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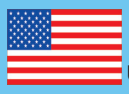

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Editorial Board of *World Journal of Hepatology*
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381892
Fax: +86-10-85381893
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World Journal of Hepatology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381892
Fax: +86-10-85381893
E-mail: wjh@wjgnet.com
http://www.wjgnet.com

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REVIEW

Concept of the pathogenesis and treatment of cholelithiasis

Vasiliy Ivanovich Reshetnyak

Vasiliy Ivanovich Reshetnyak, VA Negovsky Scientific Research Institute of General Reanimatology, Russia Academy of Medical Sciences, Moscow 107031, Russia
Author contributions: Reshetnyak VI solely contributed to this review.
Correspondence to: Vasiliy Ivanovich Reshetnyak, MD, PhD, Professor, VA Negovsky Scientific Research Institute of General Reanimatology, Russia Academy of Medical Sciences, Petrovka Str. 25-2, Moscow 107031, Russia. v_reshetnyak@yahoo.com
Telephone: +7-495-6946505 Fax: +7-495-6946505
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Abstract

Gallstone disease (GD) is a chronic recurrent hepatobiliary disease, the basis for which is the impaired metabolism of cholesterol, bilirubin and bile acids, which is characterized by the formation of gallstones in the hepatic bile duct, common bile duct, or gallbladder. GD is one of the most prevalent gastrointestinal diseases with a substantial burden to health care systems. GD can result in serious outcomes, such as acute gallstone pancreatitis and gallbladder cancer. The epidemiology, pathogenesis and treatment of GD are discussed in this review. The prevalence of GD varies widely by region. The prevalence of gallstone disease has increased in recent years. This is connected with a change in lifestyle: reduction of motor activity, reduction of the physical load and changes to diets. One of the important benefits of early screening for gallstone disease is that ultrasonography can detect asymptomatic cases, which results in early treatment and the prevention of serious outcomes. The pathogenesis of GD is suggested to be multifactorial and probably develops from complex interactions between many genetic and environmental factors. It suggests that corticosteroids and oral contraceptives, which contain hormones related to steroid hormones, may be regarded as a model system of cholelithiasis development in man. The achievement in the study of the physiology of bile formation and the pathogenesis of GD has allowed expanding indications for therapeutic treatment of GD.

Key words: Gallstone disease: Epidemiology; Pathogenesis of cholesterol stones; Treatment

Peer reviewers: Canhua Huang, Professor, The State Key Lab of Biotherapy, Sichuan University, No. 1, Keyuan Rd 4, Gaopeng ST High Tech Zone, Chengdu 610041, Sichuan Province, China; Professor, Dr. Takuji Tanaka, The Tohkai Cytopathology Institute: Cancer Research and Prevention, Minami-Uzura, Gifu City 500-8285, Japan

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INTRODUCTION

Gallstone disease (GD) (cholelithiasis) is one of the most prevalent gastrointestinal diseases, with a substantial burden to health care systems[1]. Gallstones (GS) may form because of many different disorders[2]. GD is a chronic recurrent hepatobiliary disease, the basis for which is the impaired metabolism of cholesterol, bilirubin and bile acids, which is characterized by the formation of gallstones in the hepatic bile duct, common bile duct, or gallbladder[3]. GD and cardiovascular disease, common diseases worldwide, are strongly associated and have considerable economical impact[4-6]. Among gastroenterological diseases, GD is one of the world's most expensive medical conditions[7]. In the United States, there are more than 500 000 cholecystectomies, the total cost of which exceeds 5 billion dollars[8]. GS are considered avoidable causes of death[9].

EPIDEMIOLOGY

GD is a common disorder all over the world[10]. The prevalence of GD varies widely by region. In Western countries, the prevalence of gallstone disease reportedly ranges from approximately 7.9% in men to 16.6% in women[11]. In Asians, it ranges from approximately 3% to 15%, is nearly non-existent (less than 5%) in Africans[12,13], and ranges from 4.21% to 11% in China[14]. The prevalence of gallstone disease is also high in some ethnic groups, e.g., 73% in Pima Indian women; 29.5% and 64.1% of American Indian men and women, respectively; and 8.9% and 26.7% of Mexican American men and women, respectively[11,15,16]. With an overall prevalence of 10%-20%, GD represents one of the most frequent and economically relevant health problems of industrialized countries[17]. There is a steady-state trend for higher GD morbidity, which is associated with the improved diagnosis of the disease. One of the important benefits of early screening for gallstone disease is that ultrasonography can detect asymptomatic cases, which results in early treatment and the prevention of serious outcomes[1,18]. The reference standard to detect GS was represented, not only by the ultrasonographic scan of the gallbladder, but also by the direct examination of the explanted liver[2].

The Hispanic and indigenous populations of the United States show particularly high morbidity rates[19,20]. Epidemiological survey data in the United States suggest that approximately 20 million Americans suffer from GD. At the same time, GD is, on the contrary, less characteristic for the peoples of southeast Asia, Africa and the far north[21].

In Russia, the prevalence of GD among the examinees ranges from 3% to 12%. The prevalence of gallbladder and biliary tract diseases among the digestive ones is 15.8% in Russian adults, while this index is as high as 22% in Moscow.

ETIOLOGY OF GALLSTONE DISEASE

GD is a multifactorial disease. In the general population, one of the main risk factors for developing GD is gender: gallstones are more common in women than in men. Other factors are age, genes and race. Additional factors are obesity, rapid weight loss, glucose intolerance, insulin resistance, high dietary glycemic load, alcohol use, diabetes mellitus, hypertriglyceridemia, drugs and pregnancy[2]. Four major groups of factors that contribute to the formation of cholesterol gallstones to some degree may be identified[22,23]: (1) those that contribute to cholesterol supersaturation of bile; (2) those that contribute to cholesterol precipitation and crystallization core formation; (3) those that result in impairment of basic gallbladder functions (contraction, absorption, secretion, *etc*); and (4) those that lead to impairment of the enterohepatic circulation of bile acids.

Factors that contribute to bile cholesterol supersaturation

Age: Gallstone detection rates increase with age, which makes it possible to consider it one of the risk factors for GD[24]. No significant differences have been found in the frequency of gallstone formations in childhood and adolescence. Cholelithiasis in children is an unusual finding but is not exceptional and is associated with nonspecific symptoms[25,26]. After 20 years of age, the rate of gallstone formation increases with each decade[27]. If GD occurs in 7%-11% of cases in a group of subjects under the age of 50 years, then calculi are detectable in 11%-30% of subjects aged 60-70 years and in 33%-50% of those over 90 years of age. The amount of cholesterol in the bile is supposed to increase with age[28]. This is caused by dyslipoproteinemia that results in a linear increase in cholesterol excretion into the bile and by the reduced synthesis of bile acids due to the dropped activity of the enzyme cholesterol 7α-hydroxylase (CYP7A1)[29]. The xenobiotic receptor, pregnant X receptor (PXR), has a role in the pathogenesis of cholesterol GD[30]. PXR prevents cholesterol GD *via* its coordinated regulation of the biosynthesis and transport of bile salts in the liver and intestine. Cholesterol precipitation is prevented by increases in concentrations of biliary bile salts and a reduced cholesterol saturation index (CSI)[30]. Loss of PXR sensitized mice to lithogenic diet-induced cholesterol GD, characterized by decreases in biliary concentrations of bile salts and phospholipids and increases in the CSI and formation of cholesterol crystals. The decreased bile acid pool size in PXR-/- mice that received lithogenic diets was associated with reduced expression of CYP7A1, the rate-limiting enzyme of cholesterol catabolism and bile acid formation. The reduced expression of CYP7A1 most likely resulted from activation of PXR and induction of fibroblast growth factor 15 in the intestine[30].

There is a negative correlation between age and the amount of synthesized bile acids and a positive correlation between cholesterol levels and age. Furthermore, hemoperfusion of the gallbladder wall is noted to be reduced with age due to the presence of sclerotic changes. This contributes to the dysfunction of the gallbladder, its infection and inflammation with exudation into the lumen of the organ.

Gender: The female gender is a generally recognized risk factor of GD[10,24,31-33]. Marschall HU and Einarsson C[34] assume that age and sex are profoundly associated with the incidence of gallstone disease; the metabolic risk factors for gallstone disease are different between men and women[1,29]. In reproductive-aged women, the risk of cholelithiasis is 2-3 times higher than that in men[10]. The reasons for this have not been fully elucidated. Pregnancies also contribute to formation of stones in the gallbladder[10,22,33]. GD is particularly common in multiparas (parity 4 or more). Gender differences and frequent GS detec-

tions in pregnant women are linked with hormonal background[10]. Elevated estrogen levels are known to increase cholesterol excretion into the bile by causing its supersaturation with cholesterol. During pregnancy, in addition to the elevated level of estrogens, gallbladder evacuation function suffers, giving rise to bile sludge and gallstones. Hormone replacement therapy (HRT) with estrogen-containing agents in postmenopausal women[35] and the use of hormonal oral contraceptives[19] may increase the risk of symptomatic GS. Use of HRT is positively associated with an increased risk of symptomatic GS in this population. This confirms trial data and additionally shows effects of duration of use and increased risk associated with past use[36]. Opinions regarding the association between gallbladder disease and oral contraceptives differ[19]. This may be associated with the fact that the effect of estrogens is dose-dependent. Therefore, the currently available low-dose estrogen-gestagen combination oral contraceptives have a lower risk for GD[10].

Regarding gender, despite of the higher absolute frequency of GS in females with cirrhosis, the risk of cholelithiasis in cirrhotic males is much higher than in the healthy population[2]. Fornari *et al*[37] claimed that cirrhosis is a risk factor for GD in males and suggested that a high level of estrogens could play a role by an impairment of gallbladder emptying, as observed also in pregnant women. Age, sex and body mass index (BMI), relevant factors for GS development in the general population, are much less important in patients affected by cirrhosis where the main factor to be considered is the degree of impairment of underlying liver disease[2].

Genetic factors: There is growing evidence that GS formation may be genetically determined[38]. The risk of GS formation is 2-4 times higher in individuals whose relatives suffer from GD[32,39]. In cases of family GD, genetic factors play a prevailing role and are characterized by autosomal dominant inheritance[31,40]. Genetic susceptibility contributes to the etiology of gallbladder diseases, as shown by multiple epidemiological studies. Murine experiments have shown that there is a lithogenicity gene[41]. A major gallstone susceptibility locus (*Lith6*) was identified in 2003 by quantitative trait locus mapping in mice. Two attractive positional and functional candidate genes in apolipoprotein B mRNA-editing protein (APOBEC1) and peroxisome proliferator-activated receptor gamma (PPARG) are located in this interval. In the investigated German samples, no evidence of association of APOBEC1 and PPARG with gallstone susceptibility was detected. Systematic fine mapping of the complete *Lith6* region is required to identify the causative genetic variants for gallstone in mice and humans[42]. From quantitative trait locus mapping in inbred mice, Kovacs P *et al*[43] identified the *Nr1h4* gene encoding the nuclear bile salt receptor FXR (farnesoid X receptor) as a candidate gene for the cholesterol gallstone susceptibility locus *Lith7*. Genome wide scans of inbred strains of mice have linked the genes encoding the hepatocanalicular cholesterol transporter. ATP binding cassette (ABC) G5 and G8 (ABCG5/G8) are sterol export pumps which regulate biliary cholesterol absorption and excretion. Supersaturation of bile with cholesterol is a primary step in the formation of cholesterol gallstones. The function of this transporter and the results of the genetic study taken together indicate that in gallstone-susceptible carriers of the *ABCG8 19H* allele, cholesterol cholelithiasis is secondary to increased hepatobiliary cholesterol secretion[44]. The formation of GS, supersaturated with cholesterol in bile, is determined by genetic and environmental factors. The linkage and association studies identified the cholesterol transporter ABCG5/G8 as a genetic determinant of GS formation, or *LITH* gene, in humans. The interaction of susceptible gene polymorphisms with age, sex and BMI in GD is unclear. Carriers of *ABCG5 604Q* or *ABCG8 D19H* polymorphisms have an increased risk of GD independent of age, sex and BMI[45]. The *T400K* polymorphism in *ABCG8* may be associated with the incidence of GD in males[46]. The genes associated with the development of GD are assumed to be located mainly on chromosomes 3, 4, 9 and 11[47]. The increased expression of 3-hydroxy-3-methylglutaryl-coenzyme-A-reductase, the enzyme that regulates the synthesis of cholesterol in the body, has been earlier suggested to play the most major role[48]. Gene variants in the lipid metabolism pathway contribute to the risk of biliary tract stones and cancers, particularly of the bile duct[49]. With certain gene polymorphisms, there is an increased risk for systemic metabolic disturbances, leading to the higher secretion of cholesterol into the bile and to gallbladder dysfunction[17,44,46]. Genetic polymorphisms in apolipoprotein genes may be associated with alteration in lipid profile and susceptibility to GD[5,50]. The *APOA1-75 G/A* polymorphism is associated with gallstone disease and shows sex-specific differences. On the other hand, *APOA1 M2*(+/-) and *APOC3 SstI* polymorphisms may not be associated with gallstone disease. Haplotype analysis is a better predictor of risk for GD[51]. It was recently presented that a common polymorphism in the low-density lipoprotein receptor-related protein-associated protein (*LRPAP1*) gene might be associated with GD[52]. Mutations of the gene encoding the hepatocanalicular phosphatidylcholine transporters may lead to reduced lecithin secretion into the bile and its increased lithogenicity[53,54]. Association was stronger in subjects with cholesterol gallstones (odds ratio = 3.3), suggesting that His19 might be associated with a more efficient transport of cholesterol into the bile[17]. Cholesterol 7alpha-hydrolase (CYP7A1) is an enzyme that catalyzes the first, rate-limiting reaction of a cholesterol catabolic pathway. Recently, a common c.-278A > C polymorphism (rs3808607: G > T) has been described in the CYP7A1 gene, associated with altered plasma lipid levels. Authors concluded that CYP7A1 promoter polymorphism is not a valuable marker of GD susceptibility in a Polish population[52].

Mucin, a major component of mucus, plays an important role in GS formation. The molecular mechanisms of

mucin overproduction, however, still remain unknown. Several mucin genes (*MUC*) have been implicated in various diseases and gel-forming mucin genes (*MUC2, MUC5AC, MUC5B,* and *MUC6*) were recognized to be the important components of digestive mucus. Furthermore, epidermal growth factor receptor (EGFR) might regulate the function of *MUC5AC*. *MUC5AC* is overexpressed in GD, despite of the decrease in the expression of EGFR mRNA. *MUC5AC* may be related to mucus hypersecretion[55]. The SNPs at *MUC1* and *MUC2* are significantly associated with GS in men but not in women. These genes can work jointly to further increase susceptibility to GS in a Chinese population[56].

Being overweight and obesity: Being overweight and obesity are important risk factors of cholelithiasis[24,31,33,57]. Obesity is accompanied by increased synthesis and excretion of cholesterol into bile. At the same time, the amount of produced cholesterol is directly proportional to being overweight[8]. Weight cycling, independent of BMI, may increase the risk of GD in men. Larger weight fluctuation and more weight cycles are associated with greater risk[58]. The beta3-adrenergic receptor (*ADRB3*) is a transmembrane receptor highly expressed in adipose tissue and thought to be involved in the regulation of lipolysis. *ADRB3* is also highly expressed in gallbladder tissue where it may be involved in gallbladder contraction. Klass *et al*[59] indicate that the *ADRB3 Trp64Arg* polymorphism is associated with gallstone disease, thereby representing a genetic marker that identifies subjects at higher risk for gallstone formation. Low-calorie diets used in obese patients give rise to ointment-like bile and stones in 25% of cases. In the case of bypass surgery for obesity, the likelihood of cholelithiasis is even higher: 50% of patients are found to have GS within 6 mo postoperatively. Weight loss is accompanied by the elevated levels of mucin and calcium in the cystic bile, thereby giving rise to biliary sludge and stones in the gallbladder.

