospective study of a Caucasian population showed a 40% increase in the risk of fracture compared to healthy controls[12]. CD seems to be associated with a slightly higher risk than UC does for osteoporosis and subsequent fractures, although this has been disputed in some studies[13,14]. The mechanism for development of osteoporosis in IBD patients seems to be multifactorial[15]. The slightly higher incidence of osteoporosis in CD could be attributed to the presence of ileal disease or small intestinal resection causing vitamin D malabsorption, malnutrition or estrogen deficiency[16]. Some studies have shown a genetic predisposition to osteoporosis in IBD patients[17]. The identified genes involve the pro-inflammatory cytokine interleukin-6[18,19]. It is important to identify IBD patients with osteoporosis, as treatment with bisphosphonates has been found to be effective[20,21].

There have been no large published studies regarding an association between osteoporosis and IBD in Asian populations[22]. It is important to investigate such an association because IBD among Asians seems to be genetically and phenotypically different to that in the West[23].

MATERIALS AND METHODS

Patients

Consecutive patients with previously diagnosed IBD from a single tertiary care center in Sri Lanka were eligible for inclusion in the study. IBD was diagnosed using standard criteria[24]. Inclusion criteria were age > 20 and < 70 years and the presence of IBD. Exclusion criteria were pregnancy; uncontrolled diabetes; renal, hepatic, cardiovascular or psychiatric disease; rheumatoid arthritis; ankylosing spondylitis; primary sclerosing cholangitis; or treatment with teriparatide, calcitonin, bisphosphonates, fluoride, androgens, anabolic steroids or active metabolites of vitamin D within the past 6 mo.

Controls

For each case, three age- (± 5 years) and sex-matched healthy controls were selected from among individuals who were selected randomly from the community for a large population study that screened for non-communicable diseases. The controls were screened for diabetes and were not taking active metabolites of vitamin D or calcium supplements.

Steroid use

Steroid use was defined as the continuous use of systemic steroids for > 3 mo. Others were considered steroid naïve.

Ethics

The study protocol was approved by the Ethics Committee of the Faculty of Medicine, University of Kelaniya. Written informed consent was obtained from all participants.

Study design

A comparative study involving IBD cases and age- and sex-matched community controls at ratio of 1:3.

DEXA scanning

Both cases and controls underwent pDEXA with the accuDEXA (ADXA-finger) (Schick, New York, NY, USA) using the right index finger. The bone mineral density (BMD) and *T* scores were recorded.

Diagnosis of osteoporosis and osteopenia

Osteoporosis and osteopenia were diagnosed using WHO criteria[25]. Osteoporosis was defined as a *T* score of -2.5 or below, while osteopenia was diagnosed with a *T* score between -1 to -2.49.

Statistical analysis

Previous studies have reported a 56% prevalence of osteoporosis among Caucasian IBD patients, and we assumed a 40% prevalence of osteoporosis among controls. We calculated that a sample size of 111 IBD patients and 333 controls was required to have 80% power to detect this difference at a significance level of 0.05. Quantitative data were compared using the *t* test, and categorical data were compared using a χ^2 test. Multiple logistic regression was used to identify independent risk factors for osteoporosis. The analysis was carried out using the statistical program SPSS version 16 (Chicago, IL, USA).

RESULTS

One hundred and eleven IBD patients [male:female = 43:68; mean age 42.5 years; 83 (74.8%) with UC, 28 (25.2%) with CD, and 333 age- and sex-matched healthy controls (male:female = 129:204; mean age 43.8 years) were recruited (Table 1). The site of disease was mainly proctitis for UC and colonic for CD (Table 2). Osteopenia was significantly more common among IBD patients (13.51%) than the controls (4.5%) (P = 0.001). Osteopoenia (T < -1) was also significantly more common in IBD patients than in controls (35.1% *vs* 22.5%, P = 0.008). The prevalence of osteoporosis was not significantly different between patients with UC (14.45%) and CD (10.71%) (P = 0.616). On bivariate analysis, age, female sex, menopause, presence of IBD, severity of disease and use of systemic steroids were found to be associated independently with the

Table 1 Characteristics of inflammatory bowel disease (IBD) patients and controls *n* (%), mean ± SD

	IBD patients (*n* = 111)	Controls (*n* = 333)	*P* value
Male:Female	43:68	129:204	1.000
Age (yr)	42.5 ± 14.19	43.8 ± 11.2	0.368
Postmenopausal women	29 (42.6)	83 (40.7)	0.778
Fractures	12 (10.8)	20 (6)	0.073
BMI (kg/m^2)	21.3 ± 4.45	23.8 ± 4.47	< 0.001
Disease duration (yr)	5.66 ± 5.72		
Corticosteroid use	74 (66.7)	3 (0.9)	< 0.001
Current smokers	4 (3.6)	29 (8.7)	0.094

Table 2 Disease location in IBD patients

	Frequency	Percent
Ulcerative		
Distal	46	56.8
Left sided	23	28.4
Total	12	14.8
Total	81	100.0
Crohn's disease		
Upper GI	2	6.7
Small bowel	4	13.3
Colon	21	70.0
Small bowel & colon	3	10.0
Total	30	100.0

Table 3 Summary of multiple logistic regression analyses

Variable	Regression coefficient	*P* value	OR (95% CI)
Constant	-7.478		
Age	0.78	0.001	1.081 (1.032-1.133)
Sex[1]			
Female (pre-menopausal)	0.898	0.213	2.456 (0.597-10.108)
Female (menopausal)	1.271	0.024	3.563 (1.179-10.763)
Using steroids[2]	2.082	< 0.001	8.021 (2.693-23.891)

[1]Comparison group is males; [2]Comparison group is not using steroids.

occurrence of osteoporosis. In the multivariate logistic regression model, age, sex, menopausal status and use of steroids were significant predictors of osteoporosis (Table 3). IBD was not a significant predictor of osteoporosis. With each advancing year of age, there was a 1.081 times increase in the likelihood of the development of osteoporosis. Premenopausal women were 2.5 times more likely to have osteoporosis than men, and menopausal women were 3.6 times more likely to have osteoporosis than men. Steroid use increased the risk of osteoporosis by eightfold.

DISCUSSION

The prevalence of osteoporosis and osteopenia in our IBD patients was significantly higher than in community controls. However, on multiple logistic regression analysis, only use of systemic steroids, age and menopause were found to be significant independent risk factors for osteoporosis. The presence of IBD and its severity were not, nor were the number of relapses, duration of illness, or treatment other than systemic steroids. The increased frequency of osteoporosis in our IBD patients was likely to have been caused by use of systemic steroids rather than by IBD itself. This is different to western studies, and we cannot explain this difference.

We did not find a significant difference in prevalence of osteoporosis between patients with UC and CD, although we admit that the number of CD patients in our sample was small, with mainly colonic involvement. This is in agreement with some but not all western data[26,27]. Our finding that there was no association between the occurrence of osteoporosis and severity of IBD, number of relapses, duration of illness, and treatment other than systemic steroids, agrees with the findings of Western studies[28,29].

We also noted a difference in the fracture risk between the two groups: 10.8% in the IBD group and 6% in the control group. However, this did not reach statistical significance, as our study was probably not adequately powered to investigate this complication. This finding is not surprising and could be attributed to steroid use as in western studies[30].

There are several methodological weaknesses in our study. We designed this as a comparative study rather than a case-control study, as that would have been difficult to perform in an Asian country where the prevalence of IBD is much lower than in the West. We also used pDEXA scanning instead of central DEXA to diagnose osteoporosis. However, although central DEXA scanning is accepted widely as the gold standard for diagnosis of osteoporosis, there have been many studies showing that pDEXA is a good alternative, especially in the community setting[7,8].

In conclusion, IBD does not appear to be an independent risk factor for the occurrence of osteoporosis in this population. The increased frequency of osteoporosis in our IBD patients is likely to be related to the use of systemic steroids. However, our finding that osteoporosis is more common in IBD patients, even though it may only be related to steroid use, has a direct bearing on patient management, as new guidelines advise the routine use of bisphosphonates in IBD patients with a BMD of < -1.5[31].

COMMENTS

Background

Inflammatory bowel disease (IBD) is a well-recognized risk factor for osteoporosis in Caucasian patients. However, there have been very few studies on Asian patients that have investigated this problem. To the best of our knowledge, there have been no studies on this topic in Southern Asians. However, since there are obvious genetic differences between the two populations it an important area of study that has been neglected.

Research frontiers

The genetics of IBD is a rapidly expanding field. To support this type of work, good phenotypic data from different cohorts of patients across continents are important. In studying osteoporosis, it is important to have similar data that will help in subsequent genetic studies.

Innovations and breakthroughs

In the present study, the authors showed that IBD was not an independent risk

factor for osteoporosis, but rather the use of systemic steroids was a risk factor for the development of osteoporosis.

Applications

It is important to know that not all Asian patients with IBD need routine bisphosphonates, as these are expensive drugs. This study will help to target whom to treat. Also, in future genetic studies, phenotypic racial differences will be important in the search for specific genes.

Terminology

Osteoporosis is a metabolic bone disease that is characterized by reduced bone mineral density. It is usually asymptomatic until it results in fractures. It is diagnosed using dual energy X-ray absorptiometry. IBD is a chronic disease of unknown etiology that comprises Crohn's disease and ulcerative colitis.

Peer review

This study dealt with the prevalence and risk factors of osteoporosis in adult Sri Lankan IBD patients. It is a well conceived and analyzed study.

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BRIEF ARTICLES

Assessment of the hepatic microvascular changes in liver cirrhosis by perfusion computed tomography

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Abstract

AIM: To assess the hepatic microvascular parameters in patients with liver cirrhosis by perfusion computed tomography (CT).

METHODS: Perfusion CT was performed in 29 patients without liver disease (control subjects) and 39 patients with liver cirrhosis, including 22 patients with compensated cirrhosis and 17 patients with decompensated cirrhosis, proved by clinical and laboratory parameters. CT cine-scans were obtained over 50 s beginning with the injection of 50 mL of contrast agent. Hepatic microvascular parameters, mean transit time (MTT) and permeability surface area product (PS) were obtained with the Perfusion 3 software (General Electric, ADW 4.2).

RESULTS: The overall differences of MTT and PS between control subjects, patients with compensated cirrhosis and those with decompensated cirrhosis were statistically significant (P = 0.010 and P = 0.002, respectively). MTT values were 15.613 ± 4.1746 s, 12.592 ± 4.7518 s, and 11.721 ± 4.5681 s for the three groups, respectively, while PS were 18.945 ± 7.2347 mL/min per 100 mL, 22.767 ± 8.3936 mL/min per 100 mL, and 28.735 ± 13.0654 mL/min per 100 mL. MTT in decompensated cirrhotic patients were significantly decreased compared to controls (P = 0.017), whereas PS values were remarkably increased (P = 0.001).

CONCLUSION: The hepatic microvascular changes in patients with liver cirrhosis can be quantitatively assessed by perfusion CT. Hepatic microvascular parameters (MTT and PS), as measured by perfusion CT, were significantly altered in decompensated cirrhosis.

Key words: Tomography; X-ray computed; Hepatic microcirculation; Cirrhosis

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INTRODUCTION

Mean transit time (MTT) and permeability surface area product (PS) of contrast material are two important parameters of perfusion computed tomography (CT). MTT is defined as the time that a contrast agent takes to go through the liver, from entry to exit and averaged over all possible paths. The PS is considered as the speed of transfer of a contrast agent from the capillary endothelium to the intercellular space (one-way). These parameters are, to a certain extent, two descriptors of parenchymal microvascular changes.

In chronic liver diseases, a capillarization of the sinusoids is observed, which is characterized by endothelial defenestration, collagen deposition in the extravascular Disse's space, formation of basal laminae[1-3], and increased intrahepatic vascular resistance[4,5]. All these microvascular changes in cirrhosis have great influence on the development of the hepatic function[6-8]. In order to evaluate the quantitative and qualitative microvascular alterations in liver cirrhosis, these hepatic perfusion parameters (both MTT and PS) were measured in patients with liver cirrhosis by perfusion CT and the results compared to those of healthy volunteers.

MATERIALS AND METHODS

Patients

Sixty-eight adult patients were studied, including 29 controls and 39 patients with cirrhosis (22 with compensated cirrhosis and 17 with decompensated cirrhosis, divided by two experienced physicians specialized in hepatology). Four additional patients with cirrhosis were excluded because of surgically confirmed hepatocellular carcinoma. Three cirrhotic patients were excluded due to a disagreement on the assignment to the compensated or decompensated cirrhosis group. The 29 controls without liver disease (8 women, 21 men; age range: 28-78 years; average age: 49.5 years) performed perfusion CT examination of the abdomen for unrelated causes. In these 29 controls, lack of liver disease was documented by history, physical examination, laboratory screening, and Doppler sonography of the liver. Twenty-two patients (4 women, 18 men; age range: 31-78 years; average age: 58.6 years) had compensated cirrhosis. Among them, one had alcoholic cirrhosis, two had primary biliary cirrhosis, three had cryptogenic cirrhosis and 16 had posthepatitic cirrhosis. Seventeen patients (5 women, 12 men; age range: 31-78 years; average age: 55.9 years) had decompensated cirrhosis. The origin of cirrhosis in these cases was alcohol in one, hepatitis C virus in one, cryptogenic in one, primary biliary cirrhosis in one, and hepatitis B in the remaining 13 patients. All diagnoses were confirmed by appropriate clinical and laboratory examinations, No patient had portal thrombosis at ultrasonography. The study was approved by the ethics committee at our institution and was in conformity with the ethical guidelines of the 1975 Declaration of Helsinki[9]. The patients gave informed consent to participate in the study.

Imaging

After an overnight fast, multiple-slices dynamic sequences were carried out. A fixed 5 mm thick slice, which was selected to include the right hepatic lobe, spleen and portal trunk, was repeatedly scanned with cine mode. Scanning was carried out using a low radiation dose (120 kV, 60 mA), cine-scan mode, standard reconstruction algorithm, 35 cm display field of view (LightSpeed RT, GE Medical Systems, Milwaukee, WI) with a 50 s of continuous scanning time set at 8 s after the injection of contrast material. A bolus infusion of 50 mL of contrast material [Omnipaque (iohexol); 350 mg I/mL; Beijing, China] was given at 5.5 mL/s *via* a 20 gauge intravenous catheter in the antecubital vein. Patients were advised to hold their breath as long as possible, and oxygen inhalation at 2 L/min was provided to facilitate long breathholding during scanning.

Data analysis

These images were transferred to a workstation (GE Advantage workstation 4.2) for data analysis by using Perfusion 3 software. Three regions of interest (ROI) were set on the abdominal aorta, the portal vein trunk, and the right liver lobe respectively, by two radiologists with 10 years of experience in gastrointestinal and hepatobiliary imaging. The former two ROIs were set as input function. The latter ROI of the right liver lobe was drawn on the whole visible right lobe carefully, avoiding blood vessels and margin of liver parenchyma. The value of the latter ROI was measured and averaged for the hepatic perfusion parameters.

Statistical analysis

The results of the two perfusion parameters (MTT, PS) were expressed as the mean ± SD. All data were analyzed by SPSS 11.5 software. The independent samples *t* test was used to determine differences between cirrhotics and controls. Data of the three groups (normal control subjects, patients with compensated cirrhosis, and patients with decompensated cirrhosis) were compared using one-way analysis of variance. Bonferroni's correction was applied. Statistical significance was defined as a $P < 0.05$.

RESULTS

Differences between the controls and the cirrhotics

The results summarizing the MTT and PS of the controls and the cirrhotics are shown in Figure 1 and Table 1. The MTT in cirrhotic livers (12.212 ± 4.632 s) was significantly shorter than in controls subjects (15.613 ± 3.942 s), the difference being statistically significant ($P = 0.002$). PS was increased in cirrhosis compared with controls (24.964 ± 8.298 against 18.945 ± 7.235 mL/min per 100 mL), and it was also significant ($P = 0.003$).

Differences among the three groups

The hepatic perfusion parameters for the control subjects group, compensated cirrhosis group, and decompensated cirrhosis group are shown in Figure 2 and Table 2. Compared with the control subjects, the MTT of patients with compensated cirrhosis were decreased (12.592 ± 4.7518 s *vs* 15.613 ± 3.942 s), and it diminished further in decompensated cirrhosis patients (11.721 ± 4.568 s) compared to compensated patients. The difference between controls and compensated cirrhotics was not significant ($P = 0.059$), nor was the difference between compensated and decompensated cirrhotics ($P = 1.000$). However, there was a markedly significantly difference between the control subjects and the decompensated cirrhotic patients ($P = 0.017$). One of the subjects, a 75 years old individual, had a MTT of 27.7 s, the highest value observed in the whole study. Another patient, with posthepatitic decompensated cirrhosis, had a rather high MTT value of 22.3 s. Other two patients in the compensated cirrhosis group showed much lower values (3.32 s and 3.79 s). The results of the PS were 18.945 ± 7.235 mL/min per 100 mL, 22.767 ± 8.3936 mL/min per 100 mL and 27.806 ± 7.4730 mL/min per 100 mL in the three groups (controls, compensated cirrhotics and decompensated cirrhotics, respectively). The PS in compensated cirrhotic patients did not show statistically significant difference compared to control subjects ($P = 0.25$), nor was significant the

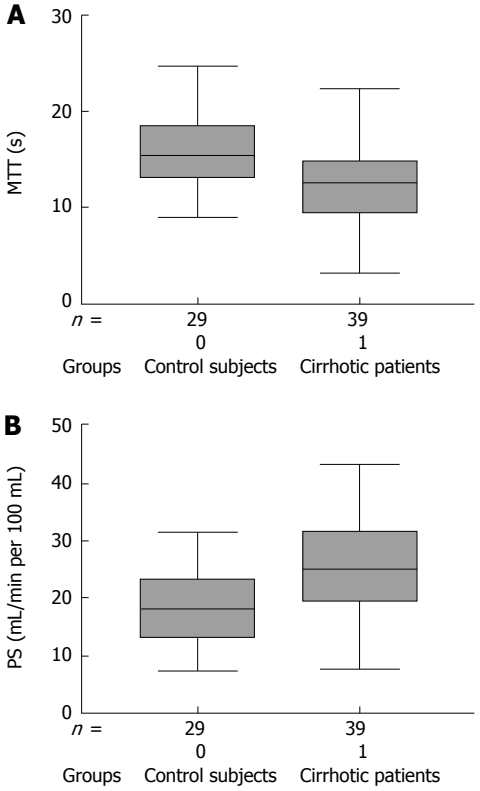

Figure 1 Distribution of hepatic microvascular parameters in controls and cirrhotics. Box-whisker plots are shown, in which the lower box boundary indicates the 25th percentile, the line within the box marks the median and the top box boundary indicates the 75th percentile. Error bars below and above the boxes indicate 10th and 90th percentiles. Other data are represented as individual dots. The graphs show box-whisker plots of mean transit time (MTT) (A) and permeability surface area product (PS) (B). *n*: Number of patients.

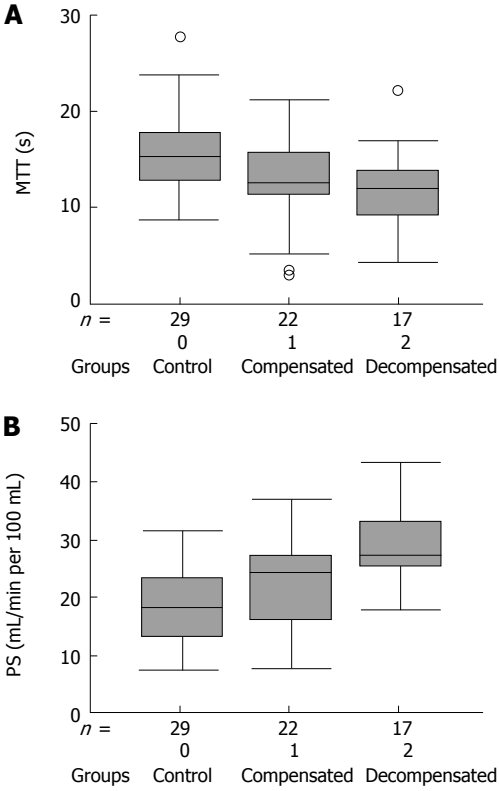

Figure 2 The box-whisker plots show the distribution of hepatic microvacular parameters in controls as well as in compensated and decompensated cirrhosis patients. The values of MTT (A) and PS (B) are shown.

Table 1 Correlations between control subjects and cirrhotic patients on perfusion parameters (mean ± SD)

Parameters	Controls	Cirrhotics	*t* value	*P* value
MTT (s)	15.613 ± 3.942	12.212 ± 4.632	3.186	0.002
PS (mL/min per 100 mL)	18.945 ± 7.235	24.964 ± 8.298	-3.121	0.003

Mean transit time (MTT) in cirrhotics was shorter than in the controls (P = 0.002). Permeability surface area product (PS) was higher in cirrhotics than in controls (P = 0.003).

Table 2 Comparison among control subjects and compensated and decompensated cirrhosis patients on perfusion parameters (mean ± SD)

Group	*n*	MTT (s)	PS (mL/min per 100 mL)
Control	29	15.613 ± 4.175	18.945 ± 7.235
Compensated	22	12.592 ± 4.752	22.767 ± 8.394
Decompensated	17	11.721 ± 4.568	27.806 ± 7.473

MTT: Controls *vs* compensated, P = 0.059; controls *vs* decompensated, P = 0.017; compensated *vs* decompensated, P = 1.000. PS: Controls *vs* compensated, P = 0.25; controls *vs* decompensated, P = 0.001; compensated *vs* decompensated, P = 0.139.

difference between compensated and decompensated cirrhotics (P = 0.139). However, compared with controls, the PS of decompensated cirrhotic patients increased remarkably, the difference reaching statistical significance (P = 0.001).

DISCUSSION

A simple, noninvasive technique to separately quantify the changes of arterial and portal venous components in cirrhosis has always been a long-standing ambition of both pathologists and physicians. Most authors have reached an agreement on the notion that the increase of intrahepatic vascular resistance leads to a decrease of the portal fraction of liver perfusion[4,5]. This decreased portal perfusion is partially compensated by an increase of arterial flow, with the total liver perfusion being reduced in cirrhotic patients[4,10,11]. More importantly, since the changes of the hepatic microcirculation in cirrhosis influence the progression of the disease, such a technique would be of greater value in hepatology. Various methods exist for the determination of hepatic microcirculation in clinical practice[12-15]. Most of them, however, are invasive or of controversial significance[12,16]. It's possible to measure both arterial and portal venous flow at the level of the main vessels using Doppler sonography, but this gives an indirect measurement of the circulation at parenchymal level. It is much harder to assess alterations in capillary blood, i.e. at the level of the microcirculation. Nuclear medicine techniques have been hindered by limited spatial and temporal resolution, and used only to estimate the arterial relative to the portal venous blood flow[17-19], since they can hardly differentiate the overlapping fractions of hepatic artery and portal vein perfusion within the liver tissue.

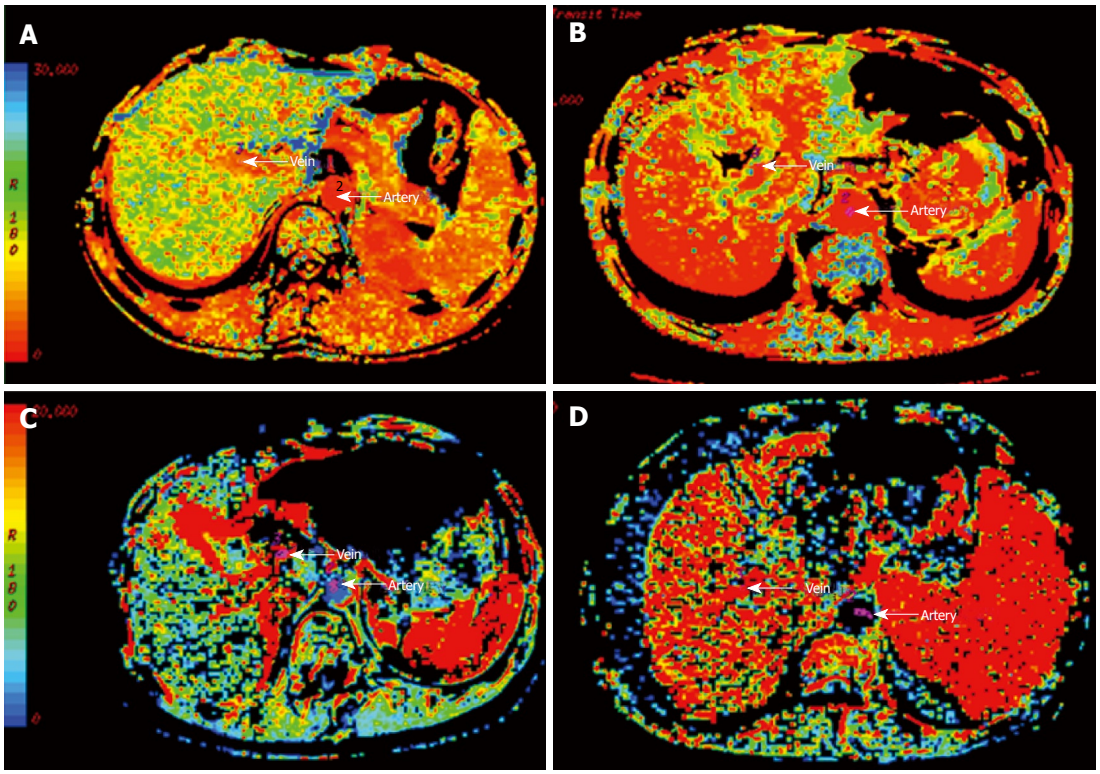

Figure 3 MTT and PS maps between controls and cirrhotic patients. MTT in a control subject (A) was higher than in a cirrhotic patient (B). PS in a control subject (C) was lower than in a cirrhotic patient (D).

Reports have shown that the hepatic microcirculation can be estimated by the hepatic clearance of sorbitol[8,13] due to its high extraction fraction in normal subjects. The clearance of sorbitol is flow-limited and reflects hepatic microcirculation through functional sinusoids. However, sorbitol has been used as a marker of functional perfusion, which raises the issue of the decrease of the hepatocyte extraction of sorbitol in cirrhosis[20]. Moreover, the sorbitol clearance method cannot separate the measurements of arterial and portal flows. In addition, it needs to collect urine to determine the renal clearance of sorbitol after several hours, which may cause logistic problems in a busy clinical department[20].

CT is suited for the measurement of perfusion. Because of the high spatial and temporal resolution, modern CT scanners have been used widely in clinical studies, diagnosis and treatment. The methods for quantifying tissue perfusion by dynamic CT were described previously[14,21]. Many studies have been conducted using this similar method with the use of iodinated contrast agents[5,22,23]. This technique measures the slope of the time-attenuation curve, and only the peak time-points of the aortic and portal time-attenuation curves have been applied. The procedure can quantify the arterial and portal venous blood flow at tissue level, but the use of single-slice CT hampers its limited temporal resolution and Z-axis spatial resolution[22,24,25]. Some respiratory motion artifacts are unavoidable, and they considerably influence the quantification. We used here a 16 slices spiral-CT with super high Z-axis spatial resolution and temporal resolution, which avoided the motion artifacts in an efficient way. The perfusion parameters were measured by the deconvolution method, which uses a well-established compartmental modeling technique[26]. Several points of the time-attenuation curves were used. Arterial and portal venous perfusion was detected quantitatively. In particular, the MTT and the PS could be quantified. These parameters were important and critical to assess the function of liver, as shown by many studies in humans and animals.

The MTT mainly reflects the blood capillary's time. Some early studies almost exclusively depended on *ex vivo* evaluations using the same multiple indicator dilution technique described previously[8,13]. A study[25] on MTT had already relied upon CT, and its results were in agreement with the findings of the indicator dilution techniques. These investigators observed that the small-molecular-weight contrast agent used in their study had an increased transit time in cirrhotic patients. Some other studies[27,28] reported no difference in MTT between controls and cirrhotic patients. In the present study, however, the MTT values observed in control subjects (Figure 3A) were higher than in cirrhotic patients (Figure 3B). The MTT of contrast agent measured in the cirrhotic livers was significantly shorter than among controls ($P = 0.002$) (Figure 1A). He *et al*[29] had already reported similar results, but the difference was not statistically significant. We hypothesize that there may be some differences as to etiopathogenesis, disease severity and gender distribution among the cirrhotic patients in previous studies[5,30,31]. The patients enrolled in our study (women/men: 9/30) had a cirrhosis mostly

due to hepatitis B virus (29/39) and only few had alcoholic cirrhosis (2/39), whereas no patients with hepatitis B virus infection and nine alcoholics of 18 cirrhotic patients (women/men: 6/12) were included in a previous study[25]. Histopathological examinations have shown that in the cirrhotic liver several sinusoids become capillarized and many terminal hepatic venules are distended[32,33]: the combination of these changes may lead to low-resistivity, high-speed vascular flow, with increased inflow and outflow. In addition, intrahepatic portal veins and hepatic veins cross cirrhotic areas, leading directly or indirectly into the central venous compartment. All of the above causes may have brought about the shorter MTT in cirrhotic patients.

The PS shows the permeability of blood capillaries. As shown in this study, PS may be quantified noninvasively using perfusion CT scans acquired from control subjects and patients with cirrhosis. We observed that the PS of controls (Figure 3C) was lower than in cirrhotic patients (Figure 3D): PS was in fact significantly increased in cirrhosis ($P = 0.003$), (Figure 1B). This change may be explained by the results obtained with multiple indicator dilution techniques in human cirrhosis or rat cirrhotic liver. The increased microvascular resistance in the cirrhotic liver reduces capillary blood flow; the reduction in capillary blood flow is compensated by liver arterialization, increasing the capillary perfusion[4,10,11].

In addition, we observed that MTT and PS measured by the perfusion CT tended to change significantly in patients with decompensated cirrhosis (Table 2). Compared to controls, the MTT of patients with compensated cirrhosis was decreased, while the PS was increased: however, these differences failed to reach a statistically significant level in our study. We cannot exclude that some microvascular changes might indeed occur also in cases with compensated cirrhosis (Figure 2), and that the differences between compensated cirrhotic patients and the other groups may become significant simply by increasing the size of the study population. In contrast, the microvascular perfusion parameters (both MTT and PS) were significantly altered in decompensated cirrhosis.

However, because it was difficult to control all the aspects of the study, there are several limitations associated with this study. First, the radiation dose may be one of the most important issues when using the perfusion CT, especially with cine-scan mode. Secondly, during CT imaging, patients are requested to hold their breath for long periods of time. Besides, a further limitation of our study is the small overall sample size. Perhaps, the differences between compensated cirrhosis group and the other groups would turn significant if the overall sample size increased.

Despite these limitations, our study with perfusion CT has proven that the perfusion parameters (MTT and PS) are significantly changed in patients with cirrhosis. The decreased MTT and increased PS of an iodinated contrast agent correlate with the severity of liver cirrhosis. They may be two acceptable indicators of the degree of hepatic microcirculation alteration in patients with cirrhosis. In addition, the quantification by perfusion CT may also improve our understanding of the effects on the hepatic microcirculation of vasoactive drugs and interventional procedures for the treatment of cirrhosis[34-36].

These findings underscore the possibility of using perfusion CT as a marker of hepatic microcirculation. Perfusion CT may be used as a noninvasive tool to quantify hepatic microvascular parameters in cirrhotic liver.

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COMMENTS

Background

Hepatic microcirculation is of great importance in hepatology. Its changes in cirrhosis may heavily influence the progression of the disease. Various methods exist for the determination of hepatic microcirculation in clinical practice. However, most of them are invasive or controversial.

Research frontiers

Mean transit time (MTT) and permeability surface (PS) area product of contrast material for quantifying hepatic vascular changes by dynamic computed tomography (CT) were rarely measured in previous studies. The research hotspot is whether hepatic microvascular changes can be quantified by perfusion CT and what kind of modifications can be measured.

Innovations and breakthroughs

Previous applications of perfusion CT to the cirrhotic liver focused on hepatic haemodynamics. Most studies have reached an agreement on the fact that the increase of intrahepatic vascular resistance leads to a decrease of the portal fraction of liver perfusion. This decreased portal perfusion is partially compensated by an increase of arterial inflow, while the total liver perfusion is reduced in cirrhotic patients. However, hepatic microvascular changes were ignored. Few studies on MTT and PS have already relied upon CT. In order to explore hepatic microvascular parameters in patients with liver cirrhosis by using the perfusion CT, the authors compared hepatic microvascular parameters in patients with cirrhosis with those found in healthy volunteers. MTT and PS were significantly altered in liver cirrhosis. In order to obtain some meaningful correlations of the MTT and PS values with the severity of cirrhosis, they measured these parameters in three groups: control subjects, and patients with compensated or decompensated cirrhosis. They found that these parameters were significantly altered in patients with decompensated cirrhosis.

Applications

Hepatic microvascular parameters may be used to quantitatively assess hepatic microcirculation: the findings correlate with the severity of liver cirrhosis. These data may improve our understanding of the hepatic microcirculation effects of vasoactive drugs and interventional procedures for the treatment of cirrhosis.

Terminology

MTT: Mean transit time, a parameter defined as the time that a contrast agent takes to cross the liver, from entry to exit, averaged across all possible paths. PS: Permeability surface area product, which is considered as the speed of a contrast agent from the capillary endothelium to the intercellular space (one-way).

Peer review

The authors examined the differences in MTT and PS between normal individuals and patients with cirrhosis; they propose that perfusion CT may be utilized to quantify changes in microcirculation among stages of cirrhosis. Overall, an interesting study.

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BRIEF ARTICLES

Urokinase perfusion prevents intrahepatic ischemic-type biliary lesion in donor livers

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Abstract

AIM: To evaluate whether urokinase perfusion of non-heart-beating cadaveric donor livers reduces the incidence of intrahepatic ischemic-type biliary lesions (IITBLs).

METHODS: A prospective study was conducted to investigate potential microthrombosis in biliary microcirculation when non-heart-beating cadaveric livers were under warm or cold ischemic conditions. The experimental group included 140 patients who underwent liver transplantation during the period of January 2006 to December 2007, and survived for more than 1 year. The control group included 220 patients who received liver transplantation between July 1999 and December 2005 and survived for more than 1 year. In the experimental group, the arterial system of the donor liver was perfused twice with urokinase during cold perfusion and after trimming of the donor liver. The incidence of IITBLs was compared between the two groups.

RESULTS: In the control group, the incidence of IITBLs was 5.9% (13/220 cases) after 3-11 mo of transplantation. In the experimental group, two recipients (1.4%) developed IITBLs at 3 and 6 mo after transplantation, respectively. The difference in the incidence between the two groups was statistically significant ($P < 0.05$).

CONCLUSION: Double perfusion of cadaveric livers from non-heart-beating donors with urokinase may reduce the incidence of IITBLs.

Key words: Cadaver donor; Liver transplantation; Ischemic-type biliary lesion; Urokinase

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INTRODUCTION

Intrahepatic ischemic-type biliary lesions (IITBLs) after orthotopic liver transplantation are the most frequent cause of non-anastomotic biliary strictures in liver grafts[1-4]. They affect 2%-19% of patients after liver transplantation and have become a leading cause of liver re-transplantation in China[5,6]. Several risk factors for IITBLs have been identified, including ischemia-related injury, immunologically induced injury, and cytotoxic injury[7-9]. Although IITBLs have a multifactorial origin, ischemia-reperfusion injury and hepatic arterial thrombosis are considered to be the major causes[10-12]. In recent years, however, impaired biliary microcirculation has led to an increasing concern. Improving microcirculation can increase oxygen supply and might prevent biliary injuries. Urokinase is a proteolytic enzyme produced by the kidney, which is found in the urine. Urokinase acts on the endogenous fibrinolytic system by converting plasminogen to plasmin, which, in return, degrades fibrin clots. Since January 2006, our center has used a procedure based on the therapeutic principle of urokinase to prevent potential microthrombosis in biliary microcirculation. We have used non-heart-beating cadaveric donor grafts in which warm or cold ischemic insult was induced and which were perfused with urokinase; this procedure has produced good results.

In the present study, we evaluated prospectively 140 liver transplantation patients who received grafts with urokinase perfusion.

MATERIALS AND METHODS

Ethics

This study was approved ethically by Beijing Chaoyang Hospital. All patients provided written consent. All donors were volunteers and signed consent documents before donation.

Clinical data

Between July 1999 and December 2005, 220 patients (176 male, 44 female; age, 14-71 years) received orthotopic liver transplantation in the Department of Hepatobiliary Surgery, Beijing Chaoyang Hospital, which is affiliated to Capital Medical University (Beijing, China). All patients survived for more than 1 year. They received ABO-compatible non-heart-beating cadaveric donor livers without urokinase perfusion. The arterial system of the donor livers was perfused with 2000 mL cold histidine-tryptophan-ketoglutarate (HTK) solution (4℃). The portal system was perfused with 2000 mL HTK solution, followed by 2000 mL University of Wisconsin (UW) solution for preservation (4℃). The biliary system was decompressed using the trocar technique. The common bile duct was perfused with normal saline at low pressure. The warm ischemia time for the donor livers was 2-8 min, and the cold preservation time was 2-13.5 h. The secondary warm ischemia for the biliary tract lasted 25-90 min. Primary diseases of the patients included posthepatitic cirrhosis in 148, primary hepatic carcinoma in 56, alcoholic cirrhosis in seven, fulminant hepatitis in four, hepatolenticular degeneration in three, and primary sclerosing cholangitis in two.

Since January 2006, all patients received donor livers with urokinase perfusion. Until December 2007, 140 patients (108 male, 32 female; age, 16-69 years) underwent liver transplantation and survived for more than 1 year. We used 2000 mL HTK solution that contained 2 MU urokinase for perfusion through the arterial system. After trimming of the donor liver, the arterial system was perfused again with 1 MU urokinase. The residual urokinase was washed out using 500 mL HTK solution before implantation. The warm ischemia time for donor livers was 2-10 min, and the cold preservation time was 2.5-15 h. The secondary warm ischemia for the biliary tract lasted 20-115 min. Primary diseases of the recipients included posthepatitic cirrhosis in 96, primary hepatic carcinoma in 34, alcoholic cirrhosis in three, fulminant hepatitis in two, hepatolenticular degeneration in two, primary sclerosing cholangitis in two, and autoimmune liver disease in one.

The immunosuppressive regimen for all the patients included cyclosporin A (CsA) (Novartis, Switzerland) or Prograf (FK506) (Astellas, Ireland), mycophenolate mofetil (CellCept; Roche, United States), and steroids. The trough CsA level was maintained at 200-300 μg/L and that of FK506 was maintained at 8-12 μg/L at 3 mo after operation. We compared the incidence of IITBLs in the patients who received grafts with or without donor urokinase perfusion.

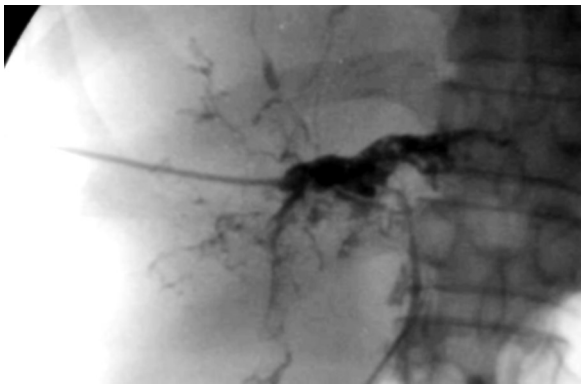

Figure 1 Percutaneous transhepatic cholangiography shows intrahepatic ITBL. Variant degrees of diffuse stricture and segmental dilatation of the intrahepatic bile duct in the biliary tree.

Statistical analysis

The difference between the two groups of patients was analyzed by a χ^2 test. $P < 0.05$ was considered statistically significant. Analysis was performed using SPSS version 11.5 (Chicago, IL, USA).

RESULTS

The incidence of IITBLs was 5.9% in patients who received grafts without urokinase perfusion (13/220 patients) 3-11 mo (mean, 5.1 mo) after transplantation, and 1.4% in the patients who received grafts with urokinase perfusion (2/140 patients) at 3 and 6 mo after transplantation. The difference in the incidence between the two groups was statistically significant ($P < 0.05$). The main symptom noted in the 15 IITBL patients in both groups was progressive hyperbilirubinemia. Cholangitis was observed as a complication in three of the 15 patients. The results of a biochemical assay showed that the level of total bilirubin increased progressively. The level of direct-reacting bilirubin increased the most, followed by the level of biliary enzymes such as alkaline phosphatase and γ-glutamyltransferase. A slight increase in the levels of aspartate aminotransferase and alanine aminotransferase levels was detected.

Of the 13 IITBL patients who received grafts without urokinase perfusion, four were diagnosed with IITBLs by T-tube cholangiography; two by percutaneous transhepatic cholangiography; four by endoscopic retrograde cholangiography; and three by magnetic resonance cholangiography (MRCP). In the two IITBL patients who received grafts with urokinase perfusion, IITBLs were diagnosed by MRCP. The imaging of the 15 IITBL patients showed varying degrees of diffuse stricture and segmental dilatation of the intrahepatic bile duct, with withered-branch-like changes in the biliary tree (Figure 1). The stricture or embolic lesion of the hepatic artery and its major branches was excluded by Doppler ultrasonography, and acute or chronic rejections were excluded by liver biopsy.

Eight of 13 IITBL patients who received grafts without urokinase perfusion required liver re-trans-

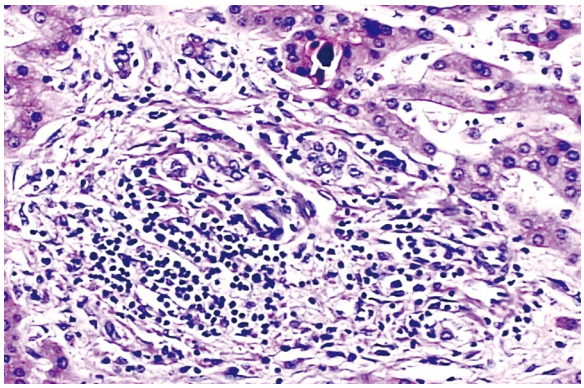

Figure 2 Cholestasis of hepatocytes, infiltration of inflammatory cells and proliferation of fibrous tissue in the portal area, fibrosis and luminal stenosis of bile ducts. (HE stain, × 200).

plantation, and the other five patients died from liver failure while still on the waiting list. The two IITBL patients who received grafts with urokinase perfusion underwent liver re-transplantation. The injured grafts were removed from the 10 re-transplantation patients and observed pathologically. Gross findings showed patchy necrosis of the biliary endomembrane, thickened and sclerosed duct wall, and biliary sludge in the narrowed lumen. Microscopic findings showed severe cholestasis of hepatocytes, spotty or patchy necrosis, infiltration of inflammatory cells and mild to moderate proliferation of fibrous tissue in the portal area, necrosis and exfoliation of a great number of endothelial cells in small biliary lumens, formation of bile thrombus, and fibrosis and luminal stenosis of some bile ducts (Figure 2).

DISCUSSION

IITBLs have become a leading cause of liver re-transplantation in China[13,14]. This is closely associated with the fact that non-heart-beating cadaveric livers constitute a major source of donor livers. Several studies conducted in China and other countries have suggested that cadaveric donor grafts with warm ischemic injury leads to a higher incidence of IITBLs than grafts from brain-dead donors. Abt *et al*[15] have reported that the ratio of IITBLs in biliary complications was 66.6% with non-heart-beating cadaveric livers and 19.2% with livers from brain-dead donors. Nakamura *et al*[16] have reported that the incidence of IITBLs was only 1.4% in recipients grafted with brain-dead donor livers. Zhang *et al*[17] have reported that in 235 patients transplanted with cadaveric livers and 36 transplanted with living-donor livers, the incidence of biliary complications was 19.1% and 5.6%, respectively. The incidence of IITBLs was 7.29% and 0%, respectively, and the difference was statistically significant. Researchers in China and other countries have agreed that the difference was mainly caused by warm ischemia.

What kinds of pathological or physiological changes associated with IITBLs may occur in a donor liver during warm ischemia? Generally, the initiation of intrinsic coagulation requires stoppage or slowing of blood flow, high blood viscosity, and injury of vascular endothelial cells. After these three requirements are met, most non-heart-beating cadaveric donors are usually in a hypercoagulable state during warm ischemia. Thus, blood coagulation or microthrombi may develop rapidly in the arterioles and peribiliary capillary plexus of the donor liver within a short period. The blood clots or microthrombi are not washed out easily by subsequent cold perfusion under normal pressure, which results in inadequate perfusion of the peribiliary capillary plexus. Even after blood circulation in the donor liver returns to normal, the arterioles and peribiliary capillary plexus still lack perfusion with arterial blood. This may lead to ischemia and hypoxia of the bile duct wall; formation of biliary sludge caused by degeneration, necrosis or even exfoliation of a great number of endothelial cells; and luminal stenosis caused by gradual fibrosis of the muscular layers. By periodically sampling livers from rats dying suddenly from cardiac arrest for light microscopy, we observed blood coagulation in most arterioles in the portal area in hepatic tissue samples collected after 25 min of cardiac arrest. For the reasons described above, we adopted the strategy of double perfusion with urokinase, which aimed to achieve maximum dissolution of microthrombi, ensure better effects of cold perfusion and reperfusion, and prevent the development of IITBLs. In the present study, we found that the difference between the two groups was significant, which indicated that urokinase perfusion can effectively reduce the incidence of IITBLs.

The *in vitro* use of urokinase may not have any effect on the coagulation function of patients and is not dose-limited. Thus, urokinase usually can be administered *in vitro* at a higher concentration than when injected intravenously. For better blood circulation in arterioles and the peribiliary capillary plexus, a higher concentration of urokinase could be used to reperfuse the liver after trimming. A urokinase-free preservation solution should be used to perfuse the artery to wash out excess urokinase before completing the whole perfusion process. The activity and function of urokinase at 0-4℃ have not been reported yet. In separate tests, a significant thrombolytic effect was observed when fresh blood clots in tubes at 4℃ were immersed in urokinase at the above-mentioned concentrations.

The results of the present study confirm that: (1) liver transplantation from non-heart-beating cadaveric donors may lead to a higher incidence of IITBLs and a higher rate of re-transplantation; and (2) double perfusion of cadaveric livers from non-heart-beating donors with urokinase may reduce the incidence of IITBLs.

COMMENTS

Background

Biliary complications are a major cause of morbidity and graft failure in patients after orthotopic liver transplantation (OLT). The most troublesome is the so-called intrahepatic ischemic-type biliary lesion (IITBL), which is one of the most important reasons for liver re-transplantation. Therefore, it is clinical significance to reduce the incidence of IITBLs in order to decrease the re-transplantation rate and improve long-term life quality.