Diet: A high intake of cholesterol increases its bile level[31]. A low-fiber diet slows transit of the intestinal contents, which promotes the increased formation and absorption of secondary bile acids and the enhanced lithogenic properties of bile[22]. Refined carbohydrates increase cholesterol saturation of bile while small doses of alcohol have the opposite effect. Epidemiological studies in the United States have demonstrated that a daily intake of 2-3 cups of coffee reduces the risk for GS formation[60]. Long-term parenteral nutrition promotes gallbladder dilatation and hypokinesia and gives rise to gallstones[48].

Liver and pancreatic diseases: In liver cirrhosis, GS are detectable in 30% of patients[61,62]. It is stated that subjects with HBsAg[63] and viral hepatitis C have an increased risk for GS formation. Hepatic metabolic dysfunction and bile duct lesions are mentioned among its possible causes[57]. In primary biliary cirrhosis, bile duct stones (more commonly pigment ones) are encountered in 39% of patients. The incidence of GD increases in fatty hepatosis[64]. Patients with diabetes mellitus are at a higher risk for GD, which is linked with hypercholesterolemia observed in this disease[31,65]. Immune resistance associated with the polymorphism of genes encoding receptors in adipocytes: retinoid X receptor and peroxisome proliferators-activated receptor promotes the occurrence of cholelithiasis, as shown by the Chinese investigators' data[66].

Drug: Estrogens, prednisolone, cyclosporine, azathioprine, sandostatin[67], clofibrate, nicotinic acid and a number of other long-term drugs increase the risk for GD[68,69]. Oral contraceptives increase the incidence of GD in younger women, especially in the early period of their use of oral contraceptives[70]. Sixty-eight point eight percent of SLE patients on corticosteroid therapy had cholelithiasis[71]. The data, presented in these articles, suggest that corticosteroids and oral contraceptives, which contain hormones related to steroid hormones, may be regarded as a model system of cholelithiasis development in man.

Long-term corticosteroid therapy is well known to cause dyslipoproteinemia, characterized by elevated plasma total cholesterol, triglycerides and low-density lipoprotein cholesterol. The major catabolic pathway for cholesterol is its transformation into bile acids, involving P450 cytochrome and subsequent bile excretion from the body. The elevated level of total cholesterol may change a bile acid/cholesterol ratio and lead to the formation of GS in patients with SLE or in patients who use oral contraceptives.

Cytostatic therapy during organ transplantation increases the risk of cholelithiasis. Stone formation is noted in 13%-60% of acromegaly patients taking octreotide (sandostatin) and becomes particularly high when it is discontinued[67,72]. Ceftriaxone frequently causes transient biliary precipitation and its probability increases if the child is over 12 mo of age, the dose is over 2 g/d, or the duration is over five days. Ceftriaxone, a third-generation cephalosporin, is widely used for treating infection during childhood. It is mainly eliminated in the urine, but approximately 40% of a given dose is unmetabolized and secreted into bile[73]. The risk for cholelithiasis increases in constitutive obesity and in the case of long-term high-dose insulin therapy and insulin resistance[74]. Gallstones appear to be a marker for insulin resistance, even in non-diabetic, nonobese men[75].

Long-term therapy with each of these agents enhances cholesterol excretion into bile and results in its supersaturation with cholesterol through competitive inhibition of bile acid synthesis from cholesterol on cytochrome P_{450}[71] (Figure 1).

The defect in the key enzyme of the classical pathway of bile acid synthesis, cholesterol 7α-hydroxylase (CYP7A1), has been associated with a decrease in bile acid production *via* the classical pathway, which is compensated by activation of the alternative acidic pathway[76].

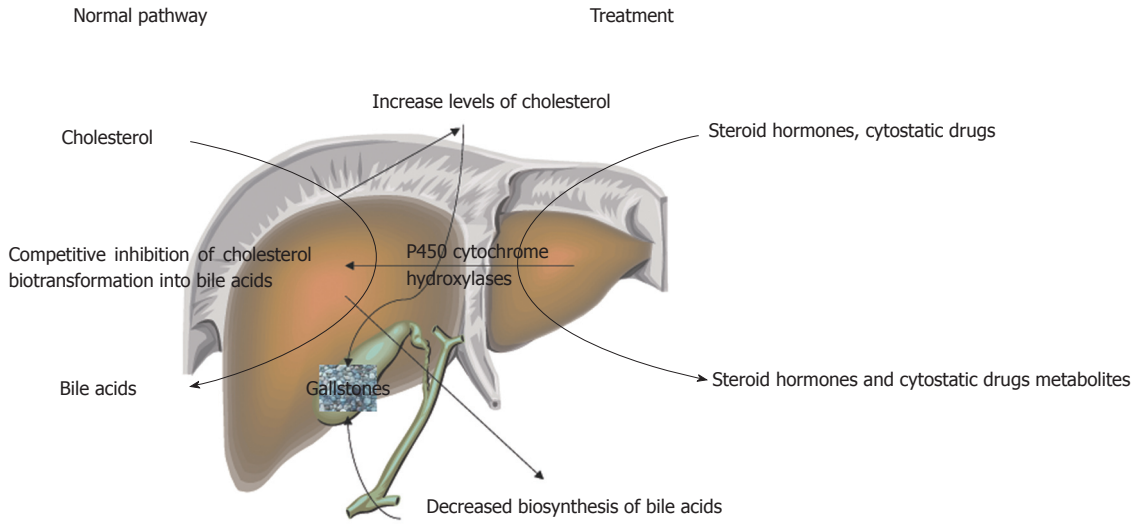

Figure 1 Diagram of steroid hormones-induced inhibition of cholesterol catabolism.

In these individuals, hepatic cholesterol contents are increased and, in adults, LDL hypercholesterolemia and cholesterol GS are commonly present[77]. Genetic variation in genes involved in steroid biosynthesis, metabolism and signal transduction have been suggested to play a role in GD. An association for cholelithiasis risk between short alleles for both *c.1092+3607* (*CA*) *5-27* and *c.172* (*CAG*) *5-32* repeat polymorphisms of the estrogen receptor-beta and androgen receptor was found in individuals of Greek descent[78]. Occurring cholesterol metabolic disturbances are attended by decreased gallbladder motor activity, which also promotes GS formation.

Low socioeconomic status and a poor hygiene level are currently stated among the risk factors of GD[79].

By using logistic regression multivariate analysis, authors[32] from Saudi Arabia note the following significant risk factors for GD: female sex, family history of gallstone disease and past history of pancreatitis. Moreover, age, education, blood pressure, smoking, coffee intake, being overweight, diabetes mellitus, number of pregnancies and use of oral contraceptives were not significant risk factors[32]. The data presented by the authors does not correspond well with the above mentioned and raises a question about the correlation of race and gallstone disease development. Apparently, a multicenter multinational investigation is required.

Factors that contribute to cholesterol precipitation and crystallization core formation

Mucin-glycoprotein gel is one of the most important and identified pronucleators. Mucins are high-molecular-weight glycoproteins containing oligosaccharide side-chains attached to serine or threonine residues of the apomucin backbone by O-glycosidic linkages[80]. Mucins can be divided into two classes: gel forming and membrane-associated. Bile mucin has two main domains: one rich in serine, threonine and proline, which contains the majority of the covalently-bound carbohydrates; and another, nonglycosylated domain, enriched in serine, glutamic acid, glutamine and glycine, which binds hydrophobic ligands such as bilirubin. In health, mucin is constantly secreted by the gallbladder mucosa; however, its secretion increases if lithogenic bile is present. Secretory mucins are gel forming and may increase bile viscosity. The biochemical composition of hepatic bile is modified during residence in the gallbladder, contributing to sludge formation. An increased expression of gel-forming mucin, such as MUC5AC and MUC2, was found in patients with hepatolithiasis[81]. Wang and coworkers[82] described a positive correlation between *MUC1* and *MUC5AC* expression, indicating a gene-gene interaction that might affect the accumulation of mucin gel and cholesterol GS formation. Bile mucin is derived from pure hepatic bile, gallbladder-concentrated bile, and mucin secreted by the bile duct epithelium. In patients with biliary sludge, mucin concentration was higher in bile collected by endoscopic retrograde cholangiography than in gallbladder bile[80]. The biochemical composition of hepatic bile is modified during residence in the gallbladder, contributing to sludge formation.

Bilirubin is frequently found in the center of cholesterol stones, which allows us to think that cholesterol crystals may precipitate as protein-pigment complexes in the gallbladder.

Factors that lead to impaired gallbladder function (contraction, absorption, secretion)

Cholesterol precipitates are constantly formed in the normal gallbladder. Its contraction removes cholesterol crystals and mucus clumps, preventing the formation of stones[83]. This is also favored by the slightly acidic medium of bile. Gallbladder filling and emptying could be impaired in patients with GD[84]. GS formation is associated with poorer contractility and larger gallbladder volume[85]. It is likely that an increase in gallbladder volume could result in impaired gallbladder motility and bile stasis, which may encourage GS formation[86]. Cholestasis in the gallbladder with its preserved concentrating func-

tion substantially increases the risk of stone formation.

Gallbladder emptying is difficult in flatulence, pregnancy[87], on switching to complete parenteral nutrition, in prompt weight loss, long-term starvation[29], celiac disease, iron-deficiency anemia[88] and gallbladder cholesterosis[89]. With age, there is a reduction in the sensitivity and number of receptors to cholecystokinin, motilin and other stimuli of the motor activity of the gallbladder receptor apparatus. There is evidence for certain cholecystokinin receptor A gene polymorphisms that increase the rate of cholelithiasis due to impaired gallbladder motility[90]. Increased expression of the gene encoding the synthesis of type II receptor to pituitary polypeptide that activates adenylate cyclase in the tissue of the gallbladder, resulting in its impaired motility, is involved in the development of GD[91].

Somatostatin, atropine and methylscopolamine lower gallbladder contractility. Morphine exerts a cholecystokinetic effect but concurrently induces spasm in the sphincter of Oddi.

A few investigators attribute gallbladder smooth muscle hypokinesia to excess cholesterol in the cytoplasmic membranes of myocytes. The defective contraction of muscle cells with excessive cholesterol levels in the plasma membrane is due to an increased expression of caveolin-3 proteins Cav-3 that results in the sequestration of CCK-1 receptors in the caveolae, probably by inhibiting the functions of Galpha (i3) proteins[92].

Contractility of the gallbladder may be impaired by its denervation after surgery of the hepatopancreatoduodenal area or gastrectomy with bypass[93-96]. A notable reduction in the number of neurons in the gallbladder wall was observed in Chagas patients, in comparison with non-Chagas subjects[97].

Factors that lead to impaired enterohepatic circulation of bile acids

Small bowel diseases accompanied by severe malabsorption (gluten enteropathy, Crohn's disease, *etc.*) result in impaired bile acid absorption[22]. The rate of stone formation amounts to as high as 26.4% in Crohn's disease with predominant localization in the terminal small bowel.

At the same time, there is no difference in the rate of GS formation between men and women. There is no age-dependence characteristic of GD[48]. Cholesterol stones are generally formed in Crohn's disease; however, there is evidence that pigment stones may be formed in this disease.

Ileectomy: Subtotal and total hemicolectomies increase the risk of GS formation.

Biliary fistulas: External drainage or biliary fistulas resulting from the pathological process, such as in xanthogranulomatous cholecystitis, promote massive loss of bile acids, which is not offset even by their intensive compensatory synthesis. Resection, diseases of the small bowel, with the pathological process being located in the terminal portion, and biliary fistulas lead to impaired enterohepatic circulation of bile acids and, as a result, to dyscholia and GD.

Composition of gallstones

Stones in the gallbladder and/or bile ducts are a morphological substrate of GD. The major components of virtually all types of GS are free unesterified cholesterol, unconjugated bilirubin, bilirubin calcium salts, fatty acids, calcium carbonates and phosphates, and mucin glycoproteins.

Three main categories of gallstones can be identified according to their predominant chemical composition, cholesterol and pigment stones[2]: (1) cholesterol stones, constituting as high as 75% of all gallstones in GD[10,98]; (2) pigment stones; and (3) mixed stones.

White or yellowish cholesterol gallstones are present in the gallbladder; they are round or oval in shape, light (they do not sink in water) and, when ignited, burn with a bright flame. When sectioned, they are radial in structure due to the radial alignment of cholesterol crystals. Cholesterol and mixed stones comprise mainly of cholesterol monohydrate (it is at least 70% in the cholesterol stones[22]) and a mixture of calcium salts, bile acids, pigments and glycoprotein, which may be present in the center of a gallstone and generate radial or concentric precipitates. Scanning and transmission electron microscopic studies of the microstructure of lithogenic bile have indicated that lamellar vesicles with incorporated lipophilic and hydrophilic compounds are not only a precursor, but also a major structural component of cholesterol stones[99]. Methods of study that determine the spatial relationships between the major components of lithogenic bile during crystallization are of great importance. The data on the structural relationship between glycoproteins and cholesterol in the GS are obtained from histochemical studies using light microscopy.

Color cathodoluminescence scanning electron microscopy (CCLSEM) studies of cholesterol GS (Figure 2) have shown that their major components are cholesterol and protein constituents (Figure 2A and B, respectively). Bilirubin is arranged as individual embedments onto the surface of the section of a stone (Figure 2C)[71,100,101].