Research frontiers

The incidence of IITBL varies between 2% and 19% after OLT. Although the exact pathophysiological mechanism of IITBL is still unknown, several risk factors of this often cumbersome complication have been identified, strongly suggesting a multi-factorial origin. Therefore, the etiology, development and prophylaxis of IITBL have been hot topics of research.

Innovations and breakthroughs

Improving biliary microcirculation might prevent biliary injury. In this study, urokinase was perfused through the arterial system during harvesting and after trimming of donor liver in non-heart-beating cadaveric donors. This procedure has produced good results and provides the possibility of reducing IITBLs after OLT.

Applications

This study may provide a method for clinical research into the prophylaxis of IITBLs after liver transplantation. This technique for prevention of IITBLs is easy to establish, and the results of this study confirm that urokinase perfusion may reduce the incidence of IITBLs from non-heart-beating cadaveric donors.

Terminology

ITBL is defined as non-anastomotic destruction of the graft's biliary tree after OLT, and is characterized by bile duct destruction, subsequent stricture formation, and sequestration.

Peer review

This is a good descriptive study in which the authors analyze the preventive effect of urokinase perfusion on IITBLs in non-heart-beating donors after OLT. The results are interesting and the conclusion is encouraging.

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BRIEF ARTICLES

Clinical application of subjective global assessment in Chinese patients with gastrointestinal cancer

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Abstract

AIM: To investigate the role of subjective global assessment (SGA) in nutritional assessment and outcome prediction of Chinese patients with gastrointestinal cancer.

METHODS: A total of 751 patients diagnosed with gastrointestinal cancer between August 2004 and August 2006 were enrolled in this study. Within 72 h after admission, SGA, anthropometric parameters, and laboratory tests were used to assess the nutritional status of each patient. The outcome variables including hospital stay, complications, and in-hospital medical expenditure were also obtained.

RESULTS: Based on the results of SGA, 389 (51.8%), 332 (44.2%), and 30 (4.0%) patients were classified into well nourished group (SGA-A), mildly to moderately malnourished group (SGA-B), and severely malnourished group (SGA-C), respectively. The prevalence of malnutrition classified by SGA, triceps skinfold thickness (TSF), mid-upper arm muscle circumference (MAMC), albumin (ALB), prealbumin (PA), and body mass index (BMI) was 48.2%, 39.4%, 37.7%, 31.3%, 21.7%, and 9.6%, respectively. In addition, ANOVA tests revealed significant differences in body mass index (BMI), TSF, PA, and ALB of patients in different SGA groups. The more severely malnourished the patient was, the lower the levels of BMI, TSF, PA, and ALB were ($P < 0.05$). χ^2 tests showed a significant difference in SGA classification between patients receiving different types of treatment (surgery *vs* chemotherapy/radiotherapy). As the nutritional status classified by SGA deteriorated, the patients stayed longer in hospital and their medical expenditures increased significantly. Furthermore, multiple regression analysis showed that SGA and serum ALB could help predict the medical expenditures and hospital stay of patients undergoing surgery. The occurrence of complications increased in parallel with the increasing grade of SGA, and was the highest in the SGA-C group (23.3%) and the lowest in the SGA-A group (16.8%).

CONCLUSION: SGA is a reliable assessment tool and helps to predict the hospital stay and medical expenditures of Chinese surgical gastrointestinal cancer patients.

Key words: Gastrointestinal cancer; Subjective global assessment; Surgery; Nutritional assessment; Hospital stay; Medical expenditures; Complication

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INTRODUCTION

Cancer, one of the serious global health problems today, is considered by the public as a frightening, painful, and untreatable disease that implies death. Approximately 10 million people get cancer and 5 million people face death every year throughout the world. It is estimated that the number of new cancer patients will reach 15 million in 2020[1,2]. It was reported about 20% cancer patients die of malnutrition or its relative complications rather than the malignant disease itself[3]. Many researchers have suggested that the nutritional status of cancer patients after diagnosis is associated with cancer recurrence and survival rate[4-6], and is generally accepted as an important prognostic factor that determines patients' outcomes including treatment response, survival, and hospital stay[7-13]. Furthermore, some studies showed that good nutrition in patients with cancer can improve their quality of life[14-16]. The objective of nutritional assessment is to accurately define the nutritional status of patients, diagnose clinically relevant malnutrition, and monitor changes in nutritional status. Comprehensive and accurate information on nutritional status of patients with gastrointestinal cancer helps decide whether surgery or chemotherapy can be delayed. A number of tools have been developed for the assessment of nutritional status[17].

Subjective global assessment (SGA) is an easy, noninvasive, and cost-effective method for the assessment of nutritional status by identifying whether the patients are malnourished or at a risk of becoming malnourished[18]. Although SGA has been originally developed to identify poor nutritional status in patients undergoing gastrointestinal surgery[19], it can be used to quantify the prevalence of malnutrition in patients with chronic and end-stage renal failure during hemodialysis or peritoneal dialysis[20-22]. In addition, SGA is a powerful predictor of postoperative complications in general surgery[23], liver transplantation[24], and in patients on dialysis[25]. Although SGA has been used widely for more than two decades all over the world, few studies are available on its clinical value in Chinese cancer patients. This study was to investigate whether SGA can reliably identify malnourished patients and predict the clinical outcomes of Chinese gastrointestinal cancer patients.

MATERIALS AND METHODS

Ethics

This study was approved by the relevant research board and the ethics committee in Shanghai, China. All patients gave their informed consent to participate in this study.

Patients

Adult patients diagnosed with gastrointestinal cancer (including stomach, colon, or rectal cancer) from August 2004 to August 2006 were enrolled in this study. Eligibility criteria included (a) patients diagnosed by pathology or cytology, (b) patients scheduled to undergo treatment modalities including radiotherapy or chemotherapy or surgery, (c) patients older than 18 years, (d) patients able to read and comprehend Chinese, and (e) patients giving their informed consent. Patients with cognitive impairment, mental disorder, or communication problems were excluded from this study. The final number of recruited subjects was 751 (including 591 newly diagnosed and 160 previously diagnosed cancer patients). Of them, 384 (51.1%) were gastric cancer patients, 367 (48.9%) were colorectal cancer patients. The male/female ratio was 455/296 with a median age of 69 years (range 23-92 years). Of the 591 newly diagnosed cancer patients, 505 underwent surgery and 86 underwent chemotherapy or radiotherapy due to tumor metastasis, while the 160 previously diagnosed cancer patients received radiotherapy or chemotherapy during their hospital stay.

Nutritional assessment

An initial assessment of nutritional status in all recruited patients was made within 72 h after admission. To avoid possible variance among observers, SGA was performed by trained researchers. Anthropometric data including body weight, height, triceps skinfold thickness (TSF), mid-upper arm circumference (MUAC), and laboratory data including albumin (ALB) and prealbumin (PA) were collected.

Subjective global assessment

SGA of nutritional status in patients was performed based on their medical history and physical examination. Changes in weight, dietary intake, functional capacity, gastrointestinal symptoms, metabolic stress, loss of subcutaneous fat, muscle wasting, and ankle/sacral edema of the patients were recorded. After careful assessment, the changes in medical history and physical examination were classified as grade A, B, or C (Table 1). Finally, the assessment results were accumulated. If the total number of grade C was more than 5, the nutritional status of patients was classified as severely malnourished. If the total number of grade B was more than 5, the nutritional status of patients was classified as mildly to moderately malnourished. If the total number of grade C and B was less than 5, the nutritional status of patients was classified as well nourished[26]. Therefore, based on the results of SGA, patients were assigned to one of the three categories: A (well nourished), B (mildly to moderately malnourished), or C (severely malnourished).

Anthropometric measurement

Body height and weight, and other anthropometric parameters were measured by SGA. Body mass index (BMI) was calculated based on body height and weight. BMI less than 18.5 was regarded as malnourished. MUAC and TSF were measured with intertape and adipometer. MAMC was calculated following the formula: MAMC = MUAC (mm) - 3.14 × TSF (mm). TSF $\leqslant$ 10.17 mm in males and $\leqslant$ 13.41 mm in females, or MAMC $\leqslant$ 20.52 cm in males and $\leqslant$ 18.81 cm in females was the diagnostic criterion for malnutrition. These standards of anthropometric parameters

Table 1 Parameters and diagnostic criteria for subjective global assessment (SGA)

Parameters	Grade A	Grade B	Grade C
Food intake	No deficiency	Definite decrease in intake or liquid diet	Severe deficiency in intake or starvation
Weight loss (during the past 6 mo)	No weight loss or weight loss > 10% during the past 6 mo but weight gain over the past month	Continuous weight loss of 5%-10%	Continuous weight loss > 10%
Gastrointestinal symptoms (nausea, vomit, diarrhea)	None	Mild or moderate GI symptoms for less than 2 wk	Continuous severe GI symptoms for more than 2 wk
Activities and function	No limitation	Not normal, but able to do fairly normal activities or do not know most things, but in bed or chair for less than half a day	Able to do little activity and spend most of the day in bed or chair; or much bed-ridden, rarely out of bed
Metabolic stress	No fever	Temperature > 37℃ and < 39℃ during the past 72 h	Continuous temperature ⩾ 39℃ during the past 72 h
Subcutaneous fat loss	No	Mild to moderate	Severe
Muscle wasting	No	Mild to moderate	Severe
Ankle edema/Ascites	No	Mild to moderate	Severe

Nutritional status: SGA-A (Normal); SGA-B (Mildly to moderately malnourished); SGA-C (Severely malnourished).

Table 2 Classification standards for nutritional parameters in assessing malnutrition

Nutritional parameter	Normal nutrition	Mildly malnourished	Moderately malnourished	Severely malnourished
TSF (mm)				
Male	> 10.17	9.04-10.17	6.78-9.03	< 6.78
Female	> 13.41	11.92-13.41	8.94-11.91	< 8.94
MAMC (cm)				
Male	> 20.52	18.24-20.52	13.68-18.23	< 13.68
Female	> 18.81	16.72-18.81	12.54-16.71	< 12.54
PA (mg/L)	⩾ 200	160-199	120-159	< 120
ALB (g/L)	⩾ 35	31-34	26-30	⩽ 25

TSF: Triceps skinfold thickness; MAMC: Mid-upper arm muscle circumference; PA: Prealbumin; ALB: Albumin.

for classifying nutritional status were formulated in accordance with the Chinese Anthropometric Reference Data (Table 2)[27].

Blood measurement

Blood samples were collected at anthropometric assessment, before initiation of Ⅳ fluids. ALB and PA were measured with a standard clinical analyzer. The cut-off value for PA and ALB was set at 200mg/L (measured by immune turbidimetry) and 35 g/L (measured by biuret method), respectively. The standards for classifying nutritional status in serum proteins were also formulated in accordance with the Chinese Anthropometric Reference Data (Table 2)[27].

Outcome variables related to health care

Outcome variables related to health care, such as hospital stay, medical expenditures, occurrence of complications, and pathological stage of cancer were also detected. Patients were discharged according to the hospital policy. Hospital stay (d) was recorded. All patients were followed up until discharge or death. Complications, including infectious complications (septicemia, incisional, respiratory, abdominal, pelvic, and urinary tract infection) and non-infectious complications (rupture of incision, intestinal obstruction, ascites, cerebrovascular accident, bleeding, and organ failure, *etc)*, were monitored and recorded daily. Pathological stage of cancer was described by TNM staging according to Union International Contere Cancer (UICC) version 5.0.

Statistical analysis

Data analyses were carried out using StatView 6.12 (SAS Institute, Cary, NC, USA). Data were expressed as mean ± SD. Differences in mean values were tested with one-way analysis of variance and Student's *t*- test. χ^2 test was used to compare differences in categorical data. Bivariate correlation analysis (Pearson's R) was performed to show the correlation between SGA grades and other nutritional parameters. Multiple regression analyses were carried out to assess the relation between SGA, other nutritional parameters, and health care outcome variables. $P < 0.05$ was considered statistically significant.

RESULTS

Nutritional status and cancer stage of gastrointestinal cancer patients

Based on different nutritional parameters, the number of patients with malnutrition was 362 (48.2%), 296 (39.4%), 283 (37.7%), 230 (31.3%), 145 (21.7%), and 72 (9.6%) for SGA, TSF, MAMC, ALB, PA, and BMI, respectively.

In our study, 71.1% patients were at advanced cancer stage. The number of cancer patients was 142 (18.9%), 179 (23.8%), 205 (27.3%), 225 (30.0%) at stage Ⅰ, stage Ⅱ, stage Ⅲ, and stage Ⅳ, respectively.

Comparison of nutritional status classified by SGA and other nutritional parameters

Based on the results of SGA, 389 (51.8%), 332 (44.2%), and 30 (4.0%) patients were classified into well nourished group (SGA-A), mildly to moderately malnourished group (SGA-B), and severely malnourished group (SGA-C), respectively. One-way analysis of variance revealed that SGA grade was closely related with other nutritional parameters (Table 3). Further analyses of *Post Hoc* least significant difference comparisons (LSD tests) identified that there were differences in percentage of weight loss, BMI, PA, and ALB between each two

Table 3 Comparison of nutritional parameters in different SGA grades

Nutritional parameters	The grade of SGA			*F*	*P*	Correlation coeffient (*r*)	*P*
	SGA-A	SGA-B	SGA-C				
Weight loss (%)	2.2 ± 2.9	9.7 ± 7.0	23.2 ± 12.6	296.0	0.000	0.65	0.00
BMI	23.4 ± 3.0	21.2 ± 2.8	19.0 ± 3.3	70.8	0.000	0.40	0.00
TSF (mm)							
Male	16.0 ± 8.5	10.5 ± 6.2	9.2 ± 6.5	31.9	0.000	0.34	0.00
Female	25.0 ± 10.2	17.4 ± 8.3	14.4 ± 10.0	26.6	0.000	0.38	0.00
MAMC (cm)							
Male	21.8 ± 2.3	21.9 ± 2.1	20.6 ± 2.5	2.4	0.095	0.03	0.50
Female	18.2 ± 2.5	18.6 ± 2.4	16.8 ± 2.5	4.1	0.018	0.02	0.71
ALB (g/L)	37.7 ± 4.2	35.7 ± 5.7	30.5 ± 6.6	36.9	0.000	0.29	0.00
PA (mg/L)	246.7 ± 41.5	221.7 ± 49.2	159.6 ± 52.9	59.5	0.000	0.37	0.00

Table 4 Comparison of SGA grades between patients before surgery and chemotherapy/radiotherapy

Treatment	*n*	Grade of SGA (%)			χ^2	*P*
		SGA-A	SGA-B	SGA-C		
Surgery	505	275 (70.7)	214 (64.5)	16 (53.3)	5.91	0.05
Chemotherapy and radiotherapy	246	114 (29.3)	118 (35.5)	14 (46.7)		
Total	751	389	332	30		

of the three SGA groups ($P < 0.05$). Therefore, in general, when the patients were classified by the SGA grade as more severely malnourished, the value of the other nutritional parameters, such as levels of BMI, ALB, and PA was lower. Bivariate correlation analysis showed that SGA grade was significantly correlated with the percentage of weight loss, BMI, TSF, ALB, and PA (Table 3), even though the correlation coefficient was less than 0.3 between SGA grade and ALB level.

χ^2 tests showed that SGA grade was significantly different between patients receiving surgery and chemotherapy/radiotherapy (Table 4). In addition, the percentage of weight loss (5.4% ± 6.7% *vs* 8.4% ± 8.8%, $P = 0.000$) and the serum of PA (235.3 ± 46.5 *vs* 223.8 ± 55.6, $P = 0.013$) existed obviously differences between the patients receiving surgery and chemotherapy/radiotherapy.

Could SGA and other nutritional parameters predict hospital stay?

One-way analysis of variance revealed that the hospital stay of 751 gastrointestinal cancer patients was not statistically different in different SGA groups ($F = 2.46$, $P = 0.086$). Preliminary multiple regression analysis using hospital stay as an outcome variable showed that the type of treatment was the biggest predictor for hospital stay in our study (Table 5). In general, patients receiving surgery stayed in the hospital much longer than those receiving chemotherapy/radiotherapy. Further ANOVA analysis revealed that the hospital stay was significantly longer in accordance with the increasing grade of SGA, both in patients receiving surgery and in patients receiving chemotherapy/radiotherapy (Table 6). Subgroup multiple regression analysis using hospital stay as an outcome variable, showed that SGA and serum ALB could help explain the length of hospital stay only in surgical gastrointestinal (GI) cancer patients (Table 7), but not in patients receiving chemotherapy/radiotherapy ($F = 1.22$, $P = 0.27$).

Could SGA and other nutritional parameters predict in-hospital medical expenditures?

One-way analysis of variance revealed that the in-hospital medical expenditures of different SGA groups of patients were significantly different ($P < 0.01$) (Table 6). SGA-C group had the highest expenditures, SGA-A group the lowest expenditures, and SGA-B group the medium expenditures. Multiple regression analysis using medical expenditures as an outcome variable showed that the type of treatment was the biggest predictor of medical expenditures for GI cancer patients in our study (Table 5). The multiple regression analysis revealed that SGA, serum ALB, and cancer stages (TNM) could independently influence the medical expenditures of surgical GI cancer patients (Table 7). On the contrary, no significant predictors could be found for those not undergone surgery.

Could SGA and other nutritional parameters predict occurrence of complications?

The occurrence of complications increased with the increasing SGA grade. SGA-C group had the highest occurrence of complications (23.3%), SGA-A group the lowest occurrence of complications (16.8%), and SGA-B group the medium occurrence of complications (19.1%) ($\chi^2 = 1.21$, $P = 0.546$). In addition, hospital stay of patients with complications was significantly longer than that of those without complications (26.1 ± 12.1 *vs* 15.5 ± 7.8, $t = -9.67$, $P = 0.00$).

During hospital stay, 8 patients died of various

Table 5 Factors influencing hospital stay and in-hospital costs of GI cancer patients (multiple regression analysis)

Factors	Factors influencing hospital stay			Factors influencing in-hospital costs		
	Standardized coefficients β	*t*	*P*	Standardized coefficients β	*t*	*P*
Age	0.02	0.64	0.52	-0.06	-1.37	0.17
Sex	-0.07	-1.64	0.10	-0.04	-0.90	0.37
Education background	-0.04	-1.04	0.30	-0.01	-0.25	0.80
Weight loss (%)	0.03	0.67	0.50	0.07	1.26	0.21
BMI	-0.03	-0.51	0.61	0.05	0.73	0.47
MAMC	-0.04	-0.69	0.49	-0.06	-0.82	0.41
TSF	0.03	0.42	0.68	0.01	0.18	0.86
ALB	-0.04	-1.04	0.30	-0.06	-1.43	0.15
SGA-A/SGA-C	0.18	1.68	0.09	0.18	1.45	0.15
SGA-B/SGA-C	0.17	1.80	0.07	0.09	0.86	0.39
TNM	-0.05	-1.54	0.12	0.06	1.49	0.14
Tumor site	-0.02	0.59	0.56	-0.07	-1.76	0.08
Type of treatment	-0.49	-13.99	0.00	-0.25	-6.30	0.00

Factors influencing hospital stay model F = 19.20, $P < 0.05$; Factors influencing in-hospital costs model F = 5.62, $P < 0.01$.

Table 6 Comparison of hospital stay and medical expenditures of patients with different SGA grades

	Grade of SGA (%)			*F*	*P*
	SGA-A	SGA-B	SGA-C		
Hospital stay (d)	17.1 ± 9.7	17.3 ± 9.0	21.1 ± 14.6	2.46	0.086
Surgery	20.8 ± 8.6	21.2 ± 7.8	29.1 ± 15.1	7.07	0.001
Chemotherapy and radiotherapy	8.2 ± 5.1	10.3 ± 6.4	12.1 ± 6.8	5.02	0.007
Medical expenditure (RMB)	6522.4 ± 6670.9	8353.7 ± 9575.9	12550.0 ± 10579.7	9.85	0.000
Surgery	7987.9 ± 6963.9	10025.8 ± 10009.6	17654.2 ± 11678.5	11.51	0.000
Chemotherapy and radiotherapy	3033.5 ± 3430.5	5358.0 ± 7945.0	6268.0 ± 3632.5	4.58	0.011

Table 7 Factors influencing hospital stay and in-hospital costs of surgical GI cancer patients (multiple regression analysis)

Factors	Factors influencing hospital stay			Factors influencing in-hospital costs		
	Standardized coefficient β	*t*	*P*	Standardized coefficient β	*t*	*P*
Age	0.05	1.01	0.32	-0.06	-1.22	0.22
Sex	-0.11	-1.80	0.07	-0.07	-1.17	0.24
Education background	-0.04	-0.89	0.37	-0.04	-0.88	0.38
weight loss (%)	0.02	0.26	0.80	-0.01	-0.05	0.96
BMI	-0.05	-0.62	0.54	0.06	0.81	0.42
MAMC	-0.05	-0.66	0.51	-0.07	-0.86	0.39
TSF	0.12	1.35	0.18	0.06	0.66	0.51
ALB	-0.10	-2.11	0.04	-0.16	-3.17	0.002
SGA-A/SGA-C	0.41	2.36	0.02	0.43	2.51	0.01
SGA-B/SGA-C	0.39	2.52	0.01	0.31	2.06	0.04
TNM	-0.01	-0.26	0.08	0.10	2.11	0.04
Tumor site	-0.06	1.21	0.23	-0.09	-1.90	0.06

Factors influencing hospital stay model F = 2.35, $P < 0.01$; Factors influencing in-hospital costs model F = 3.92, $P < 0.01$.

complications (5 in SGA-B group, 2 in SGA-A group, and 1 in SGA-C group). SGA grade was not related with the number of deaths in our study.

DISCUSSION

Severe malnutrition is associated with increased morbidity and mortality of gastrointestinal cancer, decreased treatment efficacy, and increased hospital stay[28]. Nutritional status is conventionally assessed by anthropometric measurement and laboratory assessment[29]. In this study, the prevalence of malnutrition for the same group of subjects ranged 9.6%-48.2%. The highest prevalence of malnutrition

was detected by SGA, the lowest by BMI. The purpose of nutritional assessment in cancer patients is to discover mild or moderate malnutrition before the patients become overtly wasted in order to prevent further deterioration and improve their quality of care. In clinical settings, some of the anthropometric measurements and laboratory assessments are not ideal because they are neither accurate nor convenient.

Although the British Association for Parenteral and Enteral Nutrition (BAPEN) has recommended that the measurements used for screening malnutrition should be based upon the changes in BMI and the percentage of weight loss, our study demonstrated that only a small number of patients were diagnosed with malnutrition by BMI, suggesting that BMI cannot precisely assess malnutrition in Chinese cancer patients. The established cut-off point of malnutrition for BMI largely depends on studies in younger patients[30], and therefore, cannot be directly applied to the elderly population, which may explain why only a small number of patients were diagnosed with malnutrition by BMI in this study. It has been shown that a BMI value of 20 should alert clinicians to suspect malnutrition in the elderly[29]. It was reported that the optimal range of BMI in elderly people should be increased from 20 kg/m^2 to 25 kg/m^2 in order to identify the elderly at a risk of malnutrition[31]. On the other hand, some elderly patients spend most of their day time in bed or totally bedridden, so it is not always easy or sometimes even impossible to measure their weight or height changes.

In addition, SGA was not significantly correlated with ALB level compared to other anthropometric parameters. The ALB level alone is not a good representative marker of nutritional status of cancer patients as shown in our study. It has been shown that ALB level may be considered as an indicator of illness or as a prognostic factor for complications and mortality, but not as a major indicator of nutritional status[32]. Our study showed that ALB level was an important factor for prolong hospital stay and medical expenditures of surgical cancer patients. In patients with malignancy diseases, the ALB level can be affected by nutritional status and the malignant disease itself, or by inflammatory reactions due to any causes, such as severe liver disease, dehydration, and edema[29]. In fact, serum ALB, a negative acute phase protein[33], is decreased in response to acute or chronic inflammation by altering the normal hepatic protein metabolism and inducing capillary leak[34-36]. Irrespective of the value of biochemical indicators, ALB level measurement is more time consuming and expensive than SGA.

SGA, one of the better available tools, can assess nutritional status, not only because it is patient centered by combining clinical history and physical examination, but also because it is associated with patient outcomes[37-39]. This is why SGA has been used widely in Western countries yielding trustworthy results. In the present study, the values of BMI, TSF, PA, and ALB were lower in more severely malnourished patients, which is consistent with previous findings[23,29]. It has been shown that SGA grade is closely correlated with TSF, MAMC, and ALB[29]. SGA can be used as a benchmark to validate new assessment methods, such as bioelectrical impedance analysis[40] and mid-upper arm anthropometry[41].

Although SGA is now considered a clinical method for assessing nutritional status, it was originally developed to identify patients with poorer outcomes following surgery. Baker *et al*[23] showed that patients classified as 'malnutrition' suffer more infections, use more antibiotics, and have a longer hospital stay. We hypothesized that SGA grade of patients at admission could help to predict the occurrence of complications, hospital stay, and in-hospital medical expenditures of Chinese gastrointestinal cancer patients, and found that the more severely malnourished patients had a longer hospital stay, a higher occurrence of complications, and higher in-hospitalization costs. Multiple regression analysis displayed that SGA grade could only predict hospital stay and medical expenditures of surgical cancer patients, but not those of chemotherapy/radiotherapy patients, indicating that type of treatment may influence the predictive value of SGA. Wakahara *et al*[42] reported that although SGA can be used to predict the hospital stay of patients with digestive diseases, cancer staging is a better prognostic index of cancer patients. However, the results of our study do not support the fact that advanced cancer would lead to worse nutritional status, longer hospital stay, and higher incidence of postoperative complications. In addition, patients with complications had a longer hospital stay than those with no complications. Since cancer patients are more prone to develop complications when their nutritional status deteriorates, more treatment modalities are needed to help them recover.

Although SGA could provide useful information for predicting certain outcome variables in our study, SGA was not related with death of patients. Eight patients (5 in SGA-B group, 2 in SGA-A group and 1 in SGA-C group) died of complications during hospital stay. The reason why only one patient died in the most severely malnourished SGA-C group was due to the small subgroup sample size. Whether SGA can predict the risk of in-hospital death remains unclear.

This study had some limitations. For example, the small sample size in SGA-C group resulted in a quite unbalanced distribution of nutritional status in different SGA classification groups, which may limit the power of data analysis. As an assessment tool, SGA consists of both history taking and physical examination of the patients[40,43]. Thus, reliable SGA grading depends on collection of correct history and physical examination. During our study, since some patients could not remember their exact body weight and detail dietary intake when information was collected to assess the nutritional status, the relevant information was obtained from the recall of patients and their relatives. Recently, quantification of SGA has been advocated as a way to improve the sensitivity and specificity of SGA in diagnosing malnutrition[44,45].

In conclusion, SGA is a safe, inexpensive and reliable method for assessing nutritional status of Chinese gastrointestinal cancer patients and only can predict their hospital stay and medical expenditures in surgical GI patients. Further study is needed on the role of SGA in predicting the occurrence of in-hospital deaths.

COMMENTS

Background

Cancer is one of the serious health problems worldwide. A large number of cancer patients die of malnutrition or its relative complications rather than the disease itself. Malnutrition has a negative impact on the well-being of patients and evolution of the disease. A timely efficient nutritional assessment would provide a better basis for deciding whether nutritional support is given. Many nutritional assessment methods are now available. Each method has its own advantages and disadvantages. An accurate, convenient, and inexpensive method should be available for clinicians.

Research frontiers

Subjective global assessment (SGA) has been originally developed to identify poor nutritional status in subjects undergoing gastrointestinal surgery, it can be used to quantify the prevalence of malnutrition in patients at the end-stage of renal failure, and is a powerful predictor of postoperative complications in general surgery, liver transplantation, and in patients on dialysis. In addition, a recent study revealed that although SGA can predict hospital stay of patients with benign digestive disease, its predictive power is limited in patients with malignant diseases.

Innovations and breakthroughs

Although SGA has been used widely for more than two decades all over the world, few studies are available on SGA in Chinese gastrointestinal cancer patients. In this study, SGA was used to assess the nutritional status of Chinese patients with gastrointestinal cancer. The results show that SGA helps predict certain outcomes such as hospital stay and medical expenditures of surgical gastrointestinal (GI) cancer patients.

Applications

As a convenient and reliable method, SGA can be used to assess the nutritional status of cancer patients since it helps predict certain outcomes of surgical GI cancer patients.

Peer review

This manuscript is valuable and offers important data for the clinical management of GI cancer patients. The results of this study demonstrate that SGA is superior over other nutritional parameters in the assessment of nutritional status of GI cancer patients.

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BRIEF ARTICLES

Sonographic evaluation of vessel grafts in living donor liver transplantation recipients of the right lobe

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Abstract

AIM: To evaluate the vessel grafts (VG) used to reconstruct the middle hepatic vein (MHV) tributaries with ultrasonography.

METHODS: Twenty-four patients undergone living donor liver transplantation were enrolled in our study. MHV tributaries larger than 5 mm in diameter were reconstructed with interposition VG. Blood flow of the graft and interposition VG was checked by Doppler ultrasonography daily in the first 2 postoperative weeks and monthly followed up after discharge. The sensitivity of VG detected by ultrasonography was assessed using surgical records as references. Student's *t* test was used to compare the velocity of VG and occluded VG in chronic patents (> 3 mo).

RESULTS: Thirty-one VG were used to reconstruct the MHV tributaries. Ultrasonography identified 96.7% (30/31) of large MHV tributaries and 90.3% (28/31) of VG. The diameter of VG was 5.6 ± 0.8 mm and the velocity of VG was 19.7 ± 8.1 cm/s. Two VG (2/31, 6.5%) were occluded on the first postoperative day in one patient who suffered from persistent ascites and had a prolonged recovery of liver function. Twenty-six VG (26/31, 83.9%) were patent 2 wk after operation. Six (6/31, 19.4%) VG were patent over 3 mo after operation. Intrahepatic venous collaterals were detected in 29.2% (7/24) patients. The velocity of VG and occluded VG was 30.1 ± 5.6 cm/s, 16.5 ± 5.8 cm/s, respectively, in chronic patents. The difference between two groups was statistically significant ($P < 0.001$).

CONCLUSION: Our results indicate that most VG are patent in the first postoperative week while only a small portion with a higher velocity remains patent after 3 mo. Intrahepatic venous collaterals can be observed in some patients after occlusion of VG.

Key words: Living donor liver transplantation; Ultrasound; Vessel graft; Venous collateral; Middle hepatic vein

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INTRODUCTION

Adult-to-adult living donor liver transplantation (AALDLT) is emerging as an alternative to cadaveric liver transplantation due to shortage of graft organs[1,2]. A right lobe liver graft is now commonly used in this procedure, and the middle hepatic vein (MHV) trunk is usually preserved for the donor for his or her safety[3,4]. Because hepatic venous blood of the anterior segment usually drains to the inferior vena cava through the V5 and V8 MHV tributaries, this type of grafts is inherently prone to cause congestion of the right paramedian sector (segments V and Ⅷ), leading to tissue atrophy or necrosis[5,6]. Because establishment of optimal hepatic venous outflow is essential to a successful outcome of AALDLT, there has been emerging interest in hepatic venous congestion in the paramedian sector of the right lobe graft after AALDLT. Recently, surgical reconstruction of the major MHV tributaries using an interposition VG has been advocated to achieve optimal hepatic venous outflow

of the paramedian sector in AALDLT recipients using a modified right lobe graft. Although the indication for reconstruction of MHV is still controversial[3,7,8], MHV tributaries in the anterior segment of the modified right lobe graft are routinely reconstructed in our institution if they are larger than 5 mm in diameter. However, compared with the recent surgical interest and investigations designed to prevent hepatic venous congestion in the right paramedian sector of the right lobe grafts after AALDLT, little attention has been given to postoperative radiological surveillance for thick MHV tributaries, VG and intrahepatic venous collaterals.

Therefore, this study was to assess the value of ultrasound in the evaluation of interposition VG and intrahepatic venous collateral formation after occlusion of the VG in AALDLT.

MATERIALS AND METHODS

Patients

From January 2006 to August 2007, 51 consecutive adult patients underwent AALDLT using the right lobe grafts at West China Hospital. The indications for liver transplantation included hepatitis-B virus cirrhosis in 26 patients, acute hepatic failure in 8 patients, hepatitis-B cirrhosis associated with hepatocellular carcinoma in 16 patients, retransplant for diffused ischemic intrahepatic biliary stenosis of cadaveric graft with liver failure in 1 patient. Twenty-four (47.1%) patients (21 males and 3 females) who underwent MHV reconstruction were enrolled in our study. Their median age was 38 years (range, 22-54 years). The donors (14 males and 10 females) with a median age of 36 years (range, 22-53 years) included 6 wives, 7 brothers, 2 sisters, 4 sons, 2 daughters, 2 fathers and 1 husband. All cases were approved by the ethics committee of local authority and informed consent was obtained from each patient.

Preoperative assessment

Right liver volume was estimated by multi-slice computed tomography (CT). Candidates in whom the right liver represented more than 70% of the whole liver were excluded from prospective donors. An estimated graft volume to recipient standard liver volume ratio of 40% was the lower limit for right lobe liver transplantation. The number and diameter of thick MHV tributaries draining the right paramedian sector were evaluated by CT.

Intraoperative evaluation

Donor hepatectomy was performed through a J-shaped incision. Intraoperative ultrasound was performed to confirm the hepatic vein anatomy and verify the transection plane. Major MHV tributaries were isolated and preserved if present and greater than 5 mm in diameter. Hepatic venous congestion in the right paramedian sector was investigated intraoperatively after parenchyma transaction by clamping test[9]. Liver surface discoloration in the right paramedian sector was observed 5 min after simultaneous clamping of MHV tributaries and the right hepatic artery. If the congested area was dominant as determined by the clamping test or the diameter of MHV tributaries was larger than 5 mm, reconstruction of MHV tributaries was preceded. The autogenous great saphenous and cryopreserved cadaveric external iliac veins are most commonly used as interposition vessel grafts.

Postoperative ultrasound examination

To prevent thrombosis of hepatic artery and vessel grafts, prophylactic anticoagulation therapy was routinely used after surgery. Blood flow in the graft or interposition vessel was checked by Doppler sonography using a HDI 5000 scanner (Philips Medical Systems, Bothell, WA) or a LOGIQ 9 scanner (GE Medical Systems, Milwaukee, Wis). Contrast enhanced ultrasound examinations were done with a Sequoia 512 ultrasound scanner (Acuson Siemens, Erlangen, Germany). The contrast agent was SonoVue (Bracco Imaging, Milan, Italy) consisting of sulphur hexafluoride microbubbles stabilized by a phospholipid shell, 2.4 mL of SonoVue per exploration was injected through a peripheral vein. A low mechanical index mode was used. Scans were performed by an ultrasound doctor with more than 5-year experience in liver transplantation ultrasound. Patients received ultrasound examination daily until postoperative day 14 and once a week thereafter until hospital discharge. Intercostal views of the right upper quadrant of abdomen were obtained to examine the full extension of the hepatic parenchyma. Thick tributaries of the MHV, identified at the left margin of the graft, were used as extrahepatic interposition VG. Fundamental gray scale, color flow images, and Doppler spectra were obtained. Angle-corrected velocities were obtained with the Doppler angle less than 60°. Contrast enhanced ultrasound study was conducted in 5 patients and late parenchymal phase (60 s after injection of SonoVue) was used to observe the patency of VG. All images were acquired when the patients did not hold their breath.

VG were divided into long term patent group and occluded group according to the vessel patency 3 mo after surgery. Student's *t* test was used to compare the velocity in two groups.

RESULTS

Thirty-one vessel grafts were used to reconstruct the MHV tributaries, including V5 (n = 13), V8 (n = 4), both V5 and V8 (n = 7) (Figure 1). Ultrasonography identified 96.7% (30/31) of large MHV tributaries and 90.3% (28/31) of vessel grafts (Figure 2). The diameter of VG was 5.6 ± 0.8 mm and the velocity of VG was 19.7 ± 8.1 cm/s. Two VG (6.5%) were occluded on the first day after surgery in one patient (Figure 3) who had a prolonged recovery of graft function and persistent ascites for 2 mo. Twenty-six VG (83.9%) were patent 2 wk after operation. Six VG (19.4%) were patent over 3 mo after after operation. Intrahepatic venous communications between the MHV tributaries and the right hepatic vein were detected in 7 (29.2%) patients (Figure 4). The velocity in long term patent VG group and occluded VG group was 30.1 ± 5.6 cm/s and 16.5 ± 5.8 cm/s, respectively. The

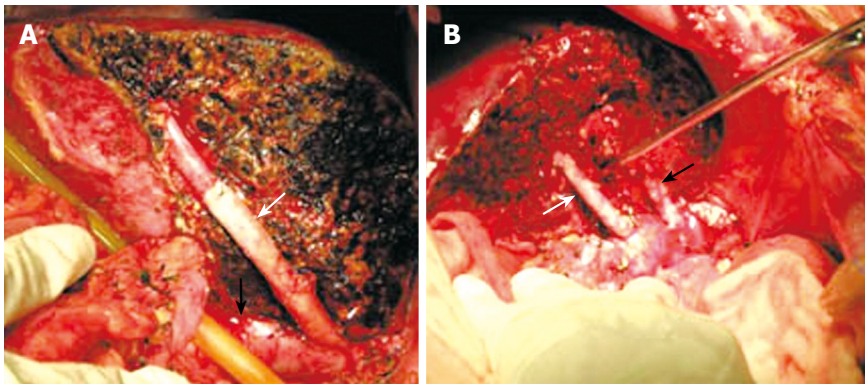

Figure 1 Intraoperative photograph showing an interposition vessel graft (white arrow) draining venous flow from MHV tributaries in segments Ⅴ-IVC (black arrow) (A) and interposition vessel grafts V5 (white arrow) and V8 (black arrow) (B) used to reconstruct MHV tributaries in segments Ⅴ and Ⅷ. MHV: Middle hepatic vein, IVC: Inferior vena cava.

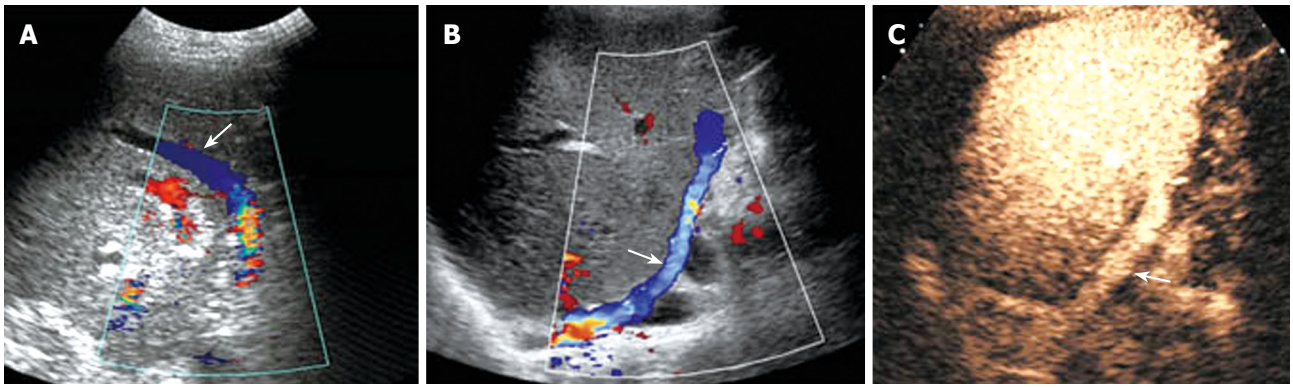

Figure 2 Color Doppler ultrasonography showing large MHV tributaries (arrow) in segment Ⅴ containing hepatofugal venous flow (A), vessel grafts (arrow) draining the IVC along the surgical margin of liver graft (B), and vessel graft (arrow) filled with contrast agent on contrast enhanced ultrasound indicating the vessel graft patency (C).

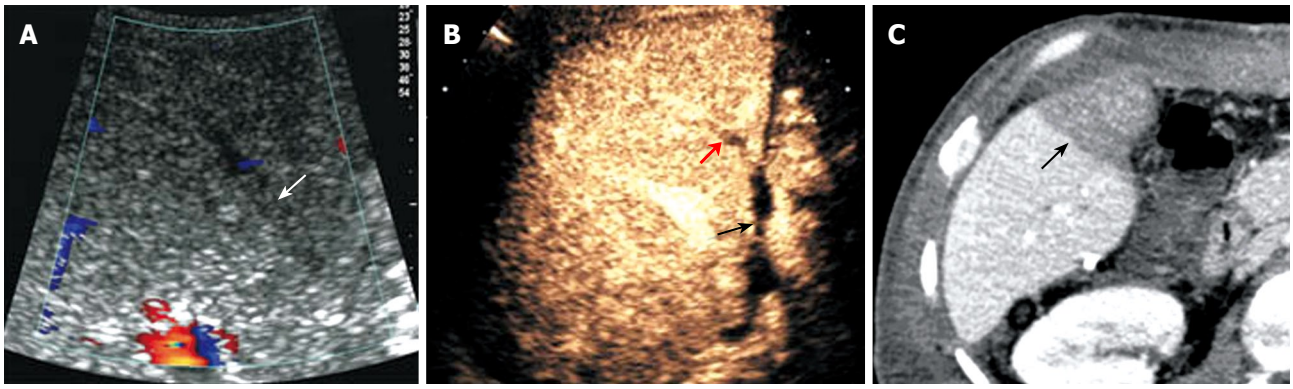

Figure 3 Color Doppler sonography showing MHV tributaries filled with hypoechoic substances representing thrombus (arrow) with no color signal in vessel grafts (A), contrast enhanced sonography showing no contrast agent in vessel grafts (arrow) and MHV tributaries (red arrow) indicating vessel occlusion (B), and contrast enhanced CT scan on postoperative day 2 showing an area of low attenuation in segment Ⅴ corresponding to the draining territory of MHV (arrow) (C) in a 42-years old male who received a modified right lobe graft from his brother.

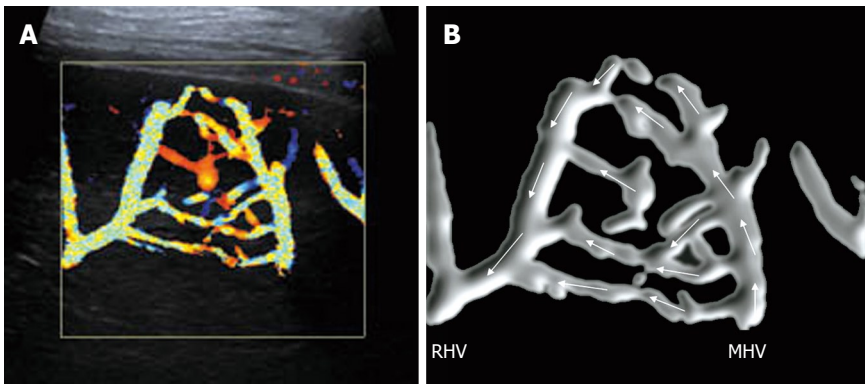

Figure 4 Color Doppler ultrasonography (A) and its sketch (B) showing MHV tributaries draining into the RHV *via* collaterals after thrombosis of vessel grafts on postoperative day 7 in a 32 years old male who received a right lobe graft from his wife.

difference between two groups was statistically significant ($P < 0.001$).

DISCUSSION

The major limitation of AALDLT is the inadequacy of graft size. To guarantee the safety of the donor, many institutes perform AALDLT using a right liver graft without the MHV trunk[1,10,11]. However, this procedure might be complicated by severe congestion of the right paramedian sector, leading to tissue atrophy or necrosis. Therefore, the MHV tributaries larger than 5 mm in diameter should be preserved to achieve optimal outflow of the right paramedian sector.

Doppler sonography is generally regarded as a primary technique for vascular surveillance after LDLT and is, sometimes, the only available bedside imaging modality in the early postoperative period[12,13]. Good spatial resolution and high sensitivity to slow flow make ultrasound useful in depicting the MHV tributaries and interposition VD. In our study, Doppler sonography demonstrated 90.3% of the VG and 96.7% of the large MHV tributaries. However, ultrasound scan was interfered by bowel and lung gas, thus failing to identify the rest three VG and one MHV tributary.

Early occlusion of VG may result in an eventful recovery of liver function. One patient in whom the VG were occluded on the first postoperative day had a prolonged recovery of graft function and persistent ascites for 2 mo. Occlusion of the VG caused venous congestion of the right paramedian sector, thus delaying the recovery of liver function. Persistent ascites can be caused by an inadequate hepatic mass[14,15]. The regenerated liver cannot reach its adequate size in the early postoperative period and venous congestion of the right paramedian sector can further decrease the functional hepatic mass size.

Unlike the hepatic vein, since VG do not support the surrounding hepatic parenchyma, these VG are under a greater pressure from the neighboring structures due to liver regeneration. Moreover, because the diminished flow also attributes to the occlusion of VG, VG have a tendency to occlude with time. In our study, only 6 VG (19.4%) remained patent 3 mo after operation and had a higher velocity than the occluded VG.

Identification of intrahepatic collaterals between MHV tributaries and right hepatic vein is important, because the presence of intrahepatic collaterals indicates the outflow of the right paramedian sector is not occluded. Whether all patients have intrahepatic venous collaterals still remains controversial. De Cecchis *et al*[16] reported that intrahepatic communicating veins are sometimes observed. Kaneko *et al*[17] demonstrated that the right branch of MHV drains into the right hepatic vein via the collaterals between them. However, Cescon *et al*[18] described that the venous flow in MHV was eliminated without intrahepatic venous communication in 24 grafts of 30 living donor liver transplantations when the MHV was clamped, Doppler ultrasound is sensitive to slow flow and provides information about the direction of blood flow. In our study, extensive subcapsular venous communications were demonstrated in 7 patients (29.2%) after the occlusion of VG at follow-up ultrasound examination with no salient echogenecity change.

Contrast enhanced ultrasound is a newly emerged technique and can greatly improve the visualization of low velocity flow[19,20]. We did not routinely use it to evaluate VG due to the high price of contrast agent. In our series, 5 patients underwent contrast enhanced ultrasound study to exclude hepatic artery thrombosis and evaluate the patency of VG. The results show that it can greatly enhance the confidence of ultrasound doctors. However, further study is needed to evaluate the diagnostic value of this new technique.

In conclusion, Doppler sonography is useful in depicting thick MHV tributaries and VG. Most interposition VG are patent in the first week after surgery and trend to occlude with time with only a small portion remains patent 3 mo after operation. Intrahepatic venous collaterals can be observed after occlusion of VG.

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COMMENTS

Background

Surgical reconstruction of major middle hepatic vein tributaries using interposition vessel grafts (VG) has been advocated to achieve optimal hepatic venous outflow of the paramedian sector in adult-to-adult living donor liver transplantation recipients using a modified right lobe graft. However, little attention has been given to postoperative radiological surveillance for thick MHV tributaries, VG and intrahepatic venous collaterals.