Pigment GS are those that contain less than 30% cholesterol. These are black (compact and small) and brown (softer and large) pigment stones. The black pigment stones account for 20%-30% of the gallstones in GD and are more frequently encountered in the elderly. They are composed predominantly of calcium bilirubinate, phosphate and carbonate without a cholesterol impurity[102-105]. They have different shapes, are more commonly very small and numerous, greenish black in color, compact, but fragile. There are also brown pigment stones, very common in east Asia, which form due to bile stasis, parasites, incomplete polymerization of calcium hydrogen bilirubinate, saturated fatty acids and bacterial infection with enzymatic hydrolysis of biliary lipids[2]. The brown stones are chiefly located in the bile duct and

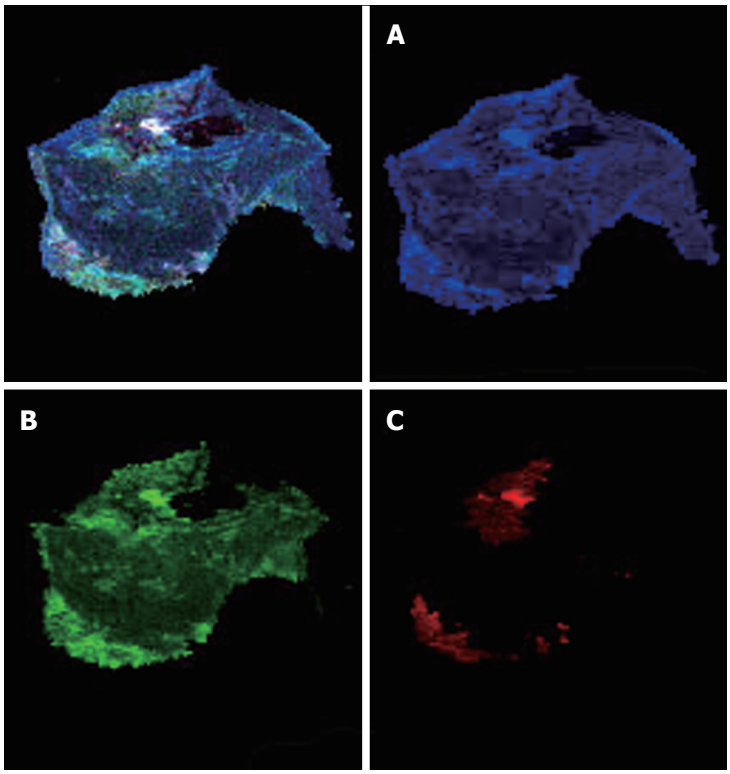

Figure 2 Color cathodoluminescence scanning electron microscopy micro images of cholesterol gallstones. The application of the computer program "Adobe Photoshop" (software) and color contrast by the color cathodoluminescence scanning electron microscopy (CCLSEM) technique permitted the determination of cholesterol, bilirubin and protein within the stone. CCLSEM micrographs of cholesterol (A), protein (B), bilirubin (C) were obtained after color separation[100]. The major components of the gallstones under examination were cholesterol (A) and protein (B). They were detected all over the entire surface of the scanned gallstone while rare bilirubin insertions (C) were seen only at the periphery of the gallstone.

amount to about 10%-20% of the stones that are formed in GD. The brown pigment stones contain calcium bilirubinate, less polymerized than that in the black pigment stones, as well as cholesterol and calcium palmitate and stearate. For pigment stones, supersaturation of bile with unconjugated bilirubin plays a major role, which results in its agglomeration[103]. Chronic hemolytic anemias are a major risk factor of bilirubin stone formation[104]. About 30% of patients with thalassemia major (TM) suffer from GD[105]. Recent studies have shown that a variant TATA-box in the promoter region of the UDP-glucuronosyltransferase 1A1 (*UGT1A1*) gene is associated with the development of cholelithiasis[105]. The coding region mutation (*G71R*) of the *UGT1A1* gene was higher in Asians than those in Caucasians. The combined TATA-box variants and *G71R* mutations of the *UGT1A1* is associated with cholelithiasis in beta-thal/Hb E[106]. It has been thought that intrahepatic stones are brown pigment stones (bilirubin carbonate stones). It became clear that the intrahepatic stones contained high levels of free bile acids and that bacterial infection, which deconjugates the glycine and taurine conjugations, is involved in the pathogenesis of GS. The fatty acid analysis demonstrated high levels of free saturated fatty acids in the GS as well as the involvement of phospholipases, which break down phospholipids in bile, particularly phospholipase A1[107].

Purely calcific stones that are composed of calcium carbonate are very rare in adults[48,108]. In contrast, calcium carbonate gallstones are relatively common in children. An increase in mucin producing epithelial cells in gallbladders from children containing calcium carbonate stones was demonstrated. This supports the hypothesis that cystic duct obstruction leading to increased gallbladder mucin production may play a role in the development of calcium carbonate gallstones in children[108].

Mixed cholesterol-calcific-pigment stones are most common: they sink in water and burn poorly; when cut, they have a lamellar pattern. The causes and factors which induce the alternation of layers and their chemical heterogeneity remain unknown. The mixed stones have various shapes and sizes. The data obtained by CCLSEM suggest that the composition and structure of single and multiple mixed GS are different[100,101]: (1) the single mixed GS display a protein-cholesterol composition in the core; (2) the multiple mixed GS exhibit a protein-bilirubin composition in the core; and (3) moreover, the single and multiple mixed GS necessarily contain a protein component that is arranged along the stone section plane. Whether bile glycoproteins are implicated in the formation of cholesterol stones is still debated. The data of qualitative and quantitative biochemical studies of the pronucleation activity of mucinic glycoproteins are in doubt and without agreement.

Knowledge of the chemical, structural and elemental composition of GS is essential for the etiopathogenesis of GD. To identify the predisposing factors for GS formation, X-ray diffraction powder analysis, atomic absorption spectroscopy and various biochemical estimations were carried out. In the present study, trace elemental analysis revealed calcium as the major constituent element, in addition to the iron, magnesium and zinc in the majority of GS. Patients with GS exhibited increased serum total bilirubin and conjugated bilirubin levels and liver function parameters (serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase and alkaline phosphatase). In patients with GS, higher concentrations of malo-

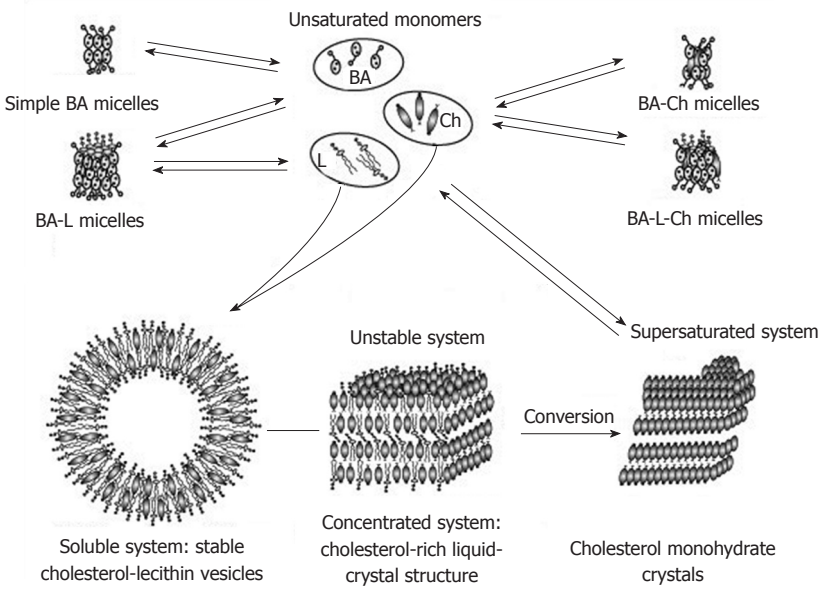

Figure 3 Formation of molecular structures in the system containing bile acids, lecithin and cholesterol. Cholesterol-supersaturated vesicles can stick together and agglomerate to form multilayer (liposomal) liquid-crystal structures. When gallbladder contractility is decreased, liposomes may be converted to solid cholesterol monocrystals. **BA: Bile acids; L: Lecithin; Ch: Cholesterol.**

ndialdehyde, significantly higher glutathione disulfide/glutathione (GSH) ratio, reduced total GSH levels and significantly decreased antioxidant enzymes activities (superoxide dismutase, catalase and glutathione peroxidase) were found than in patients without GS. Further studies are needed to establish whether the observed differences are a cause or an effect of GS formation. Such studies could ultimately result in the development of new strategies for the treatment of GS and might provide clues for the prevention of GS formation[109].

PATHOGENESIS OF CHOLESTEROL STONES

The pathogenesis of GD is suggested to be multifactorial and probably develops from complex interactions between many genetic and environmental factors[1,34]. Unphysiological biliary supersaturation from hypersecretion of cholesterol, gallbladder hypomotility and the accumulation of mucin gel contribute to the formation of cholesterol GS, while black pigment stones derive from the precipitation of calcium hydrogen bilirubinate where pigment supersaturation and deposition of inorganic salts, phosphate and calcium bicarbonate accelerate the nucleation. Pigment supersaturation is common in hemolytic disorders, enterohepatic cycling of unconjugated bilirubin and ileal disorders and/or surgery[110]. Cholesterol GD results from a biochemical imbalance of lipids and bile salts in the gallbladder bile[30].

Cholesterol stones are formed in the gallbladder due to impaired relationships between the major bile components, cholesterol, phospholipids and bile acids[111]. The pathophysiology of GS formation involves three steps: saturation, crystallization and growth. Bile cholesterol supersaturation is an obligatory, but not the only, factor that contributes to GS formation. An important role in this is played by the state of pronucleating and antinucleating factors and the functional state of the gallbladder.

The biochemical composition and physicochemical properties of bile are modified when it is located in the gallbladder. Diminished evacuatory function of the gallbladder with its preserved concentrating capacity may give rise to biliary sludge and GS. In excess cholesterol or deficiency of phospholipids and/or bile acids (a high cholesterol saturation index), bile cholesterol is transported, not only in the form of mixed micelles, but also as phospholipid vesicles. Cholesterol-supersaturated unilamellar and then multilamellar vesicles that are less stable are formed. Nuclear receptors (NRs) play a key role in the transcriptional control of critical steps of hepatobiliary transport and phase I / II metabolism of endo- and xenobiotics such as bile acids and drugs. Apart from these metabolic roles, NRs may also play a key role in the control of hepatic inflammation. Hereditary and acquired alterations of NRs contribute to our understanding of the pathogenesis of cholestasis and GD. Moreover, NRs may represent attractive drug targets for these disorders[112]. Cholesterol nucleation is known to be an initial stage in the formation of cholesterol GS[113]. The present-day interpretation of the mechanisms responsible for cholesterol transport and formation of cholesterol monohydrate crystal in the bile suggests that cholesterol molecules nucleate from the liquid-crystalline phase (a mesophase) after the aggregation and possible fusion of cholesterol-rich unilamellar vesicles[99,114,115] (Figure 3). Under certain conditions, cholesterol can aggregate and precipitate in them as cholesterol monohydrate crystals to give rise to the core of a GS.

The important factor in such mesophasic nucleation

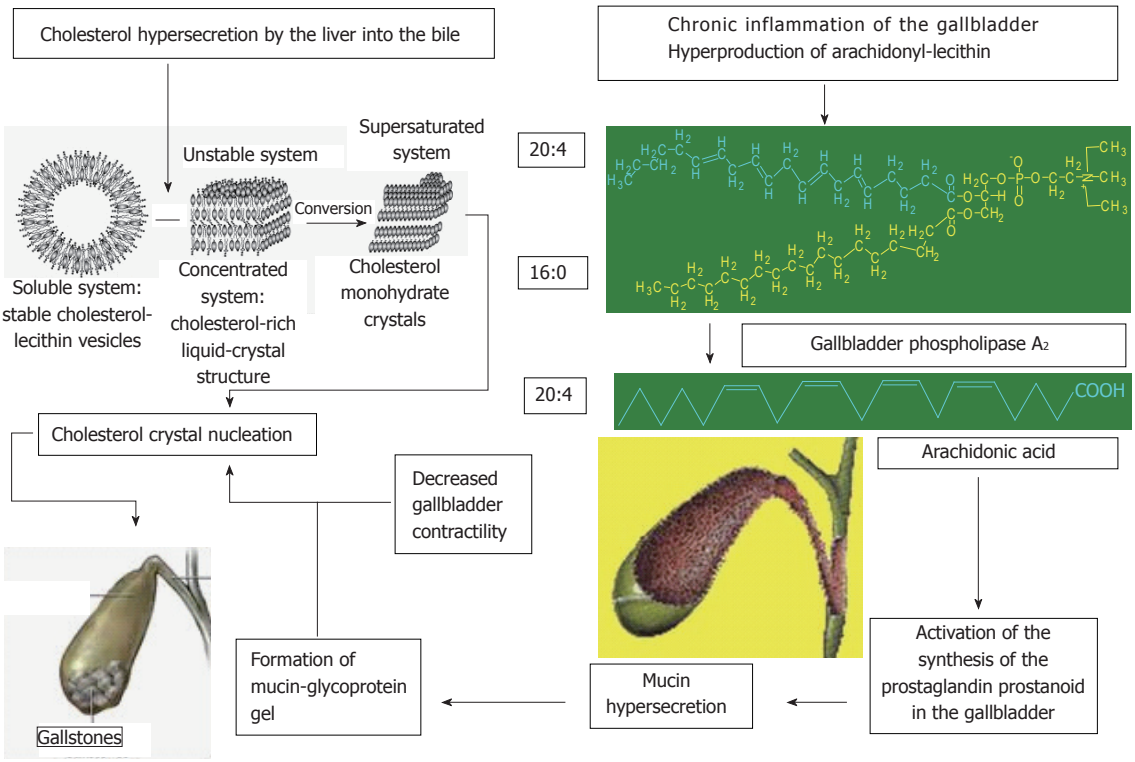

Figure 4 It shows a diagram of gallstone formation by taking into account the above impaired bile production and excretion processes.

is associated with further interaction between the monohydrate crystals and the molecules of protein and unconjugated bilirubin. All these organic substances are precursors in the lithogenic bile and structural components of most human GS[116].

Polarizing light microscopy is the main technique for visualization of cholesterol crystal formation processes in normal and lithogenic bile[117,118]. This technique has revealed that cholesterol crystallizes from bile *via* metastable intermediates[119]. Loginov *et al*[100] have shown that mixed (single and multiple) stones are composed of alternating concentric, cholesterol-rich and bilirubin-rich layers. The reason for this alternation and the periodic emergence of layers of various compositions remain unclear. By taking into account the data on the zonal stratification of bile on its drying and the relationship of the formation of cholesterol and bilirubin the deposits to the dehydration or watering of a solution, it can be presumed that the layering of stones depends on bile concentrations in a period of lithogenesis[120]. Cholesterol can crystallize even when the concentration of a bile solution is outside or slightly below the normal range. Bilirubin precipitation increases as lithogenic bile concentrates progressively. Thus, the concentrating or watering of a bile solution may be of great importance in the formation of cholesterol- and bilirubin-containing layers in the GS.

Bile proteins and bilirubin, in addition to cholesterol crystals, can be a matrix in stone formation. Mucin-glycoprotein gel is one of the most important and identified pronucleators. It should be noted the mucus of the gallbladder in normalcy constantly secretes the mucin; however, its secretion increases due to inflammation[121]. Chronic inflammation of the gallbladder wall and mucin hypersecretion are considered important factors in the pathogenesis of cholesterol GD. The results support a promoting effect of gallbladder mucin hypersecretion by lipid peroxidation leading to rapid formation of cholesterol crystals in gallbladder bile. These findings suggest that besides hypersecretion of cholesterol in bile, chronic inflammation of the gallbladder wall is implicated in the pathogenesis of cholesterol GD[121].

Bacterial infection is of great significance in the development of inflammation in GD. In health, bile is sterile as it has bactericidal activity[122]. When there are changes in bile composition or cholestasis in the gallbladder, bacteria can rise into the gallbladder through the bile duct and promote lithogenesis. Cystic bile destabilized by chronic inflammation of gallbladder wall contains high arachidonyl-lecithin levels (Figure 4). The observed increase in the activity of the phospholipase A2 secreted by bacteria leads to the hydrolysis of phospholipids and the accumulation of free fatty acids, including arachidonic acid[107]. The latter activates the generation of prostaglandins, thromboxanes and leukotrienes to cause mucin glycoproteins to be hypersecreted by the gallbladder mucosa. In infection, cholic acid is converted to lithocholic acid. The higher production of lithocholic acid in the cystic bile promotes aggregation of cholesterol monohydrate crystals.

In parallel with this, there are morphological changes in the gallbladder mucosa. The surface epithelium passes into goblet, mucus cells that secret much mucus, the columnar epithelium flattens and microvilli are lost. This results in impaired water and electrolyte absorption

processes. Mucin and mucus hypersecretion gives rise to a parietal colloid solution that is turned to viscoelastic glycoprotein-mucin gel. The latter promotes the aggregation of phospholipid vesicles and the nucleation and precipitation of cholesterol monohydrate crystals and/or bilirubin. Cholesterol monohydrate crystals, mucus glycoprotein mucin bands and calcium bilirubinate granules form the basis for biliary sludge and a pigmented matrix of the core of most cholesterol gallstones.

Hypersecretion is induced by the increased expression of one of the genes encoding the synthesis of mucin (*MUC5AC*) and by the decreased expression of the epidermal growth factor receptor gene involved in the regulation of mucin synthesis, which are observed in all patients with GD[55]. The elevated levels of glycosaminoglycans mainly due to a sulfated fraction are characteristic.

In addition to mucin, the proteins that accelerate cholesterol precipitation include N-aminopeptidase, immunoglobulins and phospholipases C. The antinucleators include apolipoproteins A1 and A2, which slow cholesterol precipitation, aspirin and other nonsteroidal anti-inflammatory drugs.

The bulk of intrahepatic stones are formed due to biliary tract infection[123]. The neck of the gallbladder hosts the biggest bacterial load in comparison with the body and the fundus. This difference might be attributed to the presence of Rokitansky-Aschoff sinuses, the main histological characteristic of the region[124]. This is frequently the opportunistic flora (Escherichia coli, streptococcus, staphylococcus and typhoid bacillus) that, by setting in motion its capsular O-antigen, can persist in the GS for decades[125]. Intrahepatic stones contain abundant free fatty acids and free bile acids due to the deconjugation with bacterial enzymes.

Bacteria are readily cultured from cholesterol stones with pigment centers, allowing for analysis of their virulence factors. Bacteria sequestered in cholesterol stones cause infectious manifestations but less than bacteria in pigment stones. Possibly, because of their isolation, cholesterol stone bacteria are less often present in bile and blood, induce less immunoglobulin G, are less often killed by a patient's serum and demonstrate fewer infectious manifestations than pigment stone bacteria[126]. The O-antigen capsule genes are bile induced and the capsule produced by the enzymes of this operon is specifically required for biofilm formation on cholesterol GS. Salmonella enterica serovar Typhi can establish a chronic, asymptomatic infection of the human gallbladder, suggesting that this bacterium utilizes novel mechanisms to mediate enhanced colonization and persistence in a bile-rich environment. GS are one of the most important risk factors for developing carriage and authors have previously demonstrated that salmonellae form biofilms on human GS *in vitro*[125]. Thus, the microorganisms induce increased mucin production and destroy both components that solubilize cholesterol in the micelles by inducing its crystallization. The performed investigations indicate that stones of various compositions are formed depending on the species of the microorganism that is responsible for biliary tract inflammation. Thus, the bacteria that produce beta-glucuronidase and mucus or beta-glucuronidase only give rise to pigment or mixed stones while the microorganisms that produce only mucus or do not produce any of these factors are more common in the cores of cholesterol stones[127].

The genetic material of Clonorchis sinensis and Ascaris lumbricoides worms may be found in the GS[128,129]. Clonorchis sinensis and Ascaris lumbricoides may be related to biliary stone formation and development[128].

Foreign bodies, such as suture materials, clips, swallowed metal or plastic fragments, or parasites, may become foci of nucleation. Surgical clips are the most common cause of iatrogenic cholelithiasis[23]. The stones' growth rate is 3-5 mm per year and in some cases it may be more[22,130].

TREATMENT FOR GALLSTONE DISEASE

The treatment of cholelithiasis is symptomatic and chiefly aims at removing the stones from the gallbladder or bile ducts. When the cause of the disease is known, the conditions resulting in cholelithiasis, such as hemolytic anemia, obesity, diabetes mellitus, *etc*, are treated.

Surgery has long remained the exclusive form of therapy for GD. The achievements in bile molecular biology and biochemistry have extended the views of intricate bile production and excretion processes and the mechanisms responsible for formation of GS and their structure. This could expand indications for medical treatment in patients with GD. Therefore, surgical and medical treatments for cholelithiasis are equally used today. The basic treatments for GD are: (1) cavitary cholecystectomy endoscopic cholecystectomy; (2) litholytic therapy (LT); (3) extracorporeal shock wave lithotripsy (ESWL); (4) extracorporeal shock wave lithotripsy + Litholytic therapy; and (5) percutaneous transhepatic LT.

The final choice of treatment policy must be eventually determined by a joint decision between a therapist, surgeon and patient. This paper will outline the basic principles of medical therapy for cholelithiasis.

The second half of the last century was marked by the emergence of new medical treatments for GD: litholytic therapy (stone dissolution) and lithotripsy (stone shattering). About 30% of patients with gallbladder stones may undergo litholytic therapy[22]. GS dissolution is based on the pathophysiology of cholepoiesis and choleresis in cholelithiasis and is carried out with bile acids. Scientists established experimentally that the ratio between the concentration of bile acids leads to a redistribution of phases in a triangular coordinate system[114] (Figure 5).

This principle underlies the dissolution of GS by using bile acids drugs. For this, litholytic drugs containing chenodeoxycholic or ursodeoxycholic acid (UDCA) are used. Preference is given to UDCA-containing agents. They are more effective and have virtually no side ef-

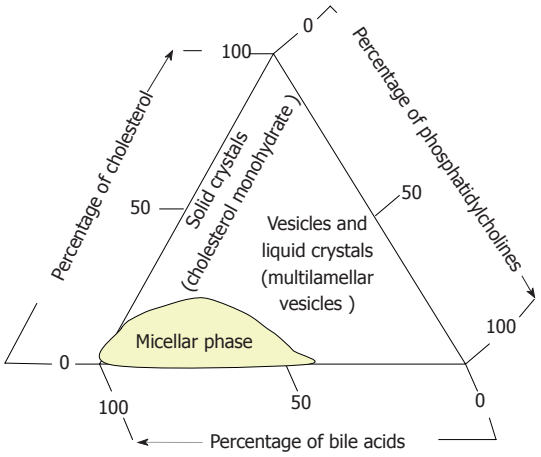

Figure 5 The phase state of the main bile components (cholesterol, phosphatidylcholines, bile acids) in the triangular coordinate system[114].

fects[48]. With administration of the agents, there is elimination of bile acid deficiency, inhibition of hepatic synthesis of cholesterol and its secretion into the bile, as well as intestinal absorption, ultimately resulting in a decreased bile cholesterol level and stone dissolution.

In health, the proportion of UDCA is not greater than 5% in the total bile acid pool, whereas it is more than 60% of all bile acids after three months or more of administration of oral UDCA-containing preparations[131]. The increased total pool of bile acids at the expense of polar UDCA causes a reduction in bile cholesterol saturation and promotes a gradual cholesterol solubilization from the gallstones. The administration of UDCA outside the intestine through the feedback system suppresses the biosynthesis of cholesterol, which also lowers the bile cholesterol saturation index. Reductions in cholesterol and potentially toxic primary acids in the total pool are followed by decreased cholesterol levels in the hepatocytic membranes[89]. This normalizes performance of the carriers of bile acids and phospholipids on the canalicular and basolateral membranes of the hepatocytes, which elevates the amount of bile acids and phospholipids in the canalicular bile and also decreases the bile cholesterol saturation index[132]. *In vitro* studies have demonstrated that UDCA reduces the levels of cholesterol and the intensity of lipid peroxidation in the myocyte cytoplasmic membrane of the gallbladder and diminishes its mucin secretion[133]. Even short-term treatment with UDCA preparations corrects impaired gallbladder motility, thus showing their choleretic activity[134,135].

For successful litholytic therapy, definite criteria should be met for selection of patients with cholelithiasis: (1) the stone should be cholesterol or mixed; (2) the size of the stones should not be greater than 1.5 cm; and (3) the gallbladder should fully preserve its function and be packed with stone not more than ¼ of the fasting volume; the cystic duct and common bile duct should preserve their patency; enterohepatic circulation of bile acids should be preserved.

The dose of a drug depends on body weight. The daily dose of bile acids should be increased in obese patients[22]. For the highest therapeutic effect, the drug should be taken in a single daily dose overnight, for its highest concentration in the gallbladder at a relative functional rest and during the maximum cholesterol synthesis[48]. Rarely, with the use of the drug there may be diarrhea. In these cases, 1/3 of the daily dose should be taken in the morning and the rest in the evening.

The efficiency of litholytic therapy is shown to depend largely on its use at the early stages of GD when compact stones have not been formed yet. Drug therapy is performed long-term (from 6 mo to 2 years or more), necessarily with ultrasound guidance and biochemical blood tests carried out every three months during therapy. When the stones are reduced in size, it is advisable to continue the therapy for 3-6 mo until they are completely dissolved. If there is no reduction in the sizes of gallstones within 12 mo of the initiation of litholytic therapy, the latter should be stopped[48]. Low-cholesterol diet and dietary intake of bran are indicated during and after the therapy[48]. Ursotherapy is not a contraindication in the treatment of pregnant women with GD[22].

When selecting the patients correctly, the efficiency of litholytic therapy with UDCA is as high as 60%-90%: (1) in the presence of "floating" cholesterol small stone, it is up to 90%; (2) with single mixed gallstones < 1 cm in diameter, it is up to 75%; and (3) with multiple mixed gallstones with the maximum diameter of < 1 cm, it is up to 60%.

The result of therapy depends on the size of a stone; cholesterol stones less than 5 mm are best dissolved irrespective of the risk factors predisposing to the disease[136]. Single stones are dissolved less well than multiple ones (the latter have a more optimal ratio of the surface of stones to the volume of the gallbladder containing bile acid preparations). The highest effect is noted in young patients. Successful therapy proves to be more frequent when GD is detected early and much rarer when there is a long history of the disease due to stone calcification. When gallbladder contractility is preserved, successful therapy is predicted to be much more optimistic[22].

Unfortunately, GS may again form after their successful dissolution. After successful oral LT, recurrent stones are annually about 10% during 5 years, more frequently during the first 2 years, and then their frequency decreases. The risk for recurrence is less in patients with a primary single stone than in those who have been earlier found to have multiple stones. For the prevention of stone recurrences, it is necessary to continue small-dose UDCA therapy, which results in a significant reduction in the bile lithogenicity index and prevents recurrent stone formation[48].

Contact litholysis

Contact litholysis is a variant of litholytic therapy. If contact litholysis is used, a substance that dissolves cholesterol stones is injected just into the gallbladder or bile ducts. Only cholesterol stones are prone to dissolution;

their size and number are of no fundamental importance. Methyltretbutyl ether and propionic ether are used to dissolve stones in the gallbladder and bile ducts, respectively. Dissolution occurs within 4-16 h. The multicenter study covering 803 patients in 21 European medical centers has shown the high efficiency of contact litholysis. Puncture was successful in 761 (94.8%) patients and stones were dissolved in 95.1% of cases. After litholysis, biliary sludge remained in the gallbladder in 43.1% patients. The technique may be successfully used to dissolve fragments remaining after ESWL[22]. This procedure can be the method of choice in treating GD patients at high intraoperative risk. It may be employed both in patients with significant clinical manifestations and biliary colic episodes and those with asymptomatic GD.

From the physiological and molecular biochemical bases of the structural and functional state of the major components of bile, it is clear that, besides bile acids, phospholipids can solubilize cholesterol. The solubilizing properties of phosphatidylcholines (lecithins) are shown to be largely due to the fatty acid that is in the second position of a phospholipid molecule. This has given an impetus to design novel agents for dissolution of cholesterol gallstones containing conjugates of bile acids and fatty acids with a chain length of 14 to 22 carbon atoms linked by an amide bond[119,137]. The amide bond prevents the compound from splitting in the intestine. The first laboratory studies have demonstrated that the conjugates of bile acids and fatty acids do show a cholesterol-solubilizing effect[119]. The conjugates of bile acids with arachidonic acid, arachidyl-amino-cholanoid, have the best solubilizing effect. It has been indicated *in vitro* and *in vivo* (in mice) that these compounds are able to prevent the formation of cholesterol crystals and to dissolve them in animals on a lithogenic diet[119,137].

ESWL has substantially extended the capabilities of medical treatment in patients with GD and could achieve a positive effect in those with gallstones up to 3 cm in diameter. The technique is based on shock wave generation. Pressure that is 1000 times greater than the atmospheric one is achieved in the focus within 30 nsec. Because soft tissues absorb little energy, its bulk falls on a stone, causing its destruction. The technique is used as a preparatory stage for further oral litholytic therapy. There are strict indications for this type of therapy.

Criteria for selection of patients for lithotripsy are as follows: (1) single radiolucent cholesterol stones not more than 3 cm in diameter; (2) multiple radiolucent stones (not more than 3) 1-1.5 cm in diameter; (3) the volume of stones is < 1/2 of that of the gallbladder; (4) a functioning gallbladder; (5) normal bile duct patency; (6) contraindications to ESWL; (7) the presence of coagulopathy or anticoagulant therapy; and (8) the presence of cavitary mass along the course of a shock wave. Approximately 20% of patients with GD meet the criteria for ESWL.

Stone shattering into small fragments occurs after 1-3 sessions. When patients are correctly selected for ESWL, stones fragmentation can be achieved in 90%-95% of cases. Lithotripsy is considered successful if stones less than 5 mm in diameter can be fragmented. ESWL yields good results when minor (< 20 mm) single stones are shattered. There are a low percentage of positive results if large dense and multiple stones are available. After lithotripsy, stone fragments are mainly excreted independently. Shock wave lithotripsy is generally used in combination with litholytic therapy that should be continued within six months after the last session of lithotripsy. The adverse reactions of lithotripsy are rare if indications are correctly chosen and the procedure is strictly followed. The most common reactions are biliary colic and, occasionally, minor signs of cholecystitis, hyperaminotransferasemia[22]. Biliary colic is eliminated by the use of spasmolytics and analgesics. Shattering of large gallstones by a few sessions in combination with litholytic therapy prevents the development of obstructive jaundice after lithotripsy.

High recurrence rates in the late period following lithotripsy are the most essential limitation to apply this technique[138]. ESWL has also shown to be effective in 90% of the common bile duct stones refractory to endoscopic treatment[139]; however, a recurrence is observed in 14.5% of patients within 10 years[140]. There are data on the relative safety and efficiency of ESWL in patients with incorporated biliary tract stones and a high surgical risk[141,142].

Potential GD-preventing drugs

Among the GD-preventing drugs, ezetimibe is noteworthy[143]. This agent prevents the formation of cholesterol stones in mice by reducing cholesterol absorption (by 35% in the animals on a lithogenic diet and by 90% in the controls) and bile cholesterol saturation index (by 60% on a lithogenic diet), intensifying bile flow, and enhancing the secretion of bile salts (by 60%), phospholipids (by 44%) and glutathione (by 100%), which is associated with the slightly increased expression of bile acid carriers. According to the preliminary data, the major effect of ezetimibe in man is to lower cholesterol absorption[144]. The drug is also effective in resorbing cholesterol stones by producing excess unsaturated micelles. Moreover, it increases the time of cholesterol crystallization in patients[145].

The long-term use of magnesium preparations has been demonstrated to prevent the occurrence of clinical forms of GD. Magnesium deficiency may cause dyslipidemia and insulin hypersecretion[146,147].

There is evidence for the administration of melatonin for the prevention of GD. Melatonin is considered to lower bile cholesterol by reducing the rate of its absorption by the intestinal epithelium and by increasing the rate of its conversion to bile acids[148]. Of great importance in the prevention of recurrent gallstones are the following factors: (1) to avoid inactivity[24,48]. Patients with GD are recommended to exercise (graduated walking of at least 1 km daily; daily exercises associated with the tension of prelum abdominal and the elevation of intraabdominal pressure); (2) to keep a dietary pattern (frequent, fractional) and low-cholesterol diet; (3) to eliminate being

overweight; (4) to avoid long-term starvation periods and intake of cholesterol synthesis-increasing drugs[22]; and (5) to have gallbladder ultrasonography at least once a year.