Research frontiers

Doppler sonography and contrast enhanced ultrasound were used to investigate the patency and velocity of VG in study. The relation between VG velocity and long term patency was discussed. The issue of intrahepatic venous collaterals was also addressed.

Innovations and breakthroughs

Although some studies have already discussed the issue of reconstructed vessels, our study focused on the patency of reconstructed vessels, which has received little attention.

Applications

By displaying the relation between the velocity and patency of VG and the formation of intrahepatic venous collaterals, sonographic surveillance may represent a strategy for prediction of the VG patency and even the paramedian sector congestion of transplanted liver.

Terminology

VG used to reconstruct middle hepatic vein (MHV) tributaries are vascular conduit between MHV tributaries and inferior vena cava. Autogenous great saphenous and cryopreserved cadaveric external iliac veins are the most frequently used VG.

Peer review

The authors investigated the pantency of vasucluar conduits (vessel graft) for MHV reconstruction with right liver graft in living donor liver transplantation. They used autogenous great saphenous and cryopreserved cadaveric external iliac veins as vascular conduit. Their data and experience are valuable because little evidence of the patency of reconstructed vessels is available, although similar studies are available.

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CASE REPORT

Generalized megaviscera of lupus: Refractory intestinal pseudo-obstruction, ureterohydronephrosis and megacholedochus

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Abstract

Dilated dysfunction involving multiple visceral organs has been reported in patients with systemic lupus erythematosus (SLE). Chronic intestinal pseudo-obstruction (CIPO) resulting from intestinal smooth muscle damage has presented in conjunction with ureterohydronephrosis and, more rarely, biliary dilatation (megacholedochus). While the molecular pathogenesis is largely unknown, observed histopathologic features include widespread myositis, myocyte necrosis in the intestinal muscularis propria with subsequent atrophy and fibrosis, preserved myenteric innervations and little vasculitis. High dose immunosuppression usually results in resolution of symptoms with recovery of smooth muscle function, indicative of an autoimmune etiology. We report a patient with SLE who presented with intestinal pseudo-obstruction, ureterohydronephrosis and megacholedochus, and present images that illustrate megaviscera simultaneously involving all 3 visceral organs. Since the co-manifestation of all 3 is unusual and has been reported only once previously, we have termed this rare clinical syndrome generalized megaviscera of lupus (GML). Although the SLE disease-activity parameters responded to aggressive immunomodulative therapy in our patient, clinical evidence of peristaltic dysfunction persisted in all involved viscera. This is a variation from the favorable outcomes reported previously in SLE patients with GML and we attribute this poor clinical outcome to disease severity and, most importantly, delayed clinical presentation. Since inflammation followed by atrophy and fibrosis are key aspects in the pathogenesis and natural history of GML, the poor response in our patient who presented late in the clinical course may be the result of 'burnt out' inflammation with irreversible end-stage fibrosis. Thus, early recognition and timely initiation of treatment may be the key to recover visceral peristaltic function in patients with GML.

Key words: Systemic lupus erythematosus; Intestinal pseudo-obstruction; Biliary tract diseases; Hydroureter; Hydronephrosis; Smooth muscle; Autoimmune myositis

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INTRODUCTION

Chronic intestinal pseudo-obstruction (CIPO) is a rare but important clinical syndrome as it causes 20% of chronic intestinal failure in adults and 15% in children[1]. It is characterized by ineffective intestinal propulsion with signs and symptoms similar to

mechanical bowel obstruction including abdominal distension, pain, nausea, vomiting, obstipation and sluggish bowel sounds, but the absence of an occluding lesion of the intestinal lumen. CIPO is idiopathic in the vast majority of cases, but secondary causes can include virtually any disease process that affects structures involved in intestinal motility, including intestinal myocytes and the extrinsic and intrinsic neural networks. The management of CIPO has been challenging, and long-term outcomes often disappointing[1]. In contrast, CIPO in systemic lupus erythematosus (SLE) patients has shown an excellent response to immunosuppressive therapy when initiated early, consistent with an autoimmune etiology[2-5]. While the pathophysiology of CIPO in SLE remains unclear, the frequent concurrence of ureterohydronephrosis (67% in one case series)[3,4,6] and histopathologic evidence of intestinal leiomyocyte damage[3-5] suggest a systemic autoimmune process targeting smooth muscle cells. Here we describe a patient with SLE who presented with refractory intestinal pseudo-obstruction, ureterohydronephrosis and megacholedochus in the setting of delayed immunosuppressive therapy. This triad of gastrointestinal, genitourinary, and hepatobiliary hollow viscera dilatation and dysmotility has been described to co-manifest in lupus only once previously[6]. We term this rare and likely under-recognized clinical syndrome Generalized Megaviscera of Lupus (GML). An understanding of the pathophysiology of these processes is needed to avoid poor outcomes resulting from either unnecessary procedures and interventions or a delay in the diagnosis and/or initiation of treatment. We review the literature and discuss the pathophysiology.

CASE REPORT

A 46-year-old Japanese-American female presented to our hospital with a 4-mo history of worsening arthralgias, abdominal discomfort, distension and obstipation. Her past medical history was notable for SLE diagnosed in 1993, which was complicated by disseminated encephalomyelitis, malabsorption syndrome, and biopsy-confirmed membranous glomerulonephritis. In the past, she has been treated with steroids and immunomodulators; however, because of adverse reactions to several of these medications, frequent changes were made to her treatment regimen. This resulted in poor compliance and resultant breakthrough episodes of disease exacerbations affecting various organ systems. Most recently, she was being treated with mycophenolate mofetil (CellCept®) and oral prednisone which she self-discontinued 8 mo prior to admission.

Physical examination revealed a distended tender abdomen with hypoactive bowel sounds. Laboratory tests were notable for elevated acute phase reactants, proteinuria, and acute renal insufficiency. An abdominal plain film X-ray series (Figure 1A-C) showed multiple air-fluid levels and distended loops of the large (up to 8 cm in diameter) and small (up to 3 cm in diameter) bowel without any obvious structural basis for luminal obstruction. Computed tomography (CT) of the abdomen (Figure 1D and E) confirmed the plain film findings but also showed ascites and fecalization of the luminal contents within the small bowel. Unlike lupus enteritis, the bowel wall in this case was neither thickened nor edematous[1]. Her symptoms and radiographic findings were consistent with an intestinal pseudo-obstruction. Supportive therapy was initiated with placement of a nasogastric tube in low intermittent suction, intravenous (IV) fluid, and pain medications. The patient was started on a course of tegaserod maleate (Zelnorm®) for 2 wk and 2 doses of neostigmine, with little improvement. In addition, she was also treated with enemas (glycerol, magnesium sucrate and water) and promotility agents (metoclopramide and erythromycin). A decompressive colonoscopy was attempted but the procedure was aborted at the level of the transverse colon because of poor visibility as a result of hard fecal matter and an increased risk of perforation. A gastrograffin enema was performed after the colonoscopic procedure, which demonstrated aperistaltic distal colon and contrast filling within a narrow lumen surrounded by stool that was cleared during the attempted colonoscopy (Figure 1C).

The abdominal CT scan also revealed intra- and extra-hepatic biliary tree dilatation (Figure 1E). To investigate this further, magnetic resonance cholangiopancreatography (MRCP) was performed and confirmed the presence of dilated bile ducts, without any structural basis for luminal obstruction (Figure 2). The common bile duct was dilated up to 2 cm without filling defects, calculi, or masses. The patient had no jaundice or right upper quadrant pain and her liver function tests remained within the normal limits throughout her hospital stay.

Regarding her acute renal insufficiency, she was started on IV fluid but her renal function did not improve despite adequate hydration. CT and MR images revealed bilateral hydroureters and hydronephrosis (Figure 1D, Figure 3B and C) without any structural obstruction. Obstructive uropathy resulting from lupus cystitis was entertained as a diagnosis and emergency placement of bilateral double-J ureteral stents was carried out in an attempt to decompress the genitourinary system. Despite optimal positioning of the stents, there was no improvement in the ureterohydronephrosis or her renal function. A voiding nephro-uretero-cystogram confirmed the persistence of dilated aperistaltic ureters bilaterally (Figure 3A and B). Treatment with cyclophosphamide (Cytoxan®) for presumed lupus cystitis also failed to produce any improvement. She continued to have modest urine output but nonetheless eventually required hemodialysis.

Full thickness biopsies from the genitourinary or gastrointestinal viscera were not performed in view of her poor clinical condition. She was treated with conventional immunomodulating therapies consisting of high dose IV methylprednisolone (Solu-Medrol®) and

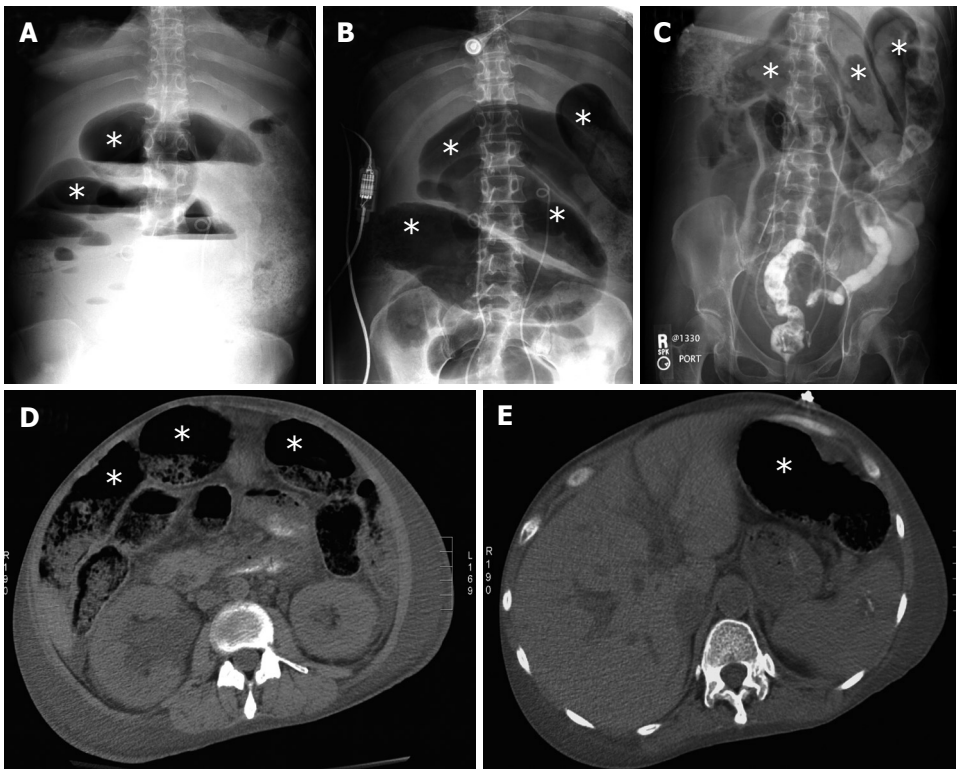

Figure 1 Megabowl: X-ray radiography (A-C) and computerized tomography scan (D, E) of the abdomen revealed the presence of distended loops (stars) of the small and large bowel (megabowl) in the absence of any luminal obstruction.

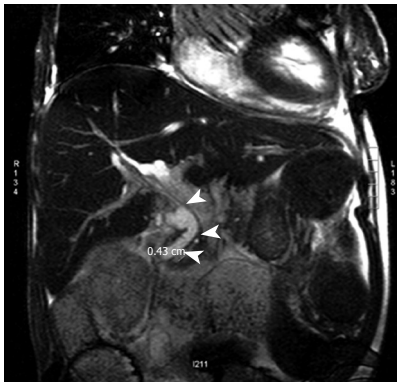

Figure 2 Megacholedochus: magnetic resonance cholangiopancreatography revealed the presence of a distended common bile duct (megacholedochus, arrowheads) without any evidence of luminal obstruction.

mycophenolic acid (Myfortic®) along with parenteral nutrition, promotility agents, antibiotics, aggressive correction of electrolytes, and hemodialysis for presumed lupus flare with multiple organ involvement. She was discharged after improvement of her ascites, arthralgias, proteinuria and serum acute phase reactants. At 6 mo follow-up there was persistent dysfunction in all 3 organ systems without recovery of peristaltic function; however at no point during the clinical course was there any evidence of encephalopathy, cardiomyopathy, or any involvement of skeletal muscles either clinically or biochemically.

DISCUSSION

We have described a patient with SLE who presented with abdominal distension, obstipation and renal insufficiency with various imaging modalities revealing diffusely dilated hollow viscera in the absence of structural obstruction involving the genitourinary, hepatobiliary and gastrointestinal systems. Since these signs and symptoms are non-specific, it is important that all other possible diagnoses are entertained. The presentation as hydroureters in isolation deserves expert consultation and exclusion of other common causes e.g., lupus cystitis. Similarly, biliary tract dilation could occur as a result of coexisting liver diseases or as a direct consequence of an autoimmune phenomenon such as vasculitis. Hepatic artery vasculitis is known to give rise to a similar radiographic appearance but is usually accompanied by symptoms, resulting in abnormalities in liver function, and responds to systemic immunomodulation. Associated hepatobiliary diseases, e.g., autoimmune hepatitis, nodular regenerative hyperplasia, cryptococcal infection, should be considered in the differential diagnosis when isolated involvement of the biliary tract is seen. Involvement of multiple hollow visceral organs should raise suspicion of a generalized autoimmune smooth muscle injury, as in our case. Intestinal pseudo-obstruction is a very rare complication in SLE patients, but hydroureters are a frequent concurrent finding in these patients. The case presented here is among the first to show an additional visceral organ dilatation - megacholedocus. These findings suggest a truly rare, but likely under-recognized clinical syndrome we term generalized megaviscera of lupus (GML), which we define as hollow visceral dilatation and dysfunction present concurrently in more than one organ system in a patient with SLE, with intestinal pseudo-obstruction the most common manifestation.

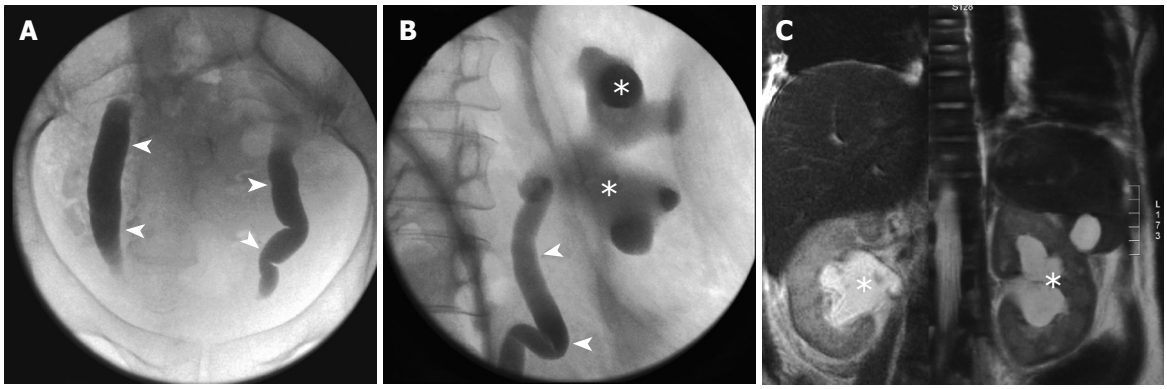

Figure 3 Hydronephrosis and megaureters: the presence of distended ureters (megaureters, arrowheads) was appreciated during a voiding nephro-uretero-cystogram (A, B) whereas hydronephrosis (stars) was detected by magnetic resonance imaging of the abdomen (C) as well as during the nephrogram (B).

We reviewed the anecdotal reports of patients with apparent GML in an attempt to further our understanding of the pathophysiology of this phenomenon. An overview of the pathological analysis of the gastrointestinal tract in lupus patients with CIPO revealed the following characteristics: widespread myocyte necrosis in the muscularis propria with active inflammatory cell infiltrate[5], severe atrophy and fibrosis of the muscularis[3,5], active serositis with serosal thickening and fibrosis[5], little or no evidence of vasculitis or injury to bowel innervation[3-5], and absence of thromboembolic disease[4,5]. It is notable that intestinal myonecrosis is observed without significant lupus vasculitis, often involving smooth muscle dysfunction in another organ system. These findings argue for the existence of a systemic circulating factor causing smooth muscle injury by a mechanism other than vasculitis (although the histopathologic basis for concomitant hydronephrosis and megacholedochus are yet to be determined). A good clinical response to immunosuppressive treatment has led some to hypothesize that the intestinal myopathy may be a direct result of an autoimmune phenomenon in the bowel wall[5], and a common autoantibody against smooth muscle cells has been proposed[4]. It has been shown that autoantibodies against proliferating cell nuclear antigen (PCNA) have been detected exclusively in SLE patients and 2 cases were presented in which patients with this antibody in systemic sclerosis developed CIPO[7]; whether PCNA autoantibodies play a role in GML, however, remains unclear. Dense T-lymphocytic infiltrates with degeneration limited to the muscularis propria is considered the histopathologic hallmark of autoimmune enteric myositis causing CIPO in young children without lupus[8-10]. The location of intestinal myocyte damage is similar in SLE-associated CIPO, but dense T-cell infiltrates have not been observed; thus it is unclear whether the same disease process is involved. Autoimmune enteric neuropathy has been considered in the etiology of SLE-associated CIPO, but neuronal structures have generally been preserved on histopathology[3,5], making this less likely. It has also been suggested that serositis can cause paralytic ileus, and this may be a secondary cause of intestinal pseudo-obstruction in SLE[4]. Further studies will be needed to elucidate the pathogenic mechanism behind the development of GML.

In prior anectodal reports, medical management that effectively led to improvement of GML, including remission of intestinal pseudo-obstruction and urinary symptoms, included a combination of high dose corticosteroids, immunomodulators and supportive care (parenteral nutrition, oral antibiotics, and pharmacological stimulation of small bowel motility)[2-5]. We had expected improvement and remission with similar management of our patient. Unfortunately, although there was improvement in the clinical and biochemical indicators of lupus exacerbation, e.g., ascites, arthralgias, proteinuria and serum acute phase reactants, she continued to show signs and symptoms of biliary dilatation, worsening of renal function, persistence of bowel distension and required parenteral nutrition and hemodialysis. This was concerning because of persistent generalized smooth muscle dysfunction causing aperistaltic megaviscera. While the clinical course is the best way to monitor treatment response, histopathologic reversal with immunosuppressive therapy has been documented for myositis-related intestinal pseudo-obstruction[11]. A delay in initiation of therapy has been associated with failure to regain functional peristalsis and was correlated with histopathologic progression to fibrosis and atrophy of the intestinal wall, and secondary impairment of the myenteric plexuses[12]. Progression to atrophy and fibrosis of the muscularis propria was also observed in a case of suspected non-compliance with immunosuppressive therapy in an SLE patient with CIPO[5]. Similarly, we presume that in the setting of delayed intervention and medication non-compliance, our patient developed advanced irreparable tissue destruction including myonecrosis, fibrosis and atrophy, resulting in failure of peristalsis to return despite treatment with high dose pulse steroids during her inpatient stay.

Our report emphasizes that timely diagnosis and intervention is crucial in the management of GML for the return of peristaltic activity in the various visceral organs involved. Appropriate imaging of the

gastrointestinal, genitourinary and hepatobiliary tracts should be obtained early in the investigation. It is also vital to recognize intestinal pseudo-obstruction as the cause of the symptoms and signs in order to avoid unnecessary and invasive interventions, which may confer extra risks and lead to further damage. Neostigmine was administered twice to our patient, and caused increased crampy abdominal pain and discomfort but failed to improve gut motility. Given that neostigmine acts at the myoneural junction upstream of the damaged myocyte, it is unlikely to help in this setting. Moreover, ureteral stenting has been utilized in lupus cystitis but is largely without benefit in the setting of aperistaltic ureters; this procedure caused multiple complications in our patient including ascending urinary tract infections and hematuria. The Gastroenterology service was consulted repeatedly to perform endoscopic retrograde cholangio-pancreatography (ERCP) for evaluation of her dilated biliary ducts. Biliary tree dilatation is without consequence, especially with normal liver function tests and reflects aperistalsis within the bile duct system. However, knowledge of this is essential to avoid unnecessary invasive procedures such as ERCP and/or biliary stenting. In our patient, a more conservative imaging modality, MRCP, was equally useful to rule out structural obstruction and avoided ERCP related risks. Overall, it seems that early recognition of generalized visceral organ dilatation in lupus patients, consistent with the syndrome GML, is helpful for gastroenterologists, urologists, and rheumatologists to initiate supportive care and early immunomodulation to restore peristaltic function as well as to avoid invasive procedures which may not address the basic pathophysiologic process involved.

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CASE REPORT

Infiltrating adenocarcinoma arising in a villous adenoma of the anal canal

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Abstract

Primary neoplasms arising in the anal canal are relatively unusual. In particular, adenomas and adenocarcinomas are distinctly rare entities in this region. We describe an infiltrating, well-differentiated adenocarcinoma arising in a villous adenoma from the distal anal canal, in an otherwise healthy patient at low risk for gastrointestinal malignancy. This is the case of an octogenarian man with a several year history of hemorrhoids and intermittent rectal bleeding, more recently complaining of continuous hematochezia. Examination revealed a blood-covered pedunculated mass with a long stalk protruding from the anus. The lesion was amputated at the bedside. Microscopic evaluation revealed an infiltrating well-differentiated adenocarcinoma, arising from a villous adenoma. This was further evaluated under anesthesia and complete excision of distal anal tissue was performed. Our report is the first describing the possible malignant degeneration of a villous adenoma in the anal canal.

Key words: Anal adenocarcinoma; Anal canal; Villous adenoma

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INTRODUCTION

The anal canal is comprised of several tissue types unique to this anatomic transition zone, ranging from colonic mucosa to keratinized epithelium with skin appendages. Anal neoplasms are a relatively uncommon occurrence in the context of all gastrointestinal tumors. Benign anal adenomas arising from the anus are particularly rare diagnoses. Furthermore, malignant neoplasms affecting the anal canal are most often of the squamous cell type and very seldom adenocarcinoma, especially in patients without known risk factors for the development of epithelial neoplasms, such as those with inflammatory bowel disease. The following describes an otherwise healthy octogenarian patient diagnosed with a well-differentiated adenocarcinoma. Although rarely reported, we concluded that this tumor may have arisen from epithelial components of the anal canal, following the sequence normal epithelium-adenoma-carcinoma (Vogelstein model) which, although has been well-described for colorectal tumors, it has only been reported twice previously.

CASE REPORT

An 81 year-old Caucasian man presented to the emergency department with the chief complaint of continuous rectal bleeding of several months duration, which was particularly associated with bowel movements.

He reported chronic intermittently bleeding hemorrhoids for about 20 years. He denied comorbid conditions and took no medications. He also denied recent weight loss, change in appetite or bowel habits. This patient reportedly underwent a colonoscopic evaluation about four years prior to the current presentation, with negative results. His family history was unremarkable for gastrointestinal benign or malignant problems. Physical examination revealed a gentleman who appeared younger than the stated age, in no acute distress. Perianal visual and digital examination showed a blood-covered 3 cm × 4 cm pedunculated mass protruding from the anus by a stalk. Significant laboratory data demonstrated microcytic anemia (hemoglobin of 6.4 mg/dL) and normal coagulation times. He was transfused with packed red blood cells and the lesion was amputated at the bedside. Microscopic analysis revealed an infiltrating well-differentiated adenocarcinoma arising from a villous adenoma (Figure 1). Computed tomography of the chest, abdomen and pelvis demonstrated no evidence of metastatic disease. The patient was subsequently taken to the operating room for examination under anesthesia and complete excision of the anal mass. Intraoperative findings revealed very large hemorrhoids, of 0.5 cm in diameter on average occupying the entire circumference of the anus (Figure 2). Gentle dilation of the anus revealed an ulcerated area of 2 cm in length at 5 o'clock in the supine position extending from the anal orifice to about 1 cm into the anal canal. Hemorrhoidectomy was performed and the ulcerated tissue was excised. Microscopic evaluation showed evidence of the prior procedure, but did not show residual neoplasm. This tumor was staged as $T_1N_0M_0$.