CONCLUSION

In conclusion, the achievement in the study of the physiology of bile formation and the pathogenesis of gallstone disease has allowed expanding indications for therapeutic treatment of GD and reducing the number of patients who undergo surgical treatment. It should be noted that notable advances have been made in studying the mechanisms responsible for the formation of GS, which could extend the capabilities of their dissolution and shattering conservatively. Because GD is a multifactorial disease, its treatment remains symptomatic. Because the etiology and pathogenesis of GD is still not well defined and strategies for prevention and efficient non-surgical therapies are missing, further studies are required[1]. This makes investigators continue so that researchers have new data to allow progress in the treatment of cholelithiasis. From a public health standpoint, it is not only important to study the background prevalence of gallstone disease regionally, but also to explore the demographic and biological markers related to the development of gallstone disease. If we can predict which factors contribute to the development of GD, we can prevent it by controlling these factors.

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REFERENCES

1 **Sun H**, Tang H, Jiang S, Zeng L, Chen EQ, Zhou TY, Wang YJ. Gender and metabolic differences of gallstone diseases. *World J Gastroenterol* 2009; **15**: 1886-1891
2 **Conte D**, Fraquelli M, Giunta M, Conti CB. Gallstones and liver disease: an overview. *J Gastrointestin Liver Dis* 2011; **20**: 9-11
3 **Belousov Yu V.** Pediatric Gastroenterology. Up-to-date guide. Moscow: Exma, 2006: 112
4 **Méndez-Sánchez N**, Zamora-Valdés D, Flores-Rangel JA, Pérez-Sosa JA, Vásquez-Fernández F, Lezama-Mora JI, Vázquez-Elizondo G, Ponciano-Rodríguez G, Ramos MH, Uribe M. Gallstones are associated with carotid atherosclerosis. *Liver Int* 2008; **28**: 402-406
5 **Sánchez-Cuén J**, Aguilar-Medina M, Arámbula-Meraz E, Romero-Navarro J, Granados J, Sicairos-Medina L, Ramos-Payán R. ApoB-100, ApoE and CYP7A1 gene polymorphisms in Mexican patients with cholesterol gallstone disease. *World J Gastroenterol* 2010; **16**: 4685-4690
6 **Temel RE**, Brown JM. A new framework for reverse cholesterol transport: non-biliary contributions to reverse cholesterol transport. *World J Gastroenterol* 2010; **16**: 5946-5952
7 **Bagaudinov KG**, Saidov SS, Garilevich BA, Zubkov AD, Abdulaev RA, Ovakimian GS. [Improvement of extracorporeal shockwave cholelithotripsy in the comprehensive treatment of cholelithiasis]. *Klin Med* (*Mosk*) 2007; **85**: 56-59
8 **Doggrell SA**. New targets in and potential treatments for cholesterol gallstone disease. *Curr Opin Investig Drugs* 2006; **7**: 344-348
9 **Goldacre MJ**, Duncan ME, Griffith M, Davidson M. Trends in mortality from appendicitis and from gallstone disease in English populations, 1979-2006: study of multiple-cause coding of deaths. *Postgrad Med J* 2011; **87**: 245-250
10 **Novacek G**. Gender and gallstone disease. *Wien Med Wochenschr* 2006; **156**: 527-533
11 **Everhart JE**, Khare M, Hill M, Maurer KR. Prevalence and ethnic differences in gallbladder disease in the United States. *Gastroenterology* 1999; **117**: 632-639
12 **Miquel JF**, Covarrubias C, Villaroel L, Mingrone G, Greco AV, Puglielli L, Carvallo P, Marshall G, Del Pino G, Nervi F. Genetic epidemiology of cholesterol cholelithiasis among Chilean Hispanics, Amerindians, and Maoris. *Gastroenterology* 1998; **115**: 937-946
13 **Shaffer EA**. Epidemiology and risk factors for gallstone disease: has the paradigm changed in the 21st century? *Curr Gastroenterol Rep* 2005; **7**: 132-140
14 **Xu P**, Yin XM, Zhang M, Liang YJ. [Epidemiology of gallstone in Nanjing City in China]. *Zhonghua Liu Xing Bing Xue Za Zhi* 2004; **25**: 928
15 **Sampliner RE**, Bennett PH, Comess LJ, Rose FA, Burch TA. Gallbladder disease in pima indians. Demonstration of high prevalence and early onset by cholecystography. *N Engl J Med* 1970; **283**: 1358-1364
16 **Everhart JE**, Yeh F, Lee ET, Hill MC, Fabsitz R, Howard BV, Welty TK. Prevalence of gallbladder disease in American Indian populations: findings from the Strong Heart Study. *Hepatology* 2002; **35**: 1507-1512
17 **Buch S**, Schafmayer C, Völzke H, Becker C, Franke A, von Eller-Eberstein H, Kluck C, Bässmann I, Brosch M, Lammert F, Miquel JF, Nervi F, Wittig M, Rosskopf D, Timm B, Höll C, Seeger M, ElSharawy A, Lu T, Egberts J, Fändrich F, Fölsch UR, Krawczak M, Schreiber S, Nürnberg P, Tepel J, Hampe J. A genome-wide association scan identifies the hepatic cholesterol transporter ABCG8 as a susceptibility factor for human gallstone disease. *Nat Genet* 2007; **39**: 995-999
18 **Ahmed M**, Diggory R. The correlation between ultrasonography and histology in the search for gallstones. *Ann R Coll Surg Engl* 2011; **93**: 81-83
19 **Stuart GS**, Tang JH, Heartwell SF, Westhoff CL. A high cholecystectomy rate in a cohort of Mexican American women who are postpartum at the time of oral contraceptive pill initiation. *Contraception* 2007; **76**: 357-359
20 **Ruhl CE**, Everhart JE. Gallstone disease is associated with increased mortality in the United States. *Gastroenterology* 2011; **140**: 508-516
21 **Tsukanov VV**, Nozdrachev KG, Tonkikh IuL, Bronnikova EP, Kupershteĭe EIu. [The mechanism of reverse cholesterol transport and cholelithiasis in Northern ethnic groups]. *Klin Med (Mosk)* 2007; **85**: 33-35
22 **Ilychenko AA.** Gallstone disease. *Lechashchiy Vrach* 2004; **4**: 27-33
23 **Chong VH**. Iatrogenic biliary stone. *Surg Technol Int* 2005; **14**: 147-155
24 **Völzke H**, Baumeister SE, Alte D, Hoffmann W, Schwahn C, Simon P, John U, Lerch MM. Independent risk factors for gallstone formation in a region with high cholelithiasis prevalence. *Digestion* 2005; **71**: 97-105
25 **Cozcolluela Cabrejas MR**, Sanz Salanova LA, Martínez-Berganza Asensio MT, Gómez Herrero H, Mellado Santos JM, Miranda Orella L, Forradellas Morales A. [Childhood cholelithiasis in a district hospital]. *An Pediatr (Barc)* 2007; **66**: 611-614

26 **Poddar U**. Gallstone disease in children. *Indian Pediatr* 2010; **47**: 945-953
27 **Bergman S**, Sourial N, Vedel I, Hanna WC, Fraser SA, Newman D, Bilek AJ, Galatas C, Marek JE, Monette J. Gallstone disease in the elderly: are older patients managed differently? *Surg Endosc* 2011; **25**: 55-61
28 **Wang DQ**. Aging per se is an independent risk factor for cholesterol gallstone formation in gallstone susceptible mice. *J Lipid Res* 2002; **43**: 1950-1959
29 Grigorieva IN. Major risk factors of cholelithiasis. *Rossiyskiy Zhurnal Gastroenterologii, Gepatologii i Koloproktologii* 2007; **6**: 17-19
30 **He J**, Nishida S, Xu M, Makishima M, Xie W. PXR prevents cholesterol gallstone disease by regulating biosynthesis and transport of bile salts. *Gastroenterology* 2011; **140**: 2095-2106
31 **Qin J**, Han TQ, Fei J, Jiang ZY, Zhang Y, Yang SY, Jiang ZH, Cai XX, Huang W, Zhang SD. [Risk factors of familial gallstone disease: study of 135 pedigrees]. *Zhonghua Yi Xue Za Zhi* 2005; **85**: 1966-1969
32 **Abu-Eshy SA**, Mahfouz AA, Badr A, El Gamal MN, Al-Shehri MY, Salati MI, Rabie ME. Prevalence and risk factors of gallstone disease in a high altitude Saudi population. *East Mediterr Health J* 2007; **13**: 794-802
33 **Ko CW**. Risk factors for gallstone-related hospitalization during pregnancy and the postpartum. *Am J Gastroenterol* 2006; **101**: 2263-2268
34 **Marschall HU**, Einarsson C. Gallstone disease. *J Intern Med* 2007; **261**: 529-542
35 **Cirillo DJ**, Wallace RB, Rodabough RJ, Greenland P, LaCroix AZ, Limacher MC, Larson JC. Effect of estrogen therapy on gallbladder disease. *JAMA* 2005; **293**: 330-339
36 **Hart AR**, Luben R, Welch A, Bingham S, Khaw KT. Hormone replacement therapy and symptomatic gallstones - a prospective population study in the EPIC-Norfolk cohort. *Digestion* 2008; **77**: 4-9
37 **Fornari F**, Civardi G, Buscarini E, Cavanna L, Imberti D, Rossi S, Sbolli G, Di Stasi M, Buscarini L. Cirrhosis of the liver. A risk factor for development of cholelithiasis in males. *Dig Dis Sci* 1990; **35**: 1403-1408
38 **Zimmer V**, Lammert F. Genetics in liver disease: new concepts. *Curr Opin Gastroenterol* 2011; **27**: 231-239
39 **Attili AF**, De Santis A, Attili F, Roda E, Festi D, Carulli N. Prevalence of gallstone disease in first-degree relatives of patients with cholelithiasis. *World J Gastroenterol* 2005; **11**: 6508-6511
40 **Katsika D**, Grjibovski A, Einarsson C, Lammert F, Lichtenstein P, Marschall HU. Genetic and environmental influences on symptomatic gallstone disease: a Swedish study of 43,141 twin pairs. *Hepatology* 2005; **41**: 1138-1143
41 **Khanuja B**, Cheah YC, Hunt M, Nishina PM, Wang DQ, Chen HW, Billheimer JT, Carey MC, Paigen B. Lith1, a major gene affecting cholesterol gallstone formation among inbred strains of mice. *Proc Natl Acad Sci USA* 1995; **92**: 7729-7733
42 **Schafmayer C**, Völzke H, Buch S, Egberts J, Spille A, von Eberstein H, Franke A, Seeger M, Hinz S, Elsharawy A, Rosskopf D, Brosch M, Krawczak M, Foelsch UR, Schafmayer A, Lammert F, Schreiber S, Faendrich F, Hampe J, Tepel J. Investigation of the Lith6 candidate genes APOBEC1 and PPARG in human gallstone disease. *Liver Int* 2007; **27**: 910-919
43 **Kovacs P**, Kress R, Rocha J, Kurtz U, Miquel JF, Nervi F, Méndez-Sánchez N, Uribe M, Bock HH, Schirin-Sokhan R, Stumvoll M, Mössner J, Lammert F, Wittenburg H. Variation of the gene encoding the nuclear bile salt receptor FXR and gallstone susceptibility in mice and humans. *J Hepatol* 2008; **48**: 116-124
44 **Grünhage F**, Acalovschi M, Tirziu S, Walier M, Wienker TF, Ciocan A, Mosteanu O, Sauerbruch T, Lammert F. Increased gallstone risk in humans conferred by common variant of hepatic ATP-binding cassette transporter for cholesterol. *Hepatology* 2007; **46**: 793-801
45 **Kuo KK**, Shin SJ, Chen ZC, Yang YH, Yang JF, Hsiao PJ. Significant association of ABCG5 604Q and ABCG8 D19H polymorphisms with gallstone disease. *Br J Surg* 2008; **95**: 1005-1011
46 **Wang Y**, Jiang ZY, Fei J, Xin L, Cai Q, Jiang ZH, Zhu ZG, Han TQ, Zhang SD. ATP binding cassette G8 T400K polymorphism may affect the risk of gallstone disease among Chinese males. *Clin Chim Acta* 2007; **384**: 80-85
47 **Qin J**, Han TQ, Yuan WT, Fei J, Jiang ZH, Zhang J, Wang Y, Huang W, Zhang SD. [Study on the position of genes responsible for gallstone disease in Chinese population]. *Zhonghua Wai Ke Za Zhi* 2006; **44**: 485-487
48 **Kharitonova LA.** Cholelithiasis in children: issues of choice of therapeutic tactics. *Russkiy Meditsinskiy Zhurnal* 2003; **11**: 787-790
49 **Andreotti G**, Chen J, Gao YT, Rashid A, Chen BE, Rosenberg P, Sakoda LC, Deng J, Shen MC, Wang BS, Han TQ, Zhang BH, Yeager M, Welch R, Chanock S, Fraumeni JF, Hsing AW. Polymorphisms of genes in the lipid metabolism pathway and risk of biliary tract cancers and stones: a population-based case-control study in Shanghai, China. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 525-534
50 **Báez S**, Tsuchiya Y, Calvo A, Pruyas M, Nakamura K, Kiyohara C, Oyama M, Yamamoto M. Genetic variants involved in gallstone formation and capsaicin metabolism, and the risk of gallbladder cancer in Chilean women. *World J Gastroenterol* 2010; **16**: 372-378
51 **Dixit M**, Choudhuri G, Saxena R, Mittal B. Association of apolipoprotein A1-C3 gene cluster polymorphisms with gallstone disease. *Can J Gastroenterol* 2007; **21**: 569-575
52 **Juzyszyn Z**, Kurzawski M, Modrzejewski A, Sulikowski T, Pawlik A, Czerny B, Droździk M. Low-density lipoprotein receptor-related protein-associated protein (LRPAP1) gene IVS5 insertion/deletion polymorphism is not a risk factor for gallstone disease in a Polish population. *Dig Liver Dis* 2008; **40**: 122-125
53 **Mbongo-Kama E**, Harnois F, Mennecier D, Leclercq E, Burnat P, Ceppa F. MDR3 mutations associated with intrahepatic and gallbladder cholesterol cholelithiasis: an update. *Ann Hepatol* 2007; **6**: 143-149
54 **Rosmorduc O**, Poupon R. Low phospholipid associated cholelithiasis: association with mutation in the MDR3/ABCB4 gene. *Orphanet J Rare Dis* 2007; **2**: 29
55 **Kim HJ**, Kim SH, Chae GB, Lee SJ, Kang CD. Increased expression of mucin 5AC mRNA and decreased expression of epidermal growth-factor receptor mRNA in gallstone patients. *Tohoku J Exp Med* 2008; **214**: 139-144
56 **Chuang SC**, Juo SH, Hsi E, Wang SN, Tsai PC, Yu ML, Lee KT. Multiple mucin genes polymorphisms are associated with gallstone disease in Chinese men. *Clin Chim Acta* 2011; **412**: 599-603
57 **Chang TS**, Lo SK, Shyr HY, Fang JT, Lee WC, Tai DI, Sheen IS, Lin DY, Chu CM, Liaw YF. Hepatitis C virus infection facilitates gallstone formation. *J Gastroenterol Hepatol* 2005; **20**: 1416-1421
58 **Tsai CJ**, Leitzmann MF, Willett WC, Giovannucci EL. Weight cycling and risk of gallstone disease in men. *Arch Intern Med* 2006; **166**: 2369-2374
59 **Klass DM**, Lauer N, Hay B, Kratzer W, Fuchs M. Arg64 variant of the beta3-adrenergic receptor is associated with gallstone formation. *Am J Gastroenterol* 2007; **102**: 2482-2487
60 **Leitzmann MF**, Willett WC, Rimm EB, Stampfer MJ, Spiegelman D, Colditz GA, Giovannucci E. A prospective study of coffee consumption and the risk of symptomatic gallstone disease in men. *JAMA* 1999; **281**: 2106-2112
61 **Zhang Y**, Liu D, Ma Q, Dang C, Wei W, Chen W. Factors influencing the prevalence of gallstones in liver cirrhosis. *J Gastroenterol Hepatol* 2006; **21**: 1455-1458
62 **Déry L**, Galambos Z, Kupcsulik P, Lukovich P. [Cirrhosis