Detailed microscopic evaluation of the mass showed villous fronds, lined by dysplastic, intestinal-type epithelium with scattered goblet cells. Several microscopic foci of invasion into the lamina propria were identified. The neoplasm demonstrated diffuse staining for cytokeratin (CK)-20 and was negative for CK7 (Figure 3). Histologic examination of the residual anal tissue excised in the operating room clearly showed reactive (not malignant) rectal-type glands present near the surface, in between areas of squamous epithelium, as well as ulceration of superficial mucosa, consistent with the prior excision. No residual adenoma or carcinoma was identified.

This case was discussed at our local tumor board conference. No consideration for further surgical or medical therapy was deemed appropriate for his optimal care. The patient was contacted two years after his intervention, and he is doing well, without recurrence of rectal bleeding or any other problems related to the tumor herein presented.

DISCUSSION

Primary anal cancer is a rare occurrence. However, its incidence has been rising over the last 25 years. Moreover, despite the small number of patients affected by these malignancies, they remain as one of the most challenging cancers to treat. Once thought to be related to chronic irritation, multiple risk factors, including human papillomavirus or human immunodeficiency virus infection, anal sexual intercourse, smoking and immunosuppression, have relatively recently been identified[1]. Anal tumors represent 5% of anorectal cancers. They have been classified in tumors of the anal canal and those of the anal margin, with mainly two histological types described. Among them, squamous carcinoma comprises approximately 95% of anal tumors[2].

Anal adenocarcinomas constitute about 5% of malignant anal neoplasms[2]. The latter tumors have been described in correlation with anal fistulae[3], hemorrhoids[4], after restorative proctocolectomy with a stapled ileal pouch-anal anastomosis without mucosectomy due to ulcerative colitis[5,6], or with Crohn's disease[7]. Together, they account for 0.1% of all gastrointestinal cancers[8].

The progression of the normal colorectal epithelium to malignancy has been extensively studied in both experimental and clinical models, and a defined normal mucosa-adenoma-carcinoma sequence has been well recognized[9-11]. However, in the anal canal this information is lacking. Benign anal adenomas are extremely rare diagnoses. Multiple adenomatous polyps arising in the transitional zone of the anus in a patient with familial adenomatous polyposis (FAP) seven years after colon resection and ileo-anal anastomosis were described by Malassagne and colleagues[12]. Anand *et al*[13] reported a tubulo-villous adenoma which arose from either an anal gland or its duct that opens into the anus. These tumors are rarely encountered in patients without predisposing risk factors, such as FAP, ulcerative colitis or Crohn's disease[13]. The above-mentioned transformation from normal epithelium to malignant tumors in the anal canal has only been previously recognized twice to the best of our knowledge. MacNeill and colleagues[14] described the case of an invasive apocrine adenocarcinoma arising in a benign adenoma in the perianal region of a 45-year-old woman. Obaidat *et al*[15] reported the occurrence of anal adenocarcinoma *in situ* associated with a tubulopapillary apocrine hidradenoma. These reports suggest that adenomatous elements from the squamocolumnar junction may demonstrate hyperplastic or malignant transformation in patients without genetic predisposition. Intraoperative examination in our case made us believe that an adenomatous polypoid lesion originated from distal anal tissue in an area of chronic inflammation and ulceration consistent with the anal transition zone. Some investigators, however, have found evidence to support alternative pathways to the adenoma-carcinoma sequence[16]. Specific mutations in key onco-suppressor genes have been found to relate to the anatomical site of the tumor and therefore, a significant proportion of rectal cancers may arise *via* alternative pathways to the Vogelstein model. Polyp behavior along with malignant propensity may actually be site dependent. Rectal polyps are thought to harbor a more aggressive phenotype. In

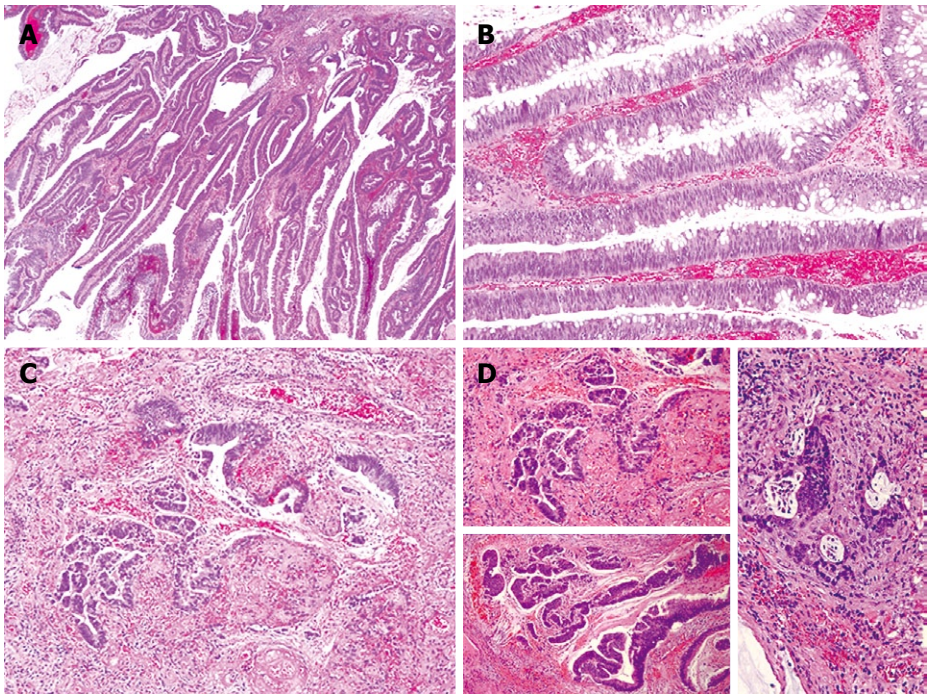

Figure 1 Microscopic sections. A: The adenoma demonstrate villous architecture with long, delicate fronds (HE, × 40); B: The villous fronds are lined by dysplastic, intestinal-type epithelium with frequent goblet cells (HE, × 100); C and D: Multiple foci of invasive carcinoma are identified, with invasion confined to the lamina propria (HE, × 100).

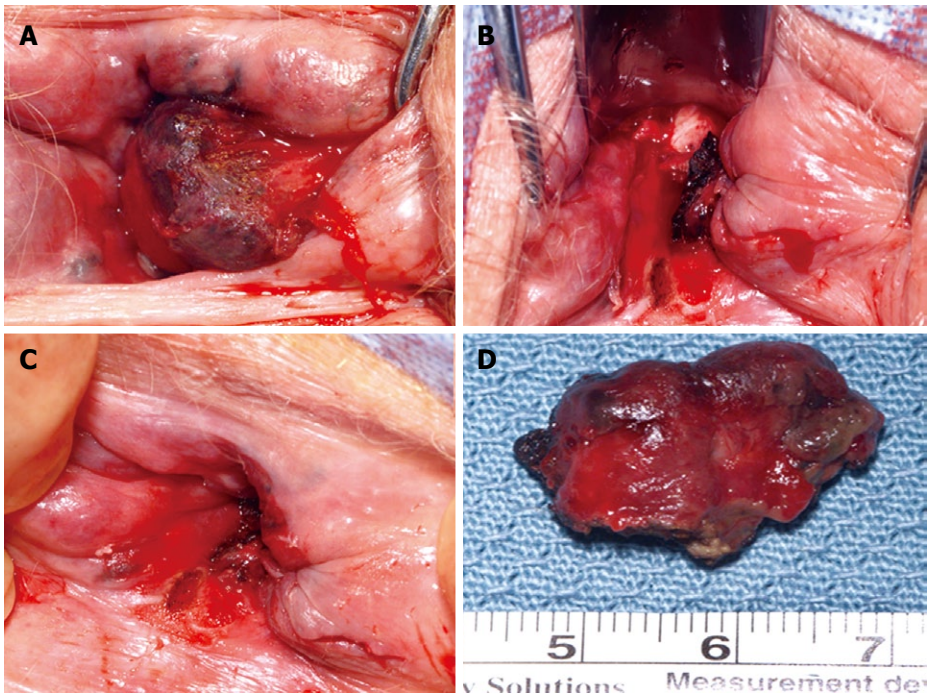

Figure 2 Intraoperative photographs. A and D: Very large hemorrhoids up to 2 cm circumferentially located around the anal canal; B: The final appearance after complete excision of the hemorrhoids and the malignant mass; C: The area that contained the adenocarcinoma, previously excised in the emergency department, and prior to operating room excision.

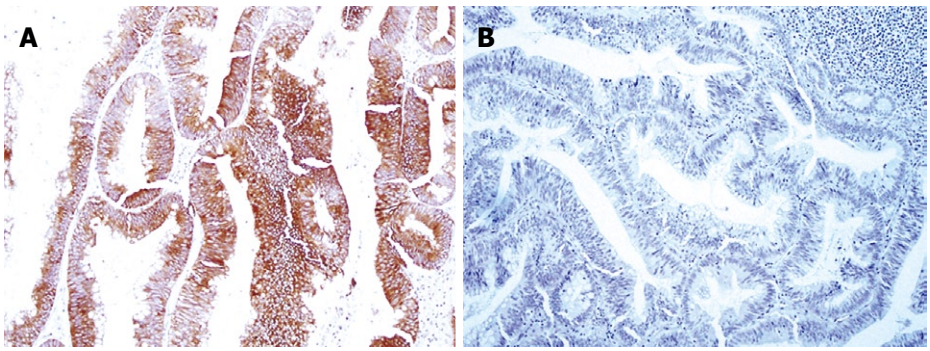

Figure 3 Immunohistochemical staining. A: Cytokeratin 20 is diffusely positive within the epithelium (× 100); B: Cytokeratin 7 is negative (× 100).

the anal area, this remains uncertain.

CK-20 positivity with negative CK7 staining is an immunohistochemistry profile most consistent with rectal-type mucosa and is not in keeping with the

apocrine or colloid types of adenocarcinoma that have been typically described as arising from the anal canal. In addition, the histologic appearance in this case of a well-formed villous adenoma with microscopic invasive carcinoma is different from typical anal gland carcinoma, which is characterized by small, tubular structures diffusely invading stroma. Clinical and pathologic findings in our case point to a true anal location for this pedunculated mass. Visual inspection showed this mass to arise distal to the dentate line, whereas histologic examination of the final excision specimen showed rectal-type glands present in between areas of normal squamous epithelium. The combination of these findings suggest the development of a tubulo-villous adenoma from rectal-type glands present in the anal mucosa, possibly as part of a reactive metaplastic phenomenon, similar to what was described in the case report from Anand and colleagues[13]. However, these authors did not report malignancy or immunohistochemistry findings. Therefore, whether the histologic profile of their case and ours is the same is unknown.

For anal adenocarcinoma, aggressive surgical resection remains the mainstay of therapy, with radiation therapy and chemotherapy used to aid in local disease control and for treatment of metastatic disease. A high rate of distant failure in this disease is responsible for the poor long-term prognosis[17]. Once invasive adenocarcinoma features were identified on pathologic examination of the polyp excised in the emergency department, this patient was taken to the operating room to ensure adequacy of resection. Some may argue that, to increase the chance of survival in this octogenarian, a larger extent of resection is needed. An analysis of 13 patients with primary anal adenocarcinoma who were treated over a 12-year period was performed[18]. With a median follow-up of 19 mo, the median survival was 26 mo, local failure rate was 37%, and the two-year actuarial survival was 62%. This study, although small, suggested that the combination of abdomino-perineal resection and combined modality therapy was a reasonable approach for this rare tumor[18]. However, this treatment strategy has to be individualized to both the patient and the tumor characteristics, as well as to the patients' wishes.

The need to perform lower gastrointestinal endoscopy in patients at low risk for colorectal malignancies is debatable. Patients such as the one presented herein, had rectal bleeding as the presenting symptom and without other alarming signs for colorectal cancer, such as recently altered bowel habit, weight loss and family or personal history of colorectal neoplasms, which were studied with colonoscopy by Sotoudehmanesh *et al*[19]. A number of unexpected findings were identified, such as adenomatous polyps (3%), ulcerative colitis (6%) and Crohn's disease (0.7%). Fissures were posterior in 106 cases (79.1%) and anterior in 27 cases (20.1%); one patient (0.7%) had both anterior and posterior fissures. The lower gastrointestinal endoscopy was abnormal in 10.4% of patients. No cases of adenocarcinoma were identified. Therefore, lower gastrointestinal endoscopy might be appropriate in this selected group of patients, if these findings are confirmed by further studies[19]. Carlo *et al*[20] specifically studied patients with hematochezia but without risk factors for neoplasia, and concluded that those patients younger than 45 years of age do not need a total colonoscopy, whereas they recommended mandatory total colonoscopy in older patients even in the presence of anal pathology. In the latter group they were able to identify proximal colonic inflammatory bowel disease, neoplastic polyps and even carcinoma.

In conclusion, the transition between normal tissue-adenoma-carcinoma has been well studied in the colorectal arena but very infrequently reported in the anal canal. Our report supports the application of the same concept for the anal region, at least for patients without major risk factors for the development of gastrointestinal malignancies. The infrequent occurrence of anal adenocarcinoma and the paucity of reports in the literature documenting these tumors make conclusions about this subject difficult to reach. The literature seems to suggest further endoscopic studies of the rest of the gastrointestinal tract in elderly patients with rectal bleeding and no risk factors for colorectal cancer.

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CASE REPORT

Extramedullary plasmocytoma associated with a massive deposit of amyloid in the duodenum

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Abstract

We report a rare case of extramedullary plasmocytoma associated with a massive deposit of amyloid in the duodenum. A 72-year-old Japanese man was admitted to our hospital presenting with a 3-mo history of epigastric pain, vomiting and weight loss. On computed tomography (CT) a wall thickening of the fourth part of the duodenum was observed. Multiple biopsies obtained from the lesion showed infiltration of plasma cells and lymphocytes, but they were not conclusive. The patient underwent resection of the lesion and, on histopathological examination, the lesion consisted of a dense and diffuse infiltrate of plasma cells and a few admixed lymphocytes with reactive follicles extending to the muscular propria. An extensive deposition of amyloid was also observed. Immunohistochemical stains revealed that a few plasmacytoid cells showed λ light chain staining, though most were κ light chain positive. These cells also were positive for CD138 and CD56 but negative for CD20 and CD79. The findings were consistent with extramedullary plasmocytoma associated with a massive deposit of amyloid in duodenum. A subsequent workup for multiple myeloma was completely negative. The patient showed no signs of local recurrence or dissemination of the disease after 12 mo follow-up. Because of the association of plasmocytoma and amyloidosis, the patient must be followed up because of the possible systemic involvement of the neoplasm and amyloidosis in future.

Key words: Extramedullary plasmocytoma; Amyloidosis; Duodenum; Plasma cell neoplasms; Immunohistochemistry

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INTRODUCTION

Plasma cell neoplasms account for approximately 1%-2% of human malignancies and they are classically categorized into four groups: multiple myeloma (MM), plasma cell leukemias, solitary plasmacytomas of the bone (SPB), and extramedullary plasmacytomas (EMP). EMP represents 3% of these neoplasms and it is defined as a soft-tissue plasma cell tumor occurring in the absence of systemic signs of multiple myeloma[1]. Most cases of EMP arise in the upper aerodigestive tract (UAD); other sites of involvement include the gastrointestinal tract, breast, thyroid, testis, bladder, retroperitoneum, and lymph nodes. In non-UAD regions, the gastrointestinal tract represents 40% of cases[2]. We reported an unusual case of localized

plasmocytoma associated with massive local deposition of amyloid in the duodenum.

CASE REPORT

A 72-year-old Japanese man was admitted to our hospital presenting with a 3-mo history of epigastric pain, vomiting and weight loss. No abdominal mass was palpated on physical examination. An upper endoscopy revealed a mass in the duodenum. CT showed wall thickening of the fourth part of the duodenum (Figure 1). Lymphadenopathy was not seen. Multiple biopsies obtained from the lesion showed infiltration of plasma cells and lymphocytes, but they were not conclusive. The patient underwent resection of the fourth part of the duodenum and proximal segment of jejunum. On gross examination of the surgical specimen, the involved segment measured 5 cm in length (Figure 2). On histopathological examination, the lesion consisted of a dense and diffuse infiltrate of plasma cells and a few admixed lymphocytes with reactive follicles extending to the muscular propria. An extensive deposition of hyaline amorphous eosinophilic extracellular material was also observed (Figure 3). With Congo red stain, amyloid appeared red in normal light and apple-green in polarized light. Immunohistochemical stains revealed that a few plasmacytoid cells showed λ light chain staining, while most were κ light chain positive (Figure 4A and B). These cells also were positive for CD138 and CD56 but negative for CD20 and CD79 (Figure 4C and D). The scattered lymphocytes represented a mixture of CD3-positive T cells and CD20-positive B cells. The extracellular hyaline materials also showed reactivity with anti-kappa (but not lambda) immunoglobulin light chains. The findings were consistent with a plasma cell neoplasm. A subsequent workup for MM (serum and urinary protein electrophoresis, radiographic examination of the axial skeleton and a bone marrow aspirate and biopsy) was completely negative. Anemia and hypercalcemia were not seen. The patient showed no signs of local recurrence or dissemination of the disease after 12 mo follow-up.

DISCUSSION

The involvement of gastrointestinal tract is common in MM but a localized plasmocytoma is rare and not suspected clinically. In previous reports[3-6] of duodenal EMP, males were more affected than females and the disease was most commonly seen in patients older than 50 years of age. These patients presented with nonspecific symptoms such as dyspepsia, vomiting, epigastric pain and weight loss. As in our case, in these previous reports radiological features were not suggestive and the diagnosis was revealed only by the findings of histopathological and immunohistochemical examination of the sample biopsy or surgical specimen, mainly the light chain restriction (demonstrating the monoclonality) and positivity for CD138 (indicating plasma cell lineage).

Treatment options for EMPs include surgical resection and radiation therapy, given the relative sensitivity of plasma cell neoplasms to radiation[2,7]. Alexiou *et al*[2] found no statistically significant difference in patient survival comparing surgery alone, surgery plus radiotherapy, or radiotherapy alone. Surgery alone, however, had the lowest recurrence rate. Our patient was not submitted to radiation after surgery. Of all of the plasma cell tumors, EMPs have the best prognosis. Progression to MM is more frequent in solitary osseous plasmacytoma than in EMP[8,9]. The prognosis of patients with EMP of the duodenum is uncertain as so few patients have been reported, but, of those cases reported in the literature, we found two that presented evolution to myeloma after surgery[10,11].

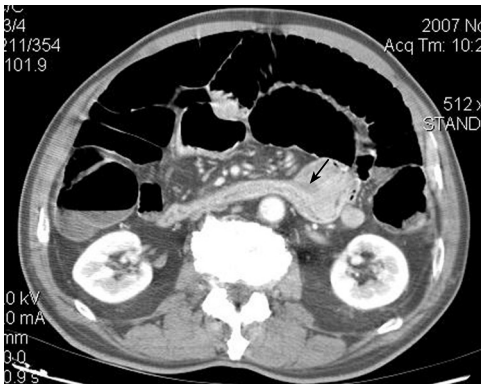

Figure 1 CT showing a mass on the fourth part of the duodenum (arrow).

Amyloidosis, a complication of plasmacytoma in the present case, is characterized histopathologically by the extracellular deposition of insoluble fibrillar proteins. In our case, amyloidosis was caused by the deposition of immunoglobulin light chains since both amyloid and plasma cells shared the same immunoglobulin light chain restriction (lambda-restriction). The absence of systemic amyloidosis in massive localized deposits, as in our case, may be explained by the secretion of abnormal, poorly soluble immunoglobulin molecules with a tendency toward local precipitation[12]. We did not find previous cases of amyloidosis associated with duodenal plasmocytoma, so the impact of associated amyloid accumulation on progression or response to treatment in our case cannot be determined. Amyloid deposition can be found in 15% to 40% of extramedullary plasmacytomas in the head and neck regions and, according to previous reports, without clinical significance[13].

In view of their indolent biological nature and their frequent involvement of anatomic sites containing mucosal lymphoid tissues, it has been proposed that extramedullary plasmacytomas represent a low-grade lymphoma of mucosal lymphoid tissues (MALT) with extensive plasmacytic differentiation. Results from a previous study support this hypothesis[14]; morphologic features of MALT lymphoma, including centrocytic-like cells, reactive follicles, and lymphoepithelial lesions, are often found in extramedullary plasmacytomas. Besides this, amyloid deposits have also been described in lym-

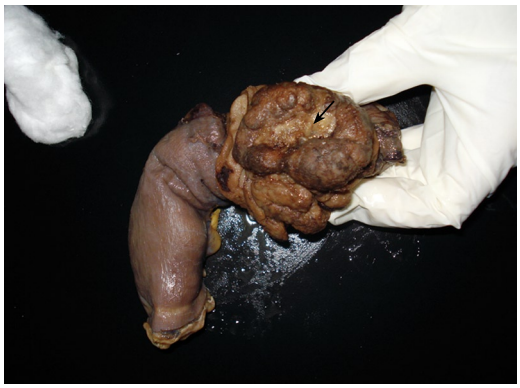

Figure 2 Gross appearance of the surgical specimen containing the tumor (arrow).

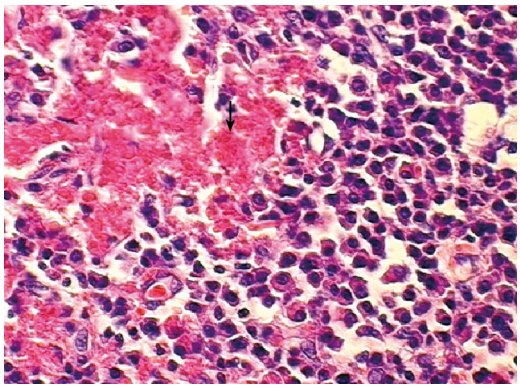

Figure 3 Histopathological examination displaying a dense and diffuse infiltrate of plasma cells and amyloid deposit (arrow). (HE × 400).

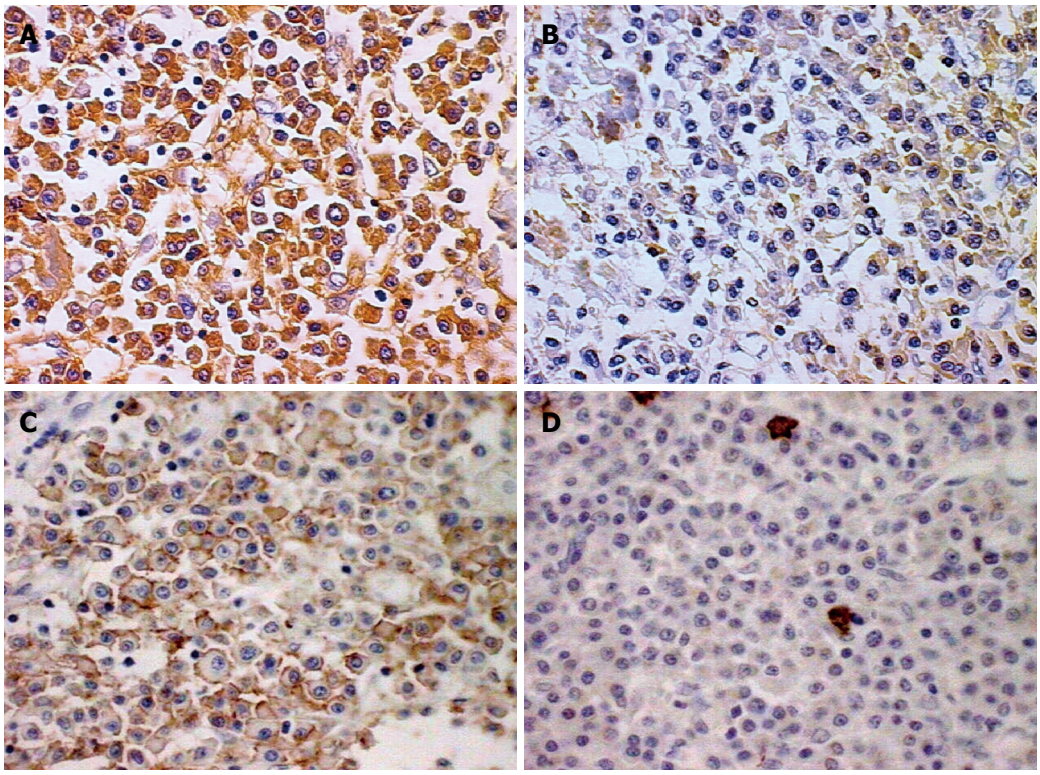

Figure 4 Immunohistochemical findings (positive cells in brown, × 400). A: Most plasmocytoid cells were positive for κ light chain; B: Few plasmacytoid cells showing λ light chain staining; Plasmocytoid cells were positive for CD56 (C) and negative for CD20 (D).

phomas[15]. However, according to others authors, some immunohistochemical findings on plasmocytoid cells are helpful in differentiating plasmacytoma from MALT lymphoma[16,17]. These authors found that plasmocytoid cells were less likely to express CD56 in lymphoma than in myeloma. So, the positivity for CD56, in our case, suggests that it may indeed represent a true primary PC dyscrasia (plasmacytoma), with phenotypic feature similar to myeloma.

In conclusion, plasmocytoma localized in the duodenum is rare and may cause intestinal obstruction. The diagnosis is performed by histopathological examination and must be distinguished from lymphomas with extensive plasmacytic differentiation. In associations of EMP and amyloidosis, the patient must be followed up because of the possible systemic involvement of the neoplasm and amyloidosis in future.

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CASE REPORT

Laparoscopic diagnosis of pleural mesothelioma presenting with pseudoachalasia

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Abstract

Pseudoachalasia due to pleural mesothelioma is an extremely rare condition. A 70-year-old woman presented with progressive dysphagia for solid and liquids and a mild weight loss. A barium swallow study revealed an esophageal dilatation and a smoothly narrowed esophagogastric junction. An esophageal manometry showed absence of peristalsis. Endoscopy demonstrated an extrinsic stenosis of the distal esophagus with negative biopsies. A marked thickening of the distal esophagus and a right-sided pleural effusion were evident at computed tomography (CT) scan, but cytological examination of the thoracic fluid was negative. Endoscopic ultrasound showed the disappearance of the distal esophageal wall stratification and thickening of the esophageal wall. The patient underwent an explorative laparoscopy. Biopsies of the esophageal muscle were consistent with the diagnosis of epithelioid type pleural mesothelioma. An esophageal stent was placed for palliation of dysphagia. The patient died four months after the diagnosis. This is the first reported case of pleural mesothelioma diagnosed through laparoscopy.

Key words: Dysphagia; Achalasia; Pseudoachalasia; Mesothelioma; Laparoscopy

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INTRODUCTION

Esophageal achalasia is an uncommon disorder, with an incidence of about one case per 100 000 persons per year. The term "pseudoachalasia" generally applies to a subgroup of less than 5% of patients presenting with clinical features of achalasia in whom an occult malignant tumor is the cause of dysphagia[1]. Benign pseudoachalasia is an even more unusual condition, accounting for less than 2% of patients with achalasia-like symptoms[2]. Adenocarcinoma of the esophagogastric junction represents the most frequent diagnosis in patients with malignant pseudoachalasia, accounting for 70% of cases[3]. Other causes include lymphoma[4-6] and primary neoplasms from the lung, esophagus, liver, pancreas, colon, kidney, prostate, and breast[7-14].

Malignant mesothelioma has been infrequently associated with dysphagia. In some reports, dysphagia was secondary to direct extension of the cancer into the esophagus and was a late, usually terminal, event[15]. The first case of pleural mesothelioma presenting as dysphagia was described by Johnson in 1983[16]. We report the case of a patient presenting with pseudoachalasia in whom the diagnosis of pleural mesothelioma was performed by laparoscopy.

CASE REPORT

A 70-year-old woman presented in December 2007 with a two-month history of progressive dysphagia for solids and liquids, and a 5-kg weight loss. There was no prior history of gastroesophageal reflux, odynophagia, or other gastrointestinal symptoms. Social history was negative for tobacco use and alcohol intake. Physical examination was unremarkable and routine laboratory tests were within normal limits. A barium swallow study revealed a mildly dilated esophagus and a smoothly narrowed esophagogastric junction (Figure 1). An

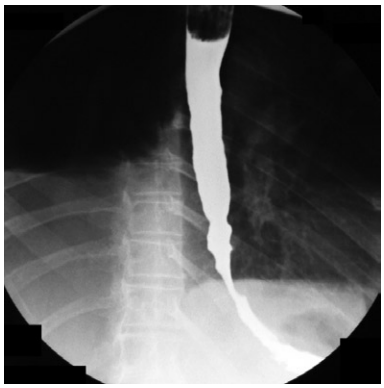

Figure 1 Barium swallow showing dilatation of the esophageal body and narrowed esophago-gastric junction.

esophageal manometry showed absence of peristalsis (Figure 2), but evaluation of the lower esophageal sphincter was precluded due to the inability to advance the catheter into the gastric cavity. Subsequent upper gastrointestinal endoscopy showed no evidence of gross mucosal lesions, but a mild resistance to the passage of the instrument through the cardia was noted. On retroflexed view, the esophagogastric junction appeared normal. A pneumatic dilation was performed using a 30 mm Rigiflex balloon dilator inflated at 10 PSI for one minute, and multiple esophageal biopsies were performed, which showed no evidence of malignancy.

Three weeks later, due the persistence of dysphagia, a thoraco-abdominal CT scan was performed that demonstrated the presence of a right pleural effusion, thickening of the mediastinal and parietal pleura, and a 4-cm long concentric thickening of the distal esophageal wall (Figure 3). A thoracentesis yielded 1500 cc of yellow fluid with a protein content of 2 g/dL and a pH of 9. Bacteriological and cytological examinations of the fluid were negative. Subsequent endoscopic ultrasound examination showed the disappearance of the distal esophageal wall stratification and thickening of the wall up to 9 mm. At that point, the decision to proceed with an exploratory laparoscopy was taken in an attempt to clarify the diagnosis. Upon incision of the peritoneal reflection, the distal esophageal wall appeared markedly thickened and tightly adherent to the diaphragm. No lymphadenopathy was found in the lesser sac. Peritoneal lavage cytology was negative. Intraoperative ultrasound confirmed the thickening of the distal esophagus.

Multiple biopsies from the esophageal muscle wall and from the contiguous diaphragm were taken. Histopathological findings from the esophageal wall were consistent with the diagnosis of pleural mesothelioma (epithelioid type). Immunohistochemistry was positive for calretinin, vimentin, Ckpan and CK 7 (Figure 4). Soon after surgery the patient complained of recurrent dysphagia and onset of pain in the right side of the chest.

Two weeks later, a right thoracoscopy in the prone position with single-lumen intubation was performed to complete the staging of the disease and to provide drainage of the recurrent pleural effusion. Multiple nodularities and plaques were noted along the diaphragmatic, parietal and mediastinal pleura surfaces, mostly in the lower half of the pleural cavity. Multiple biopsies were repeated, which confirmed the previous histopathological diagnosis. During the same operative session, a porth-a-cath was inserted in the right subclavian vein, and a 10 cm self-expanding esophageal metal stent with antireflux valve (Hanarostent®) was deployed endoscopically. A gastrographin swallow study performed the next day showed a partial stent expansion that required a single endoscopic balloon dilation within the stent. As a result of this treatment, there was a marked improvement of dysphagia but worsening of the chest pain, requiring sustained analgesia. The patient was also started on a chemotherapy regimen. She died from septic shock four months after the diagnosis without stent related complications.

DISCUSSION

Malignant pseudoachalasia is a term used to describe the clinical picture of gastroesophageal junction obstruction associated to an occult submucosal tumor or a non-contiguous tumor. Several mechanisms have been proposed to explain the pathogenesis of this type of secondary achalasia. First, the tumor might directly infiltrate the nerves within the myenteric plexus of the esophagus. Second, a paraneoplastic syndrome might affect the function of the distal esophagus[13]. Third, the tumor might replace the smooth muscle at the esophagogastric junction, reducing compliance of the esophageal wall to distension. In turn, the esophagus might generate high pressures to overcome this obstruction with dilatation as a compensatory response.

Patients with malignant pseudoachalasia are, as a group, older than patients with primary achalasia. In addition, there is a male predominance, in contrast to primary achalasia in which both sexes seem to be equally affected. Tucker[7] suggested that advanced age (> 50 years), short duration of symptoms (< 1 year) and marked weight loss (> 15 pounds) support the diagnosis of secondary achalasia over primary achalasia. When these criteria are met, patients should undergo additional imaging to rule out an occult malignancy. It should also be kept in mind that an occult malignancy cannot be reliably detected even during the course of a laparoscopy esophagomyotomy for presumed primary achalasia[3,17].

Pleural mesothelioma is a rare cause of malignant pseudoachalasia, accounting for 7.5% of all diagnoses[3]. Goldschmiedt *et al*[18] reported of a 64-year-old male presenting with progressive dysphagia and radiological and manometric findings suggestive of achalasia. Thoracentesis and pleural biopsy were performed for a left pleural effusion and no malignant cells were found. The CT scan was negative. At left thoracotomy, multiple pleural plaques covered the diaphragmatic surfaces, as well as the pleura reflection over the lung. A distal esophageal myotomy was carried out and revealed an abnormal tissue consistency at the level of the esophagogastric junction. Biopsy and immunohistochemical studies showed a malignant epithelial mesothelioma.

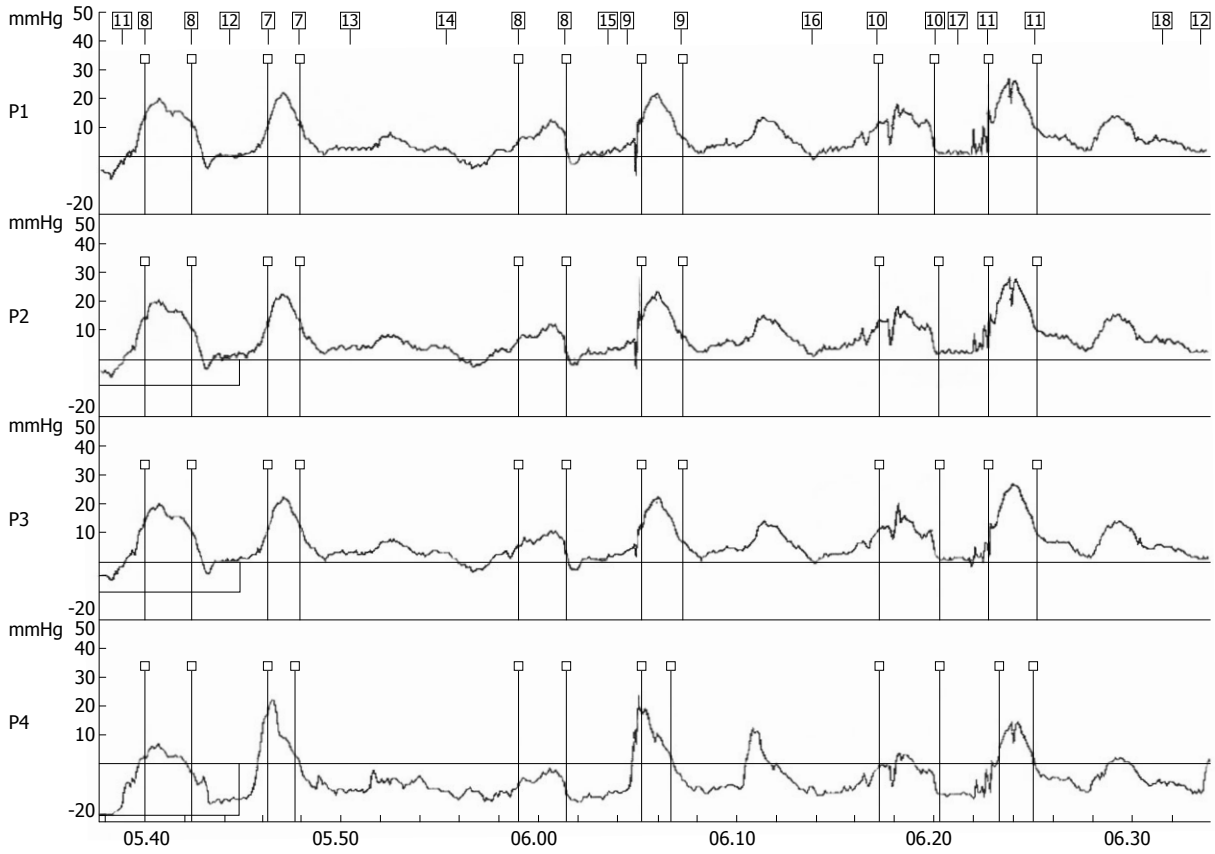

Figure 2 Stationary esophageal manometry showing synchronous waves throughout the esophageal body indicating aperistalsis.

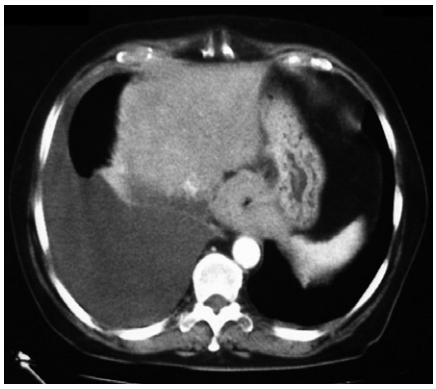

Figure 3 CT scan showing right pleural effusion and concentric thickening of the esophageal wall.

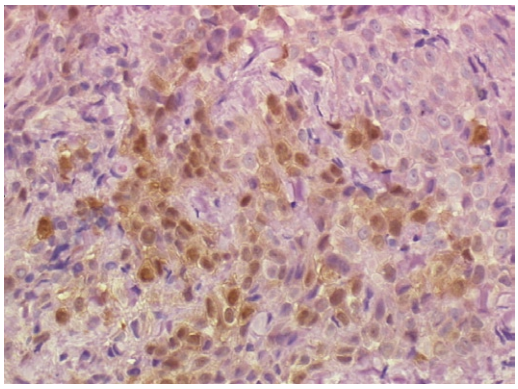

Figure 4 Biopsy of the distal esophageal wall taken at laparoscopy showing a neoplastic cell population consistent with the diagnosis of pleural mesothelioma (epithelioid type). Immunohistochemistry stains positive for calretinin (40 × HPF).

The preoperative diagnosis of pseudoachalasia secondary to pleural mesothelioma is problematic. A CT scan is often inaccurate in diagnosing the extension of the disease through the diaphragm[19]. Thoracentesis is usually the initial diagnostic procedure because most patients present with a pleural effusion. However, pleural fluid cytology is positive for malignancy in less than 50% of patients. Percutaneous pleural biopsy is also unreliable because the small specimens obtained do not allow immunohistochemical studies to differentiate the disease from metastatic adenocarcinoma[20]. There is only one case report showing the efficacy of EUS-guided fine-needle aspiration to diagnose pleural malignant mesothelioma in a patient with CT and endosonographic evidence of a paraesophageal mass[21]. Video-assisted thoracoscopy is considered the diagnostic procedure of choice because it consistently yields a diagnosis without committing the patient to a major surgical procedure through a formal thoracotomy[22]. In our patient, laparoscopy was safe and effective in providing the correct diagnosis. To our knowledge, this is the first reported case of pleural mesothelioma diagnosed through laparoscopy.

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CASE REPORT

Biliary cystadenoma

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Abstract

The diagnosis of cystadenoma is rare, even more so when located in the extrahepatic bile duct. Unspecific clinical signs may lead this pathology to be misdiagnosed. The need for pathological anatomy in order to distinguish cystadenomas from simple biliary cysts is crucial. The most usual treatment nowadays is resection of the bile duct, together with cholecystectomy and Roux-en-Y reconstruction.

Key words: Cystadenoma; Extrahepatic bile duct tumors; Choledochal cyst; Jaundice; Biliary surgery

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INTRODUCTION

Cystadenoma is a benign tumor, although prone to malignant degeneration[1], supposedly originating in intrahepatic (and more rarely extrahepatic) embryonic tissue precursors of biliary epithelium. It is a non recurrent lesion, with only 125 cases reported in literature[2]. It may appear either as a uninodular or as a multinodular cystic lesion, and may attain large proportions.

Cystadenomas account for 4.6% of intrahepatic biliary cysts. They are more recurrent in middle-aged females (40-50 years old) with an incidence rate of 4:1 with respect to males. Cystadenomas are rarely found in extrahepatic bile ducts.

The etiology of cystadenomas remains unclear, although several theories have been suggested. Some authors consider this disease a premalignant lesion. Due to the usual absence of clinical symptoms, the most frequent diagnosis is by chance, as in the excision of a cystic lesion.

In this paper, we report a case of cystadenoma at the excision of a suspected choledochal cyst in an adult female.

CASE REPORT

Our patient was a 60-year-old woman with a history of high blood pressure under treatment. While a laparoscopic cholecystectomy was performed for recurrent episodes of biliary colic, a dilated bile duct was evidenced intraoperatively.

Upon this finding, an informed magnetic cholangioresonance was requested due to dilatation of the choledochal duct. However, it did not show any proximal or distal bile duct dilatation, which was most likely a normal variant (type-1 choledochal cyst according to Todany's classification) (Figure 1). Blood tests with tumor markers were requested, and a CA19.9 of 51.8 was shown, whereas the rest of the tests were normal.

Surgery was performed as planned for the diagnosis of choledochal cyst. The patient underwent resection of the bile duct up to the pancreas joint and before the bifurcation of the hepatic duct, with terminolateral transmeso-

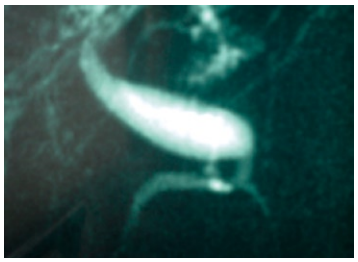

Figure 1 X-ray image showing a choledochal cyst in the bile ducts (Cholangio-NMR).

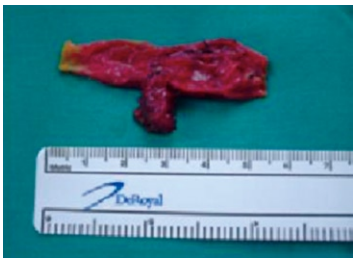

Figure 2 Macroscopy displaying significant dilatation of the bile duct and an evident cyst in the sample.

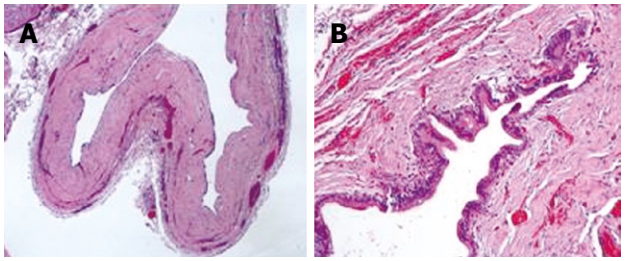

Figure 3 Microscopic view of the sample revealing a multiloculated cystic lesion (A) and a fibrous cyst wall is fibrous lined by epithelium (B).

colic Roux-en-Y hepaticojejunal anastomosis. A type-1 choledochal cyst was discovered macroscopically (Figure 2). The sample was sent to the Pathological Anatomy Service, where it was reported as a 1.4 cm extrahepatic bile duct cystadenoma, with chronic inflammation and bile duct dilatation with regenerative epithelial atypia. No evidence of malignant degeneration was found (Figure 3).

The postoperative course was eventful, with anastomotic leak noted on hepatobiliary iminodiacetic acid (HIDA) scan, central venous catheter-related septicaemia and abscess formation shown by computed tomography (CT), which were solved with percutaneous drainage. Finally, the patient was discharged from the hospital asymptomatically.

DISCUSSION

Biliary cystadenomas arise in the liver in 90% of the cases, and they are much less frequent in the extrahepatic bile ducts, as in our patient. Intra and extrahepatic concomitant cases have also been found. Despite the report of some unilocular cystadenomas, multilocular ones are by far more common. Cystadenomas are usually located in the right lobe of liver, although they may also be present in both lobes or only in the left one of liver.

Macroscopically, their surface is usually flat, and may reach great proportions. The contents are mostly liquid, tending to become mucinous texture, including biliary pigment, hemosiderine and even purulent material if overinfected. Biliary communication has been rarely reported. In some patients, the tumor projects into the bile ducts. In our case, the projection was located in the choledochal wall.

On light microscopy, the inner surface was covered with a cuboidal-to-tall epithelium and some papillary and polypous excrescences, basally oriented nuclei with prominent nucleus and thick chromatin fibers, pale acidophil cytoplasm with mucine-filled vacuoles. According to some authors, this epithelium must be surrounded by a densely cellular mesenchymal stroma with plain muscular fibers and oval cells, which are typical of epithelia[3,4]. However, others claim that hepatic cystadenomas with such features are consistent only with females, males being different in stroma formation. That is why the latter scholars suggest the name of cystadenoma with mesenchymal stroma. Outside this cellular stroma, a dense layer of collagenous tissue separates it from the hepatic parenchyma[5].

The etiology of cystadenomas is unclear. Cystadenomas without mesenchymal stroma have been induced experimentally with aflatoxins in an animal model. This might lead to a possible malignant transformation of simple hepatic cystic lesions[6,7]. Coincidences between cystadenoma, gallbladder embryonic tissue, and main bile ducts tissue have also been found[3]. Stimuli such as ischemia or carcinogenic elements also produce this kind of lesions.

Cystadenoma may display a wide range of symptoms, although it is mainly asymptomatic. The most typical symptoms are a slowly growing abdominal mass, upper abdomen pain, dyspepsia, anorexia, nausea and fever. Jaundice by compression, protrusion, invasion of bile ducts or by secretion of dense mucinous material has been reported[8]. Invasion of the bile ducts may result in pancreatitis episodes. In our patient, we could only reach a diagnosis by the anatomopathological study of the sample, as the patient was asymptomatic except for recurrent biliary colics due to gallbladder lithiasis.

The most widely used diagnosis methods are ecography and tomography. They allow us to observe the cyst formation walls, intracystic projections and possible multilocular arrangement. Since magnetic cholangioresonance provides precise images of the lesion, it is thus the current reference test for tumor study[8-10].