and cholelithiasis. Laparoscopic or open cholecystectomy?]. *Orv Hetil* 2008; **149**: 2129-2134
63 **Hsing AW**, Gao YT, McGlynn KA, Niwa S, Zhang M, Han TQ, Wang BS, Chen J, Sakoda LC, Shen MC, Zhang BH, Deng J, Rashid A. Biliary tract cancer and stones in relation to chronic liver conditions: A population-based study in Shanghai, China. *Int J Cancer* 2007; **120**: 1981-1985
64 **Loria P**, Lonardo A, Lombardini S, Carulli L, Verrone A, Ganazzi D, Rudilosso A, D'Amico R, Bertolotti M, Carulli N. Gallstone disease in non-alcoholic fatty liver: prevalence and associated factors. *J Gastroenterol Hepatol* 2005; **20**: 1176-1184
65 **Guraya SY**. Reappraisal of the management of cholelithiasis in diabetics. *Saudi Med J* 2005; **26**: 1691-1694
66 **Chang SC**, Rashid A, Gao YT, Andreotti G, Shen MC, Wang BS, Han TQ, Zhang BH, Sakoda LC, Leitzmann MF, Chen BE, Rosenberg PS, Chen J, Chanock SJ, Hsing AW. Polymorphism of genes related to insulin sensitivity and the risk of biliary tract cancer and biliary stone: a population-based case-control study in Shanghai, China. *Carcinogenesis* 2008; **29**: 944-948
67 **Paisley AN**, Roberts ME, Trainer PJ. Withdrawal of somatostatin analogue therapy in patients with acromegaly is associated with an increased risk of acute biliary problems. *Clin Endocrinol (Oxf)* 2007; **66**: 723-726
68 **Davidson MH**, Armani A, McKenney JM, Jacobson TA. Safety considerations with fibrate therapy. *Am J Cardiol* 2007; **99**: 3C-18C
69 **Schiemann U**, Ferhat A, Götzberger M, Kaiser C, Stief J, Landgraf R, Dieterle C. Prevalence of cholecystolithiasis and its management among kidney/pancreas-transplanted type 1 (insulin-dependent) diabetic patients. *Eur J Med Res* 2008; **13**: 127-130
70 **Khan MK**, Jalil MA, Khan MS. Oral contraceptives in gall stone diseases. *Mymensingh Med J* 2007; **16**: S40-S45
71 **Reshetnyak TM,** Saparin GV, Ivannikov PV, Reshetnyak VI. Corticosteroids and Cholelithiasis in Systemic Lupus Erythematosus. *Scholarly Research Exchange* 2009; **2009**: 9
72 **Sherlock S,** Dooley J. Diseases of the liver and biliary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 44-58
73 **Soysal A**, Eraşov K, Akpinar I, Bakir M. Biliary precipitation during ceftriaxone therapy: frequency and risk factors. *Turk J Pediatr* 2007; **49**: 404-407
74 **Liu CM**, Tung TH, Tsai ST, Liu JH, Tsai YK, Chen VT, Tam TN, Lu HF, Wang KK, Hsu CT, Shih HC, Chan DC, Chou P. Serum insulin, insulin resistance, beta-cell dysfunction, and gallstone disease among type 2 diabetics in Chinese population: a community-based study in Kinmen, Taiwan. *World J Gastroenterol* 2005; **11**: 7159-7164
75 **Chang Y**, Sung E, Ryu S, Park YW, Jang YM, Park M. Insulin resistance is associated with gallstones even in non-obese, non-diabetic Korean men. *J Korean Med Sci* 2008; **23**: 644-650
76 **Pullinger CR**, Eng C, Salen G, Shefer S, Batta AK, Erickson SK, Verhagen A, Rivera CR, Mulvihill SJ, Malloy MJ, Kane JP. Human cholesterol 7alpha-hydroxylase (CYP7A1) deficiency has a hypercholesterolemic phenotype. *J Clin Invest* 2002; **110**: 109-117
77 **Monte MJ**, Marin JJ, Antelo A, Vazquez-Tato J. Bile acids: chemistry, physiology, and pathophysiology. *World J Gastroenterol* 2009; **15**: 804-816
78 **Kitsiou-Tzeli S**, Giannatou E, Spanos I, Nicolaidou P, Fretzayas A, Tzetis M, Lazaris D, Kanavakis E, Tsezou A. Steroid hormones polymorphisms and cholelithiasis in Greek population. *Liver Int* 2007; **27**: 61-68
79 **Momiyama M**, Wakai K, Oda K, Kamiya J, Ohno Y, Hamaguchi M, Nakanuma Y, Hsieh LL, Yeh TS, Chen TC, Jan YY, Chen MF, Nimura Y. Lifestyle risk factors for intrahepatic stone: findings from a case-control study in an endemic area, Taiwan. *J Gastroenterol Hepatol* 2008; **23**: 1075-1081
80 **Vilkin A**, Geller A, Levi Z, Niv Y. Mucin gene expression in bile of patients with and without gallstone disease, collected by endoscopic retrograde cholangiography. *World J Gastroenterol* 2009; **15**: 2367-2371
81 **Sasaki M**, Nakanuma Y, Kim YS. Expression of apomucins in the intrahepatic biliary tree in hepatolithiasis differs from that in normal liver and extrahepatic biliary obstruction. *Hepatology* 1998; **27**: 54-61
82 **Wang HH**, Afdhal NH, Gendler SJ, Wang DQ. Targeted disruption of the murine mucin gene 1 decreases susceptibility to cholesterol gallstone formation. *J Lipid Res* 2004; **45**: 438-447
83 **Maximenko VB.** Impaired concentration and motor evacuatory functions of the gallbladder in cholecystolithiasis. *Rossiyskiy Zhurnal Gastroenterologii, Gepatologii i Koloproktologii* 2006; **4**: 24-28
84 **Cerçi SS**, Ozbek FM, Cerçi C, Baykal B, Eroğlu HE, Baykal Z, Yildiz M, Sağlam S, Yeşildağ A. Gallbladder function and dynamics of bile flow in asymptomatic gallstone disease. *World J Gastroenterol* 2009; **15**: 2763-2767
85 **Huang SM**, Yao CC, Pan H, Hsiao KM, Yu JK, Lai TJ, Huang SD. Pathophysiological significance of gallbladder volume changes in gallstone diseases. *World J Gastroenterol* 2010; **16**: 4341-4347
86 **Olokoba AB**, Bojuwoye BJ, Olokoba LB, Wahab KW, Salami AK, Braimoh KT, Inikori AK. The relationship between gallstone disease and gall bladder volume. *Niger J Clin Pract* 2008; **11**: 89-93
87 **Bolukbas FF**, Bolukbas C, Horoz M, Ince AT, Uzunkoy A, Ozturk A, Aka N, Demirci F, Inci E, Ovunc O. Risk factors associated with gallstone and biliary sludge formation during pregnancy. *J Gastroenterol Hepatol* 2006; **21**: 1150-1153
88 **Pamuk GE**, Umit H, Harmandar F, Yeşil N. Patients with iron deficiency anemia have an increased prevalence of gallstones. *Ann Hematol* 2009; **88**: 17-20
89 **Mansurov KhKh,** Mirodzhov GK. Pathogenetic therapy for gallbladder cholesterosis. *Rossiyskiy Zhurnal Gastroenterologii, Gepatologii i Koloproktologii* 2005; **6**: 45-48
90 **Srivastava A**, Pandey SN, Dixit M, Choudhuri G, Mittal B. Cholecystokinin receptor A gene polymorphism in gallstone disease and gallbladder cancer. *J Gastroenterol Hepatol* 2008; **23**: 970-975
91 **Zhang ZH**, Wu SD, Gao H, Shi G, Jin JZ, Kong J, Tian Z, Su Y. Expression of pituitary adenylate cyclase-activating polypeptide 1 and 2 receptor mRNA in gallbladder tissue of patients with gallstone or gallbladder polyps. *World J Gastroenterol* 2006; **12**: 1468-1471
92 **Xiao Z**, Schmitz F, Pricolo VE, Biancani P, Behar J. Role of caveolae in the pathogenesis of cholesterol-induced gallbladder muscle hypomotility. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G1641-G1649
93 **Yi SQ**, Ohta T, Tsuchida A, Terayama H, Naito M, Li J, Wang HX, Yi N, Tanaka S, Itoh M. Surgical anatomy of innervation of the gallbladder in humans and Suncus murinus with special reference to morphological understanding of gallstone formation after gastrectomy. *World J Gastroenterol* 2007; **13**: 2066-2071
94 **Kobayashi T**, Hisanaga M, Kanehiro H, Yamada Y, Ko S, Nakajima Y. Analysis of risk factors for the development of gallstones after gastrectomy. *Br J Surg* 2005; **92**: 1399-1403
95 **Nakamura K**, Ogoshi K, Makuuchi H. Clinicopathological study of cholelithiasis following gastric cancer surgery. *Eur Surg Res* 2005; **37**: 29-35
96 **Quesada BM**, Kohan G, Roff HE, Canullán CM, Chiappetta Porras LT. Management of gallstones and gallbladder disease in patients undergoing gastric bypass. *World J Gastroenterol* 2010; **16**: 2075-2079
97 **Crema E**, Ribeiro LB, Adad SJ, Ectchebehere RM, Martins Júnior A, Silva AA. Gallbladder neuron count in cholelithiasis patients with and without Chagas disease. *Rev Soc Bras Med Trop* 2007; **40**: 15-17
98 **vanBerge-Henegouwen GP**, Venneman NG, van Erpecum

KJ, Portincasa P. Drugs affecting biliary lipid secretion and gallbladder motility: their potential role in gallstone treatment and prevention. *Curr Drug Targets Immune Endocr Metabol Disord* 2005; **5**: 185-191
99 **Loginov AS**, Chebanov SM, Marakhovskiĭ IuKh, Goncharik II. [Microstructure of vesicular agglomerates of the lithogenic bile]. *Biull Eksp Biol Med* 1989; **108**: 251-254
100 **Loginov AS**, Chebanov SM, Petrakov AV, Saparin GV, Obyden SK, Ivannikov PV. Investigation of cholesterol, bilirubin, and protein distribution in human gallstones by color cathodoluminescence scanning electron microscopy and transmission electron microscopy. *Scanning* 1998; **20**: 17-22
101 **Loginov AS,** Chebanov SM, Saparin GV, Obyden SK, Reshetnyak VI. Microstructure of gallstones according to the color cathodoluminescence scanning electron microscopy. *Russian Gastroenterol J* 1998; **4**: 26-29
102 **Isayeva GSh.** Possible involvement of Helicobacter bacteria in the pathogenesis of hepatobiliary diseases. *Rossiyskiy Zhurnal Gastroenterologii, Gepatologii i Koloproktologii* 2008; **4**: 14-22
103 **Vasavda N**, Menzel S, Kondaveeti S, Maytham E, Awogbade M, Bannister S, Cunningham J, Eichholz A, Daniel Y, Okpala I, Fulford T, Thein SL. The linear effects of alpha-thalassaemia, the UGT1A1 and HMOX1 polymorphisms on cholelithiasis in sickle cell disease. *Br J Haematol* 2007; **138**: 263-270
104 **Leff DR**, Kaura T, Agarwal T, Davies SC, Howard J, Chang AC. A nontransfusional perioperative management regimen for patients with sickle cell disease undergoing laparoscopic cholecystectomy. *Surg Endosc* 2007; **21**: 1117-1121
105 **Origa R**, Galanello R, Perseu L, Tavazzi D, Domenica Cappellini M, Terenzani L, Forni GL, Quarta G, Boetti T, Piga A. Cholelithiasis in thalassemia major. *Eur J Haematol* 2009; **82**: 22-25
106 **Tankanitlert J**, Morales NP, Fucharoen P, Fucharoen S, Chantharaksri U. Association between promoter and coding region mutations of UDP-glucuronosyltransferase 1A1 and beta-thalassemia/Hb E with cholelithiasis. *Eur J Haematol* 2008; **80**: 351-355
107 **Uchiyama K**, Kawai M, Tani M, Terasawa H, Tanimura H, Yamaue H. Pathogenesis of hepatolithiasis based on the analysis of components of intrahepatic stones. *Hepatogastroenterology* 2007; **54**: 1798-1804
108 **Sayers C**, Wyatt J, Soloway RD, Taylor DR, Stringer MD. Gallbladder mucin production and calcium carbonate gallstones in children. *Pediatr Surg Int* 2007; **23**: 219-223
109 **Kaur T**, Kaur S. Pathophysiological conditions in cholelithiasis formation in North Indian population: spectroscopic, biophysical, and biochemical study. *Biol Trace Elem Res* 2010; **138**: 79-89
110 **Carey MC**. Pathogenesis of gallstones. *Am J Surg* 1993; **165**: 410-419
111 **Dikkers A**, Tietge UJ. Biliary cholesterol secretion: more than a simple ABC. *World J Gastroenterol* 2010; **16**: 5936-5945
112 **Claudel T**, Zollner G, Wagner M, Trauner M. Role of nuclear receptors for bile acid metabolism, bile secretion, cholestasis, and gallstone disease. *Biochim Biophys Acta* 2011; **1812**: 867-878
113 **Small DM.** The staging of cholesterol gallstones with respect to nucleation and growth. In: Paumgartner J, Stiehl A, Gerok W, editors. Falk Symposium 29. Bile acids and lipids. England: MTP Press Limited, 1981: 291-300
114 **Carey MC,** Cohen D. Biliary transport of cholesterol in vesicles, micelles and liquid crystals. In: Paumgartner G, Stiehl A, Gerok W, editors. Falk Symposium 45. Bile Acids and the Liver. England: MTP Press Limited, 1987: 287-300
115 **Cohen DE**, Kaler EW, Carey MC. Cholesterol carriers in human bile: are "lamellae" involved? *Hepatology* 1993; **18**: 1522-1531
116 **Been JM**, Bills PM, Lewis D. Microstructure of gallstones. *Gastroenterology* 1979; **76**: 548-555
117 **Chijiiwa K**, Hirota I, Noshiro H. High vesicular cholesterol and protein in bile are associated with formation of cholesterol but not pigment gallstones. *Dig Dis Sci* 1993; **38**: 161-166
118 **Holan KR**, Holzbach RT, Hermann RE, Cooperman AM, Claffey WJ. Nucleation time: a key factor in the pathogenesis of cholesterol gallstone disease. *Gastroenterology* 1979; **77**: 611-617
119 **Konikoff FM**, Cohen DE, Carey MC. Phospholipid molecular species influence crystal habits and transition sequences of metastable intermediates during cholesterol crystallization from bile salt-rich model bile. *J Lipid Res* 1994; **35**: 60-70
120 **Loginov AS**, Chebanov SM, Saparin GV, Obyden SK. The morphology and composition of cholesterol, protein, and bilirubin deposits in dried human bile: cathodoluminescence and backscattered electron imaging. *Scanning* 1998; **20**: 442-446
121 **Jüngst C**, Sreejayan N, Eder MI, von Stillfried N, Zündt B, Spelsberg FW, Kullak-Ublick GA, Jüngst D, von Ritter C. Lipid peroxidation and mucin secretagogue activity in bile of gallstone patients. *Eur J Clin Invest* 2007; **37**: 731-736
122 **Hofmann AF**. Biliary secretion and excretion in health and disease: current concepts. *Ann Hepatol* 2007; **6**: 15-27
123 **Wu SD**, Yu H, Sun JM. Bacteriological and electron microscopic examination of primary intrahepatic stones. *Hepatobiliary Pancreat Dis Int* 2006; **5**: 228-231
124 **Manolis EN**, Filippou DK, Papadopoulos VP, Kaklamanos I, Katostaras T, Christianakis E, Bonatsos G, Tsakris A. The culture site of the gallbladder affects recovery of bacteria in symptomatic cholelithiasis. *J Gastrointestin Liver Dis* 2008; **17**: 179-182
125 **Crawford RW**, Gibson DL, Kay WW, Gunn JS. Identification of a bile-induced exopolysaccharide required for Salmonella biofilm formation on gallstone surfaces. *Infect Immun* 2008; **76**: 5341-5349
126 **Stewart L**, Griffiss JM, Jarvis GA, Way LW. Bacteria entombed in the center of cholesterol gallstones induce fewer infectious manifestations than bacteria in the matrix of pigment stones. *J Gastrointest Surg* 2007; **11**: 1298-1308
127 **Stewart L**, Grifiss JM, Jarvis GA, Way LW. Biliary bacterial factors determine the path of gallstone formation. *Am J Surg* 2006; **192**: 598-603
128 **Jang JS**, Kim KH, Yu JR, Lee SU. Identification of parasite DNA in common bile duct stones by PCR and DNA sequencing. *Korean J Parasitol* 2007; **45**: 301-306
129 **Choi D**, Lim JH, Lee KT, Lee JK, Choi SH, Heo JS, Choi DW, Jang KT, Lee NY, Kim S, Hong ST. Gallstones and Clonorchis sinensis infection: a hospital-based case-control study in Korea. *J Gastroenterol Hepatol* 2008; **23**: e399-e404
130 **Mayev IV,** Dicheva DT, Buragina TA. Diagnosis and treatment of biliary sludge in patients with ulcerative disease. *Rossiyskiy Zhurnal Gastroenterologii, Gepatologii i Koloproktologii* 2007; **4**: 68-72
131 **Mekhtiyev SN,** Grinevich VB, Kravchuk YuA, Bogdanov RN. Biliary sludge: unsolved problems. *Lechashchiy Vrach* 2007; **6**: 24-28
132 **Marschall HU**, Wagner M, Zollner G, Fickert P, Diczfalusy U, Gumhold J, Silbert D, Fuchsbichler A, Benthin L, Grundström R, Gustafsson U, Sahlin S, Einarsson C, Trauner M. Complementary stimulation of hepatobiliary transport and detoxification systems by rifampicin and ursodeoxycholic acid in humans. *Gastroenterology* 2005; **129**: 476-485
133 **Jüngst C**, Sreejayan N, Zündt B, Müller I, Spelsberg FW, Hüttl TP, Kullak-Ublick GA, del Pozo R, Jüngst D, von Ritter C. Ursodeoxycholic acid reduces lipid peroxidation and mucin secretagogue activity in gallbladder bile of patients with cholesterol gallstones. *Eur J Clin Invest* 2008; **38**: 634-639
134 **Guarino MP**, Carotti S, Sarzano M, Alloni R, Vanni M, Grosso M, Sironi G, Maffettone PL, Cicala M. Short-term ursodeoxycholic acid treatment improves gallbladder bile