For some scholars, ecologically-guided fine-needle aspiration puncture (FNAP) may be a good diagnostic method, but it may present drawbacks such as the danger of dissemination and its low diagnostic value[11]. CEA levels in cyst liquid help to differentiate cystadenomas from cystadenocarcinomas. Other tests, such as endoscopic retrograde cholangiopancreatography (ERCP), gammagraphy and angiography, may give indirect signs for diagnosis. In blood tests, high levels of CA 19.9 are inconsistent with relation to the lesion. In our case, the rise of this marker occurred inside a cystadenoma[12].

Treatment must be surgical whenever possible, due to a potential malignant degeneration of these lesions. The technique chosen for bile duct sites is complete resection of the bile duct, associating cholecystectomy and recon-

struction with hepatic-jejunostomy in Roux-en-Y. When a partial resection has been done for other reasons and the sample shows evidence for a cystadenoma, complete resection of the bile duct and its reconstruction must be performed. However, this was not necessary for our case, as the bile duct was properly fully removed, and the gallbladder was previously removed.

In the hepatic lobes, enucleation must be the objective. The technique used should be personalised taking into account the placement and the patient in context[13,14].

The patient follow-up is justified in order to avoid possible surgical complications in the bile duct, such as cholangitis, gallstones, estenosis of the anastomosis, and malignant degeneration. In hepatic cystadenomas, the high level of recurrence should be monitored in the postoperative follow-up[13,15,16].

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CASE REPORT

Ascending retrocecal appendicitis presenting with right upper abdominal pain: Utility of computed tomography

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Abstract

Acute appendicitis is a common surgical condition that is usually managed with early surgery, and is associated with low morbidity and mortality. However, some patients may have atypical symptoms and physical findings that may lead to a delay in diagnosis and increased complications. Atypical presentation may be related to the position of the appendix. Ascending retrocecal appendicitis presenting with right upper abdominal pain may be clinically indistinguishable from acute pathology in the gallbladder, liver, biliary tree, right kidney and right urinary tract. We report a series of four patients with retrocecal appendicitis who presented with acute right upper abdominal pain. The clinical diagnoses at presentation were acute cholecystitis in two patients, pyelonephritis in one, and ureteric colic in one. Ultrasound examination of the abdomen at presentation showed subhepatic collections in two patients and normal findings in the other two. Computed tomography (CT) identified correctly retrocecal appendicitis and inflammation in the retroperitoneum in all cases. In addition, abscesses in the retrocecal space (n = 2) and subhepatic collections (n = 2) were also demonstrated. Emergency appendectomy was performed in two patients, interval appendectomy in one, and hemicolectomy in another. Surgical findings confirmed the presence of appendicitis and its retroperitoneal extensions. Our case series illustrates the usefulness of CT in diagnosing ascending retrocecal appendicitis and its extension, and excluding other inflammatory conditions that mimic appendicitis.

INTRODUCTION

The vermiform appendix may occupy several positions in relation to the cecum. The most common positions are descending intraperitoneal (31%-74%) and retrocecal (26%-65%)[1,2]. The location and spread of inflammation from acute appendicitis depends on the location of the appendix. If the appendix is located retrocecally, it may give rise to an abscess in the pararenal space and spread to bare area of the liver[3], or it may spread along the right paracolic gutter, and extend to the right posterior subhepatic and right subphrenic spaces[4]. More than half of the patients with ascending retrocecal appendicitis may have an atypical clinical presentation[5]. We present a case series of ascending retrocecal appendicitis with atypical clinical presentation, and highlight the utility of computed tomography (CT) in diagnosing the condition.

CASE REPORT

During the period January 2003 to December 2006, a computerized search for patients with surgically confirmed retrocecal appendicitis was made. Patients with retrocecal appendicitis and preoperative CT were selected. There were four patients who had atypical clinical presentation with right upper abdominal pain, who formed the study group. The hospital records were reviewed for clinical features, laboratory investigations, surgical findings and follow-up. Preoperative ultrasound and CT were reviewed.

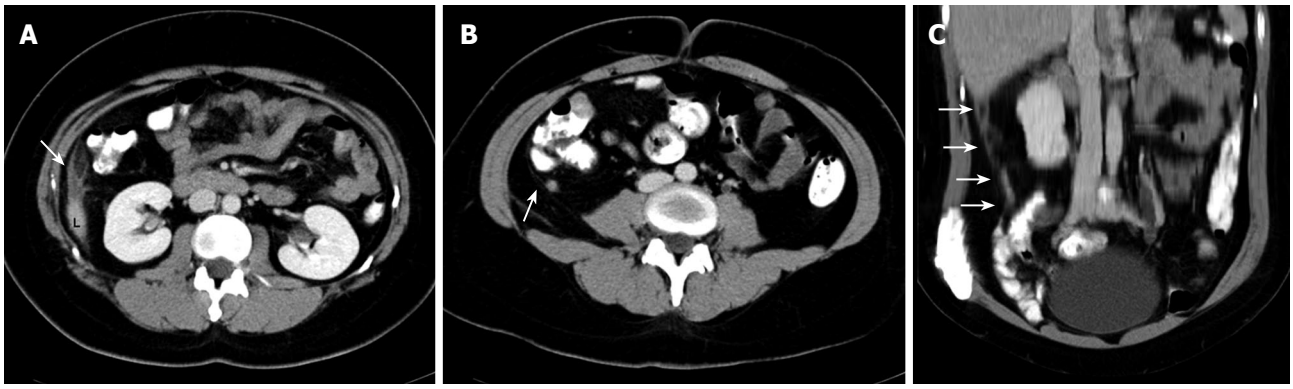

Figure 1 A 30-year-old woman presenting with a clinical diagnosis of acute cholecystitis. A and B: Contrast-enhanced computed tomography (CT) sections showing inflammatory changes (arrow) adjacent to the inferior tip of the liver (L); B: Thickened appendix (arrow) with mild inflammatory changes in the retrocecal region; C: Coronal reconstruction showing the extent of inflammatory changes (arrows) from the retrocecal region to the tip of the liver.

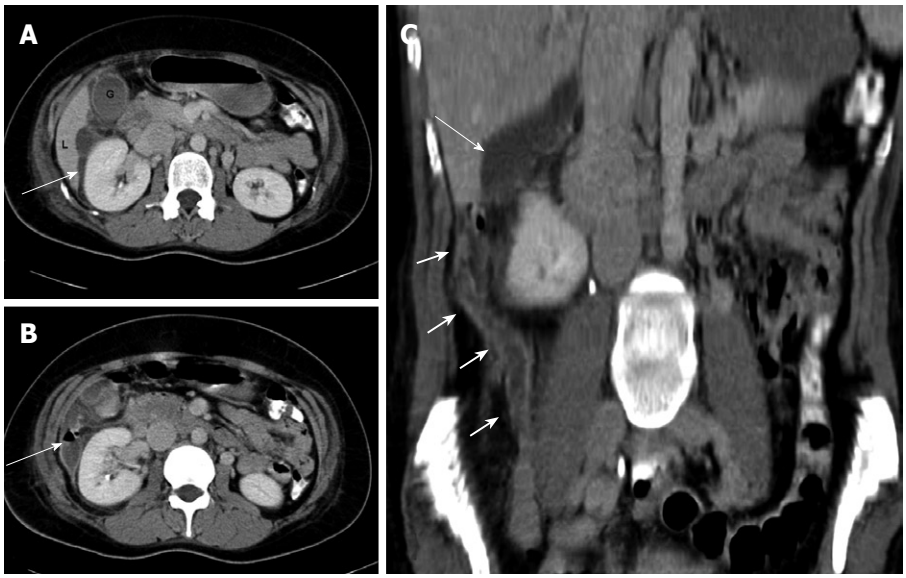

Figure 2 A 31-year-old woman presenting with right hypochondrial pain and a clinical diagnosis of pelvic inflammatory disease and right pyelonephritis. A: Contrast-enhanced CT scan showing fluid collection (arrow) in the subhepatic region, extending anteriorly to the gallbladder fossa with inflammatory stranding; B: Note the air fluid level in the collection adjacent to the right kidney; C: Coronal reconstruction showing the long thickened and inflamed appendix (short arrows) reaching the subhepatic region, and the subhepatic collection (arrow) is seen extending more cranially.

Case 1

A 30-year-old woman presented with a 3-d history of right hypochondrial pain that was constant and radiated to the back. There was right hypochondrial tenderness with negative Murphy's sign and renal punch. She had leukocytosis (13 200 cells/μL). A clinical diagnosis of acute cholecystitis was made. Ultrasound showed a normal liver and gallbladder with no significant abnormality. She was treated initially with antibiotics. A CT scan performed 2 d after presentation showed an inflamed appendix with inflammatory changes in the retrocecal and subhepatic regions (Figure 1). Open appendectomy revealed a moderately inflamed retrocecal appendix with no perforation. Postoperative recovery was uneventful.

Case 2

A 31-year-old woman presented with a 2-d history of right hypochondrial pain that radiated to the back and right shoulder tip, fever with chills, and vomiting. She also complained of foul-smelling urine and vaginal discharge. She had a termination of pregnancy 4 d earlier. The pain was increasing in severity and aggravated by movement and coughing. She was febrile with right hypochondrial rebound tenderness and a positive right renal punch. She had leukocytosis (14 080 cells/μL). The clinical impression was pelvic inflammatory disease with pyelonephritis and hepatobiliary sepsis. Focused assessment with sonography for trauma (FAST) showed no fluid collection in Morrison's pouch, and pelvic ultrasound did not show any collections. CT (Figure 2) at 3 d after admission showed loculated collections adjacent to segment 6 of the liver. A long inflamed retrocecal appendix was seen with surrounding inflammation that extended to involve the hepatic flexure and anterior pararenal space. There was also consolidation of the right lung base. At surgery, there was retrocecal appendicitis with perforation that caused a subhepatic collection. The patient made a good recovery.

Case 3

A 34-year-old man presented with right flank pain of 6 d duration. The pain was colicky and radiated to the back. There was no history of dysuria or urinary frequency, but he also had nausea and vomiting with loss of appetite for 2 d. He had leukocytosis (13 620 cells/μL). The abdomen was soft but the right renal punch was positive. The clinical impression was of right-sided ureteric colic and urinary

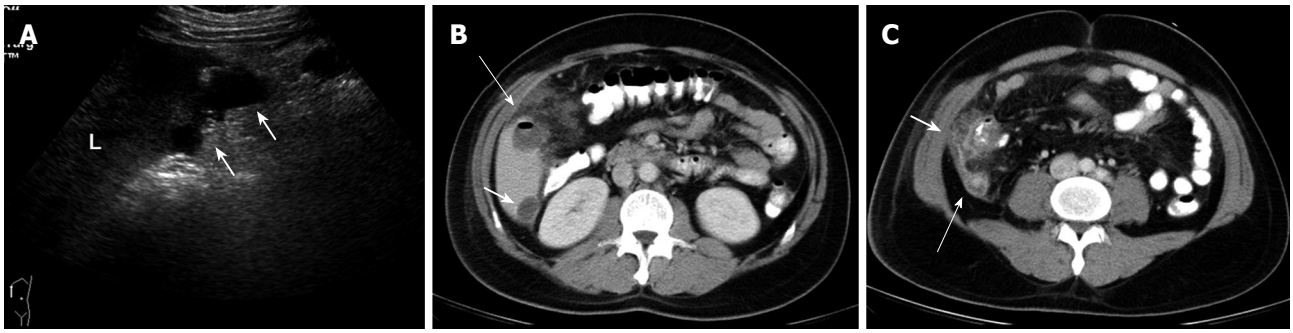

Figure 3 A 34-year-old man with colicky right flank pain and clinical diagnosis of right ureteric colic. A: Ultrasound showed a subhepatic fluid collection (arrows) and no other significant abnormality; B: CT scan performed 2 d later showed the collection in the subhepatic region (short arrow). Note the air-fluid level in the anterior collection (long arrow) with inflammatory changes; C: The section at the level of the cecum and appendix shows inflammatory changes in the retrocecal region (short arrow) and thickened appendix (long arrow).

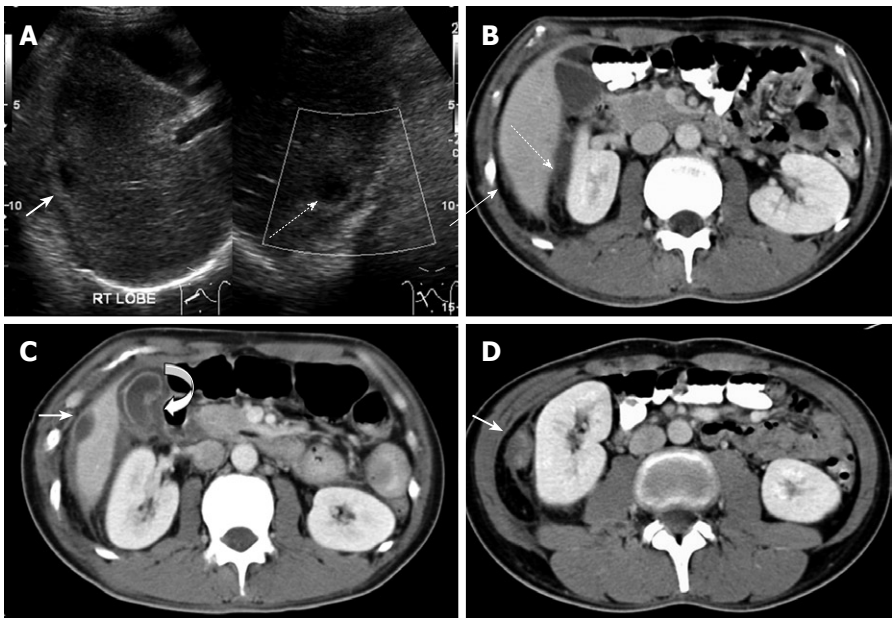

Figure 4 A 27-year-old man with recurrent right upper abdominal pain. A: Ultrasound showed a hypoechoic area in the subphrenic (straight arrow) and subhepatic (broken arrow) region; B: Confirmation by contrast-enhanced CT; C: CT also showed a thickened gallbladder wall (curved arrow), subhepatic collection (white arrow) and inflammation in the perinephric region; D: Another caudal section shows a thickened appendix with inflammatory stranding in the perinephric region.

tract infection. Ultrasound showed no urinary calculi but there was a subhepatic collection (Figure 3) with hyperechoic areas that were suggestive of an abscess. CT (Figure 3) performed on the next day showed the appendix to be swollen and inflamed. There were collections in the subhepatic, rectovesical and paravesical regions. Surgery on the same day confirmed an inflamed and perforated retrocecal appendix with extensive retrocecal collections and abscesses, and a hemicolectomy was performed. The patient recovered uneventfully.

Case 4

A 27-year-old man presented with fever and right hypochondrial pain for 1 d. There was tenderness in the right hypochondrium. He was treated nonsurgically for acute cholecystitis but defaulted from treatment and follow-up. He presented again 2 mo later with fever, vomiting and right hypochondrial pain of 2 d duration. There was tenderness in the right hypochondrium with a positive right renal punch. The total white cell count was elevated at 24700 cells/μL but liver function tests were normal. Ultrasound showed two abscesses in the right hepatic lobe and a thickened gallbladder wall, but no gallstones were seen. He was treated for liver abscesses with intravenous antibiotics and made an excellent recovery. Follow-up ultrasound scans showed resolution of the liver abscesses.

Four months later, he presented again with fever and right hypochondrial pain. Ultrasound showed no significant abnormality. He was treated with a further course of intravenous antibiotics and discharged. He was readmitted 5 mo later. CT showed subphrenic and subhepatic collections with a thickened inflamed appendix (Figure 4). In view of the recurrent collections, diverticulitis was suspected. Colonoscopy was normal and he was managed conservatively. Follow-up CT 3 mo later showed resolution of the collection, but the appendix was still thickened with periappendiceal inflammatory changes. Elective laparoscopic appendectomy was then performed. This showed a high retrocecal appendix with dense adhesions between the appendix and the liver. A final diagnosis of recurrent retrocecal appendicitis was made. The patient made a good recovery with no further episodes.

DISCUSSION

Acute appendicitis may be diagnosed easily and treated

in children and adults if there is a classical history with typical clinical signs[5]. When the appendix is in the retrocecal position, the signs and symptoms of acute appendicitis may be atypical and mimic pathology in the right flank and hypochondrium, such as acute cholecystitis, diverticulitis, acute gastroenteritis, ureter colic, acute pyelonephritis, colon cancer and irritable bowel syndrome[6].

When the clinical impression is of gallbladder, hepatobiliary or urinary tract pathology, ultrasound is often performed. This may show liver abscesses and collections in the subhepatic and right flank regions. Although ultrasound is used frequently in the assessment of suspected acute appendicitis in young children, it requires expertise and dedicated techniques, such as graded compression. The appendix could be visualized in up to 99% of suspected cases of appendicitis in children in one series[7]. However, in adults, un-enhanced CT has been shown to be more sensitive in diagnosing acute appendicitis than ultrasound is[8]. In our series, acute appendicitis was not suspected in any of the patients, therefore, the ultrasound scan performed was not dedicated to rule out appendicitis.

CT is very sensitive for evaluating the appendix, and a thickened appendix, inflamed periappendiceal fat, collections, and presence of free gas in ruptured appendix are detected readily by CT. The inflammatory changes that result from an acutely inflamed ascending retrocecal appendix may extend to the perirenal, adrenal and subhepatic regions, and on rare occasions, inferior extension along the psoas muscle into the thigh has been reported[9,10]. The inflammatory changes are seen most commonly in the retrocolic space (88%), followed by paracolic gutter (30%), pararenal space (27%), mesentery (24%), perirenal space (18%), and less often, in the subhepatic space (3%)[6]. In a recently published series of 33 patients[6] with ascending retrocecal appendicitis who were investigated with CT, only one was found to have a subhepatic collection.

It is interesting that all four of our cases involve young adult patients rather than children. A retrocecal appendix is common and one series showed the appendix to be retrocolic and retrocecal in 58% of cases[1]. A retrocecal appendix has been described also in families and is thought to be inherited as a simple dominant unit character[11]. Studies of the fetal appendix, however, show that it is almost always subcycle during this period[12]. It is possible therefore that the appendix continues to grow during childhood, extending further from the cecum later in life, although there have been no published studies documenting this process. Therefore, retrocecal appendicitis with symptoms remote from the right iliac fossa may occur also in an older age group.

Our case series illustrated a spectrum of uncommon clinical and radiological manifestations of ascending retrocecal appendicitis. This emphasizes the importance of considering the possibility of ascending retrocecal appendicitis in cases in which the signs and symptoms are referred to areas along the possible location of a retrocecal appendix, especially when initial investigations like ultrasound do not support other diagnoses, such as cholecystitis, or hepatobiliary or urinary tract pathology. CT is helpful to establish rapidly the correct diagnosis, as delays in appendectomy for over 24-36 h have been shown to increase the complication rate[13,14].

In summary, CT is useful for evaluation of patients with atypical right upper abdominal pain and nonspecific clinical findings, to rule out the possibility of retrocecal appendicitis.

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Meetings

Events Calendar 2009

January 12-15, 2009
Hyatt Regency San Francisco, San Francisco, CA
Mouse Models of Cancer

January 21-24, 2009
Westin San Diego Hotel, San Diego, CA
Advances in Prostate Cancer Research

February 3-6, 2009
Carefree Resort and Villas, Carefree, AZ (Greater Phoenix Area)
Second AACR Conference
The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved

February 7-10, 2009
Hyatt Regency Boston, Boston, MA
Translation of the Cancer Genome

February 8-11, 2009
Westin New Orleans Canal Place, New Orleans, LA
Chemistry in Cancer Research: A Vital Partnership in Cancer Drug Discovery and Development

February 13-16, 2009
Hong Kong Convention and Exhibition Centre, Hong Kong, China
19th Conference of the APASL
http://www.apasl2009hongkong.org/en/home.aspx

February 27-28, 2009
Orlando, Florida
AGAI/AASLD/ASGE/ACG Training Directors' Workshop

February 27-Mar 1, 2009
Vienna, Austria
EASL/AASLD Monothematic: Nuclear Receptors and Liver Disease
www.easl.ch/vienna2009

March 13-14, 2009
Phoenix, Arizona
AGAI/AASLD Academic Skills Workshop

March 20-24, 2009
Marriott Wardman Park Hotel
Washington, DC
13th International Symposium on Viral Hepatitis and Liver Disease

March 23-26, 2009
Glasgow, Scotland
British Society of Gastroenterology (BSG) Annual Meeting
Email: bsg@mailbox.ulcc.ac.uk

April 8-9, 2009
Silver Spring, Maryland
2009 Hepatotoxicity Special Interest Group Meeting

April 18-22, 2009
Colorado Convention Center, Denver, CO
AACR 100th Annual Meeting 2009

April 22-26, 2009
Copenhagen, Denmark
the 44th Annual Meeting of the European Association for the Study of the Liver (EASL)
http://www.easl.ch/

May 17-20, 2009
Denver, Colorado, USA
Digestive Disease Week 2009

May 29-June 2, 2009
Orange County Convention Center
Orlando, Florida
45th ASCO Annual Meeting
www.asco.org/annualmeeting

May 30, 2009
Chicago, Illinois
Endpoints Workshop: NASH

May 30-June 4, 2009
McCormick Place, Chicago, IL
DDW 2009
http://www.ddw.org

June 17-19, 2009
North Bethesda, MD
Accelerating Anticancer Agent Development

June 20-26, 2009
Flims, Switzerland
Methods in Clinical Cancer Research (Europe)

June 24-27 2009
Barcelona, Spain
ESMO Conference: 11th World Congress on Gastrointestinal Cancer
www.worldgicancer.com

June 25-28, 2009
Beijing International Convention Center (BICC), Beijing, China
World Conference on Interventional Oncology
http://www.chinamed.com.cn/wcio2009/

July 5-12, 2009
Snowmass, CO, United States
Pathobiology of Cancer: The Edward A. Smuckler Memorial Workshop

July 17-24, 2009
Aspen, CO, United States
Molecular Biology in Clinical Oncology

August 1-7, 2009
Vail Marriott Mountain Resort, Vail, CO, United States
Methods in Clinical Cancer Research

August 14-16, 2009
Bell Harbor Conference Center, Seattle, Washington, United States
Practical Solutions for Successful Management
http://www.asge.org/index.aspx?id=5040

September 23-26, 2009
Beijing International Convention Center (BICC), Beijing, China
19th World Congress of the International Association of Surgeons, Gastroenterologists and Oncologists(IASGO)
http://iasgo2009.org/en/index.shtml

September 27-30, 2009
Taipei, China
Asian Pacific Digestive Week
http://www.apdwcongress.org/2009/index.shtml

October 7-11, 2009
Boston Park Plaza Hotel and Towers, Boston, MA, United States
Frontiers in Basic Cancer Research

October 13-16, 2009
Hyatt Regency Mission Bay Spa and Marina, San Diego, CA, United States
Advances in Breast Cancer Research: Genetics, Biology, and Clinical Applications

October 20-24, 2009
Versailles, France
Fifth International Conference on Tumor Microenvironment: Progression, Therapy, and Prevention

October 30-November 3, 2009
Boston, MA, United States
The Liver Meeting

November 15-19, 2009
John B. Hynes Veterans Memorial Convention Center, Boston, MA, United States
AACR-NCI-EORTC Molecular Targets and Cancer Therapeutics

November 21-25, 2009
London, UK
Gastro 2009 UEGW/World Congress of Gastroenterology
www.gastro2009.org

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http//www.cdc.gov/ncidod/EID/eid.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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Write as mean ± SD or mean ± SE.

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Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

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