turnover in gallstone patients: a randomized trial. *Neurogastroenterol Motil* 2005; **17**: 680-686
135 **Mas MR**, Comert B, Mas N, Yamanel L, Ozotuk H, Tasci I, Jazrawi RP. Effects of long term hydrophilic bile acid therapy on in vitro contraction of gallbladder muscle strips in patients with cholesterol gallstones. *World J Gastroenterol* 2007; **13**: 4336-4339
136 **Stawarski A**, Iwańczak B, Iwańczak F. [Predisposing factors and results of pharmalogical treatment using ursodeoxycholic acid of gallbladder stones in children]. *Pol Merkur Lekarski* 2006; **20**: 199-202
137 **Keizman D**, Goldiner I, Leikin-Frenkel A, Konikoff FM. [Innovations in the medical treatment of gallstones and fatty liver: FABACs (Fatty Acid Bile Acid Conjugates)]. *Harefuah* 2008; **147**: 344-39, 373, 372
138 **Rabenstein T**, Radespiel-Tröger M, Höpfner L, Benninger J, Farnbacher M, Greess H, Lenz M, Hahn EG, Schneider HT. Ten years experience with piezoelectric extracorporeal shockwave lithotripsy of gallbladder stones. *Eur J Gastroenterol Hepatol* 2005; **17**: 629-639
139 **Amplatz S**, Piazzi L, Felder M, Comberlato M, Benvenuti S, Zancanella L, Di Fede F, de'Guelmi A, Bertozzo A, Farris P, Grasso T, Mega A, Chilovi F. Extracorporeal shock wave lithotripsy for clearance of refractory bile duct stones. *Dig Liver Dis* 2007; **39**: 267-272
140 **Carrilho-Ribeiro L**, Pinto-Correia A, Velosa J, Carneiro De Moura M. A ten-year prospective study on gallbladder stone recurrence after successful extracorporeal shock-wave lithotripsy. *Scand J Gastroenterol* 2006; **41**: 338-342
141 **Xu Z**, Wang LX, Zhang NW, Hou CS, Ling XF, Xu Y, Zhou XS. Clinical application of plasma shock wave lithotripsy in treating impacted stones in the bile duct system. *World J Gastroenterol* 2006; **12**: 130-133
142 **Shim CS**, Moon JH, Cho YD, Kim YS, Hong SJ, Kim JO, Cho JY, Kim YS, Lee JS, Lee MS. The role of extracorporeal shock wave lithotripsy combined with endoscopic management of impacted cystic duct stones in patients with high surgical risk. *Hepatogastroenterology* 2005; **52**: 1026-1029
143 **Ahmed MH**. Ezetimbe as potential treatment for cholesterol gallstones: the need for clinical trials. *World J Gastroenterol* 2010; **16**: 1555-1557
144 **Zúñiga S**, Molina H, Azocar L, Amigo L, Nervi F, Pimentel F, Jarufe N, Arrese M, Lammert F, Miquel JF. Ezetimibe prevents cholesterol gallstone formation in mice. *Liver Int* 2008; **28**: 935-947
145 **Wang HH**, Portincasa P, Mendez-Sanchez N, Uribe M, Wang DQ. Effect of ezetimibe on the prevention and dissolution of cholesterol gallstones. *Gastroenterology* 2008; **134**: 2101-2110
146 **Ko CW**. Magnesium: does a mineral prevent gallstones? *Am J Gastroenterol* 2008; **103**: 383-385
147 **Tsai CJ**, Leitzmann MF, Willett WC, Giovannucci EL. Long-term effect of magnesium consumption on the risk of symptomatic gallstone disease among men. *Am J Gastroenterol* 2008; **103**: 375-382
148 **Koppisetti S**, Jenigiri B, Terron MP, Tengattini S, Tamura H, Flores LJ, Tan DX, Reiter RJ. Reactive oxygen species and the hypomotility of the gall bladder as targets for the treatment of gallstones with melatonin: a review. *Dig Dis Sci* 2008; **53**: 2592-2603

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BRIEF ARTICLE

Transjugular intrahepatic porto-systemic shunt in the elderly: Palliation for complications of portal hypertension

Mubin I Syed, Hetal Karsan, Hector Ferral, Azim Shaikh, Uzma Waheed, Talal Akhter, Alan Gabbard, Kamal Morar, Robert Tyrrell

Mubin I Syed, Azim Shaikh, Uzma Waheed, Kamal Morar, Robert Tyrrell, Dayton Interventional Radiology, Dayton, OH 45409, United States
Mubin I Syed, Kamal Morar, Robert Tyrrell, Department of Radiological Sciences, Wright State University School of Medicine, Dayton, OH 45409, United States
Hetal Karsan, Department of Hepatology, Emory University, Atlanta, GA 30322, United States
Hector Ferral, Department of Interventional Radiology, Rush University Medical Center, Chicago, IL 60612, United States
Talal Akhter, Department of the College of Medicine, Northeast Ohio Medical University, Rootstown, OH 44272, United States
Alan Gabbard, Department of Gastroenterology, Springfield Regional Medical Center, Springfield, OH 45505, United States
Author contributions: All authors contributed equally to this paper.
Correspondence to: Mubin I Syed, MD, FSIR, FACR, Dayton Interventional Radiology, 3075 Governors Place Blvd., Ste. 120, Dayton, OH 45409, United States. mubinsyed@aol.com
Telephone: +1-937-4242580 Fax: +1-937-4242581
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Abstract

AIM: To present a dedicated series of transjugular intrahepatic porto-systemic shunts (TIPS) in the elderly since data is sparse on this population group.

METHODS: A retrospective review was performed of patients at least 65 years of age who underwent TIPS at our institutions between 1997 and 2010. Twenty-five patients were referred for TIPS. We deemed that 2 patients were not considered appropriate candidates due to their markedly advanced liver disease. Of the 23 patients suitable for TIPS, the indications for TIPS placement was portal hypertension complicated by refractory ascites alone (n = 9), hepatic hydrothorax alone (n = 2), refractory ascites and hydrothorax (n = 1), gastrointestinal bleeding alone (n = 8), gastrointestinal bleeding and ascites (n = 3).

RESULTS: Of these 23 attempted TIPS procedure patients, 21 patients had technically successful TIPS procedures. A total of 29 out of 32 TIPS procedures including revisions were successful in 21 patients with a mean age of 72.1 years (range 65-82 years). Three of the procedures were unsuccessful attempts at TIPS and 8 procedures were successful revisions of our existing TIPS. Sixteen of 21 patients who underwent successful TIPS (excluding 5 patients lost to follow-up) were followed for a mean of 14.7 mo. Ascites and/or hydrothorax was controlled following technically successful procedures in 12 of 13 patients. Bleeding was controlled following technically successful procedures in 10 out of 11 patients.

CONCLUSION: We have demonstrated that TIPS is an effective procedure to control refractory complications of portal hypertension in elderly patients.

Key words: Transjugular intrahepatic porto-systemic shunt; Portal hypertension; Elderly; Ascites; Cirrhosis

Peer reviewer: Dr. Paolo Feltracco, Department of Pharmacology and Anesthesia, Clinica di Anestesia e Medicina Intensiva, Via cesare battisti 267, Padova 35100, Italy

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INTRODUCTION

Transjugular intrahepatic porto-systemic shunt (TIPS) is a proven therapy for the treatment of complications of portal hypertension in adults with cirrhosis. However, there is limited data on the use of this modality in the elderly.

Cirrhosisis the 10th leading cause of death in the United States[1]. Variceal bleeding is the most dreaded complication of chronic liver disease with a 30 d mortality of 20%. Standard therapies including endoscopic variceal sclerotherapy/ligation/banding and pharmacological management have proved useful in controlling bleeding for up to 90% of patients. Ascites is the most common of the major complications of cirrhosis. Medical treatment for symptomatic ascites includes sodium restriction, diuretics and/or paracentesis. Definitive long term treatment of refractory ascites and or variceal bleeding involves usually either liver transplantation or use of a portosystemic shunt. TIPS is a relatively new technique whereby a portosystemic shunt is created entirely within the liver. TIPS has been performed primarily in young to middle aged adults and to a lesser extent in children. The experience of TIPS in the elderly has been limited to a case report[2]. Consequently, the technical and physiological limitations as well as the clinical results are not well described. Although the methods used to perform TIPS in the elderly is similar to that in younger adults, special consideration in the elderly include the presence of comorbidities leading to reduced overall life expectancy. In addition, a lesser potential for hepatocellular regeneration seems to exist in the elderly, which may ironically lead to longer shunt patency[3]. In this article we report the results of TIPS placement in a group of elderly patients with severe portal hypertension, not responsive to medical management.

MATERIALS AND METHODS

All patients were referred to our institutions and were deemed appropriate candidates for TIPS due to a significant complication of portal hypertension, including refractory ascites, hydrothorax and/or, bleeding not responsive to medical management. Relative contraindications included severe pulmonary hypertension or limited cardiopulmonary reserve. Between 1997 and 2010, a total of 25 patients were greater than 65 years of age were referred for a possible TIPS procedure of which 23 were deemed suitable for TIPS. In these patients, the indication for TIPS was refractory ascites alone (n = 9), hepatic hydrothorax alone (n = 2), refractory ascites and hydrothorax (n = 1), gastrointestinal bleeding alone (n = 8), gastrointestinal bleeding and ascites (n = 3). Model for end-stage liver disease (MELD) score was calculated for each patient[4]. MELD score is calculated by the United Network for Organ Sharing modification of the original formula: MELD score = 9.6 × $\log_e$ (creatinine mg/dL) + 3.8 × $\log_e$ (bilirubin mg/dL) + 11.2 × $\log_e$ (INR) + 6.4. Meld and Child Pugh score for each individual patient are shown in Table 1. Technical details are shown in Table 2. Comorbidities are shown in Table 3.

TIPS placement technique

Anesthesiology consultation was utilized to provide sedation. We started the procedure with catheterization of the hepatic vein with a balloon occlusion catheter. The hepatic vein was then occluded with the balloon and carbon dioxide was injected into the hepatic vein. As the carbon dioxide preferentially opacified the portal vein, the portal vein was able to be targeted. In four patients a computerized tomography guided percutaneous metallic marker was inserted just anterior to the right portal vein to assist targeting. A curved cannula was advanced over a guidewire from the right internal jugular vein into a hepatic vein. A sheathed needle was then advanced through the liver parenchyma into the right branch of the portal vein. The resultant portal vein tract was then dilated and catheterized. The shunt tract was dilated with an angioplasty balloon ranging from 8 mm to 10 mm, and a self-expanding metallic stent with a maximal diameter of 10 mm to 12 mm *vs* a covered stent with a maximal diameter of 10 mm was utilized. A self expanding metallic stent, WALLSTENT (Boston Scientific Natick, MA) or covered stent VIATORR (WL Gore and Associates, Elkton, MD) was deployed across the tract to support the shunt channel. Four patients underwent CT localization of the portal vein using a technique developed by Fontaine *et al*[5]. (Patients No. 6, No. 7, No. 8, No. 23). Due to advances in technology the last 17 patients received covered stents (VIATORR). Finally, portal venography and pressure measurements were performed to assess the extent of portal decompression.

Follow-up assessments were performed by examination by the gastroenterologist in addition to ultrasonography. Ultrasonography was routinely performed, after TIPS placement, at 3 mo, and then at 6 mo intervals following TIPS placement (when patients were compliant). Ultrasonographic evaluation included assessment of patency, measurement of maximum peak systolic velocity, direction of flow in the vein, and the presence of ascites/hydrothorax. Transjugular portal venography followed by shunt revision was performed in patients with recurrent symptoms or when ultrasonography demonstrated shunt dysfunction.

RESULTS

Technical results

A total of 29 out of 32 TIPS procedures including revisions were successful in 21 patients with a mean age of 72.1 (range 65-82) years old. Three of the procedures were unsuccessful attempts at TIPS and 8 procedures were successful revisions of our existing TIPS. Sixteen of 21 patients who underwent successful TIPS (exclud-

Table 1 Patient characteristics and clinical results

Patient	Sex, age (yr)	Underlying condition	Indication for TIPS	Previous treatment	Child pugh score	MELD score	Survival post shunt	Complications and follow-up
1	F (76)	Hepatitis B	Hydrothorax, refractory ascites	Numerous thoracenteses, furosemide	B (9)	9	25 mo, expired	transient encephalopathy (grade 1 to 2) 1 episode < 30 d, 6 episodes over 24 mo, pulm, edema x 1
2	M (78)	Celiac sprue	Refractory ascites	Numerous paracenteses, spironolactone	C (10)	7	41 mo, expired	Encephalopathy (grade 1 to 2) > 30 d 6 episodes over 40 mo
3	M (71)	Cryptogenic cirrhosis	Refractory ascites	Numerous paracenteses, spironolactone	B (8)	16	8 mo, expired	None
4	F (80)	Cryptogenic cirrhosis	Hepatic hydrothorax	Numerous thoracenteses, spironolactone	C (11)	11	3.5 mo, expired	Encephalopathy transient grade 1 (2 episodes at 30 d and 3 mo)
5	M (69)	Sclerosing cholangitis	Refractory ascites	Numerous paracenteses, spironolactone	C (12)	17	3 mo, expired post attempted shunt	None
6	M (65)	Alcoholic cirrhosis	Hepatic hydrothorax	Numerous thoracenteses, spironolactone, lasix	B (9)	15	19 mo liver transplant, still alive	Encephalopathy transient grade 1 (1 episode > 30 d and < 3 mo)
7	M (71)	Alcoholic cirrhosis	Refractory ascites	Numerous paracenteses, spironolactone, lasix	C (10)	13	3 mo, expired	Encephalopathy transient grade 1 (3 episodes > 30 d)
8	F (70)	Hepatitis C	Refractory ascites, skin breakdown	Numerous paracenteses, spironolactone, lasix	B (8)	9	2nd TIPS placed at 21 mo due to occlusion of 1st TIPS expired 47 mo post initial TIPS	None
9	F (66)	Hepatitis C	Acute bleeding		B (7)	9	Lost to fu pt in Spain	None
10	F (72)	Cryptogenic	Acute bleeding, refractory ascites	Paracentesis, propanolol, lactulose, protonix	B (9)	12	10 mo lost to fu	None
11	F (72)	Hepatitis C	Acute bleeding	Sclerotherapy, spleno-renal shunt × 2, numerous paracentesis, lasix, spironolactone	B (8)	9	Liver transplant 18 mo after TIPS, still alive	None
12	F (67)	Hepatitis B portal vein thrombois	Acute bleeding	Octreotide, protonix	C (10)	10	Liver transplant 16 mo after TIPS, still alive	None
13	M (74)	Hepatitis C (end stage liver disease)	Refractory ascites	Aldactone, lasix, lactulose, protonix	B (8)	15	2 wk, expired	Transient encephalopathy grade 1 (< 2 wk)
14	F (68)	Hepatitis C	Acute bleeding	Midodrine	B (7)	24	2 mo, expired	Recurrent GI bleed 3 wk required revision
15	M (69)	Cryptogenic	Acute bleeding	4 unsuccessful banding, lasix, spironolactone, lactulose, paracentesis	C (10)	21	Revision required 48 h after liver transplant 11 mo after revision, still alive	Continued bleed after 1st TIPS, stopped after revision
16	F (73)	NASH cirrhosis	Acute bleeding	Octreotide, protonix, spironolactone, lasix	B (9)	11	25 mo, still alive	Minimal ascites
17	M (71)	Cryptogenic	Acute bleeding	Band ligation-not successful	B (8)	16	Lost to fu	None
18	M (82)	Cryptogenic	Refractory ascites, acute bleeding	Numerous paracentesis, lasix, amiloride	B (9)	16	2 wk expired (AV block-DNR/DNI)	None
19	M (73)	Hepatitis C	Refractory ascites	Numerous paracenteses, Spironolactone, lasix s/p kidney and liver tx	B (9)	14	1 mo lost to fu	None
20	F (78)	Hepatitis C	Acute bleeding, refractory ascites		B (8)	18	5 d, expired	Post-procedural bleeding, encephalopathy (< 30 d), ascites
21	M (71)	Cirrhosis	Acute bleeding		B (8)	11	Status unknown, discharged to rehabilitation hospital	None
22	M (66)	Alcoholic cirrhosis, s/p liver transplant, portal vein thrombosis	Refractory ascites	Lasix	B (9)	8	Unknown	Failed attempted TIPS
23	M (76)	Alcoholic cirrhosis	Refractory ascites	Numerous paracenteses (once a week)	B(8)	12	15 mo, still alive	None

Table 2 Technical details and results

Patient	Procedure time (min)	Success	No. of stents	Stent type (mm)	Balloon size (mm)	Portosystemic gradient (mmHg)	Revision dates
1	195	No	N/A	N/A	N/A	N/A	
1	150	Yes	2	10 × 68	10	6-9	
2	160	Yes	2	10 × 68	8	8	
Revision 2	165	Yes	1	12 × 90	10	5	Revision 3 wk
Revision 2	105	Yes	1	12 × 90	10	10-12	Revision 8 mo
Revision 2	90	Yes	1	12 × 90	10	6-8	Revision 32 mo
3	140	Yes	1	12 × 90	10	3-4	
4	65	Yes	1	10 × 94	8	3-5	
5	127	No	N/A	N/A	N/A	N/A	
6	165	Yes	1	10 × 60[1]	8	12	
7	235	Yes	1	10 × 70[1]	8	13	
8	135	Yes	2	8 × 60[1]	8	12	
Revision 8	120	Yes	2	8 × 6[1]	8	10	New Parallel TIPS 21 mo
9	15	Yes	1	8 × 70[1]	8	5	
10	Unavailable	Yes	1	10 × 80[1]	10	7	
Revision 10	50	Yes	1	Unavailable	Unavailable	Unavailable	Revision 10 mo
11	50	Yes	2	10 × 80[1]	10	10	
12	70	Yes	1	10 × 80[1]	10	4	
13	150	Yes	1	10 × 80[1]	10	3	
14	35	Yes	1	10 × 60[1]	10	6	
Revision 14	Unavailable	Yes	1	Unavailable	Unavailable	Unavailable	Revision 3 wk
15	35	Yes	1	10 × 70[1]	10	13	
Revision 15	18	Yes	1	Unavailable	Unavailable	Unavailable	Revision 48 h
16	55	Yes	1	10 × 80[1]	10	2	
17	65	Yes	1	10 × 80[1]	10	8	
18	30	Yes	1	10 × 80[1]	10	4	
19	85	Yes	2	10 × 60[1]	10	4	
Revision 19	Unavailable	Yes	1	12 × 60[1]	10	Unavailable	Unavailable
20	Unavailable	Yes	1	10 × 80[1]	8	4	
21	Unavailable	Yes	1	8 × 80[1]	8	2	
22	Unavailable	No	N/A	N/A	N/A	N/A	
23	135	Yes	1	10 × 70[1]	8	8	

[1]Denotes Viatorr (W.L Gore, Elkton, MD) covered stent use.

ing 5 patients lost to follow-up) were followed for a mean of 14.7 mo. Ascites and/or hydrothorax was controlled following technically successful procedures in 12 out of 13 patients. Bleeding was controlled following technically successful procedures in 10 out of 11 patients. Two patients in whom TIPS could not be placed had portal vein thrombosis; thus, the portal vein could not be successfully catheterized. Only 4 patients were candidates for orthotopic liver transplantation. The mean duration of the procedures was 101 min (range 15-235 min). The created shunt size was 8-10 mm in diameter in all patients. Four patients received WALLSTENT bare metal stents, 17 patients received the VIATORR covered stents (Tables 1 and 2).

Clinical results

Complications: All complications were clinical complications. There were no technical complications. Within 30 d after TIPS placement, 4 patients (Patient No. 1, No. 4, No. 13 and No. 20) experienced mild transient grade Ⅰ-Ⅱ encephalopathy. In 3 out of 4 of these patients, this was controlled with medical therapy. One (patient No. 20) out of this 4 died due to failure to cure within 5 d as a result of continued gastrointestinal bleeding. One patient experienced pulmonary edema, which was readily controlled with diuretics. Early death (< 3 mo) occurred in 2 patients at 2 wk and 1 patient at 2 mo following TIPS for an early death rate of 14% (3 of 21). Of these early deaths the average MELD score was 18.3. This is compared to 12.0 for the average MELD score of the rest of the patients who underwent successful TIPS. Technical issues corrected by successful revisions (also not considered complications) of TIPS within 30 d included 2 patients (Patient No. 14, No. 15) for continued gastrointestinal bleeding and 2 for recurrent ascites (Patient No. 2, No. 19). Failure to cure (inability to durably control bleeding), which is not considered a complication occurred in 1 patient [1 of 11 (9%)] who died (Patient No. 20 at 5 d).

Follow-up

Of the 21 patients who underwent successful TIPS placement, 10 were followed until their death and 6 patients are still alive for an overall mean follow-up of 14.7 mo. In addition, 3 patients were lost to follow-up immediately after the procedure, whereas 1 patient was lost to follow-up at 1 mo and 1 patient was lost to follow-up at 10 mo.

Table 3 Comorbidites

Patient	
1	Hypothyroidism, diabetes mellitus, hypertension, chronic GI bleed, hypoxemia on home oxygen
2	Celiac sprue, recurrent chronic bleeding from esophageal varices
3	Congestive heart failure, s/p CABG, s/p mitral valve replacement, chronic atrial fibrillation, hypertension
4	Aortic stenosis
5	Noninsulin dependent diabetes mellitus, chronic renal failure, cholecystectomy, bilateral hernia repair
6	None
7	None
8	None
9	None
10	Status post lumpectomy, diabetes type 2, basal cell carcinoma of the skin, some masculinizing tumor of the ovary for which the patient has had a bilateral salpingo-oopherectomy, hypertension
11	Cholecystectomy, diabetes mellitus
12	Breast cancer in 1996 status post modified radical mastectomy, history of portal vein thrombosis in 2002 secondary to tamoxifen
13	Aortic stenosis; coronary artery disease, status post 3-vessel CABG; diabetes mellitus; and bilateral lower extremity cellulitis.
14	End-stage renal, diabetes, GERD, diverticulitis
15	Hypertension
16	Coronary artery disease (CAD), hyperlipidemia, Anemia
17	Peptic ulcer disease, obstructive sleep apnea, gastroesophageal reflux disease, myeloproliferative disorder, diabetes, aortic stenosis, status post aortic valve replacement in 1996, coronary artery disease
18	Severe aortic stenosis, chronic renal insufficiency, BPH, sinus bradycardia with mobitz type I AV block
19	Renal failure, status post transplant, cryoglobulinemia, BPH, hypothyroidism
20	Hypothyroid, coagulopathy
21	Prostate cancer, CHF, AFib, COPD, hypertension, CVA, respiratory failure, history of MRSA and VRE
22	Spontaneous bacterial peritonitis, status post liver transplant, hypertension, diabetes, portal vein thrombosis
23	CAD, hiatal hernia

Ascites, hepatic hydrothorax, and/or bleeding was controlled in 20 of 21 (95%) patients who underwent successful TIPS. Fifteen out of 21 patients maintained shunt integrity with no need for shunt revision. Patient No. 2 required repeat shunt revision for restenosis at 8 and 32 mo. All revisions in this patient were done with an uncovered stent since covered stents were not yet available. One patient (Patient No. 10) required a revision at 10 mo due to restenosis from a bile duct puncture. This was corrected with a covered stent. Patient No. 8 developed recurrent ascites at 20 mo due to an occluded shunt and therefore underwent a parallel TIPS creation with resolution of ascites. Patient No. 14 and 15 underwent successful shunt revision at 3 wk and 48 h respectively due to recurrent bleeding for patient No. 14 and persistent bleeding for patient No. 15. Patient No. 19 required shunt revision at 3 wk for recurrent ascites. One patient (No. 3) who developed recurrent ascites 5 mo post procedure was successfully treated using diuretic therapy without paracentesis. This patient's TIPS was patent. The patient (No. 5) in whom a shunt could not be placed due to chronic portal vein thrombosis developed renal and hepatic failure 6 wk post attempt and died 3 mo post attempted procedure.

Two patients (No. 1 and No. 2) developed chronic gastrointestinal bleeding later on in the course of their illness. Both of these patients had successful TIPS which were demonstrated to be patent on long-term follow up. Patient No. 1 had gastric vascular ectasias (watermelon stomach) and colonic angiodysplasia without variceal hemorrhage. This patient did have a total of six (3-5 d) admissions for grade Ⅱ encephalopathy. Patient No. 2 was diagnosed with nonvariceal gastric bleeding. A total of 16 hospital admissions (between 3-5 d admissions) from 10/98 to 06/01 occurred. Of those admissions, 6 were secondary to grade Ⅱ encephalopathy, 2 were secondary to concurrent grade Ⅱ encephalopathy and anemia, and 4 were secondary to anemia alone.

Five patients (Patient No. 1, No. 2, No. 4, No. 6 and No. 7) experienced an episode of grade Ⅰ to Ⅱ encephalopathy beyond 30 d post procedure which required admission. All patients responded to oral lactulose therapy. The rest of the patients did not experience encephalopathy beyond 30 d.

Patient No. 4, No. 11, No. 12 and No. 15 received liver transplantation at 19, 18, 16, and 11 mo respectively. These patients continued to remain alive. Of patients who underwent successful TIPS who did not undergo liver transplant, average follow up was 14.7 mo. This includes patients who are either dead or currently alive and excludes the 5 patients who were lost to follow-up.

DISCUSSION

TIPS is an accepted treatment of portal hypertension related complications which are not amenable to medical management[6]. TIPS has been successful in the young and middle aged adult population, as well as even the pediatric population[7]. Published literature regarding TIPS and the elderly population is scarce. Our report suggests that TIPS may be performed safely and successfully in the elderly population.

Because liver transplantation is often not an option for elderly patients, TIPS can provide palliative relief in

this population who may otherwise require multiple hospitalizations and/or repeated paracenteses/thoracenteses or endoscopic therapy. Consensus opinion suggests that TIPS for ascites is indicated in appropriate patients if the frequency of paracentesis is greater than 3 times per month, the patient does not tolerate paracentesis, or if the paracentesis is contraindicated/ineffective[8,9]. TIPS is also useful for hepatic hydrothorax[9-12]. For portal hypertensive bleeding, polytetrafluoroethylene (PTFE)-covered stent TIPS is indicated after failure of endoscopic therapy and/or medical treatment. Additionally, early use of TIPS is now advocated to reduce treatment failure and mortality[13].

It is imperative that appropriate evaluation is performed prior to consideration of the patient for TIPS. Unlike other age groups such as the pediatric and younger adult population, the justification of TIPS as a "bridge to transplantation" does not usually exist in the elderly. Therefore, based on prognostic criteria TIPS should not be performed in elderly patients with markedly advanced liver disease whose survival is limited.

The duration of 101 min was comparable to the TIPS procedure time reported for younger patients (60-120 min). There were no irreversible or catastrophic complications that were encountered during this small series. Complications in the younger adult population include shunt closure, hemorrhage, encephalopathy, portal vein occlusion, and liver failure. Our 30 d complications included 4 patients with mild reversible hepatic encephalopathy and 1 case of reversible pulmonary edema. Three out of 21 patients experienced early death (2 patients at 2 wk and one patient at 2 mo). It should be noted of these early death patients that the average MELD score was 18.3 (range 15-24) *vs* 12.0 in the rest of the patients who underwent successful TIPS creation. Of note the MELD score was calculated in retrospect in patients 1, 2, 3, 4, and 5 as MELD scores were not in use at that time period. Our failure to cure includes 1 case [Patient No. 20 (died at 5 d)] of recurrent gastrointestinal bleeding resulting in death.

Follow-up after 30 d did demonstrate 5 of our patients had transient grade Ⅰ-Ⅱ encephalopathy requiring 3-5 d admissions. It should be noted that our patients presenting with ascites were more likely to develop encephalopathy than our patients presenting with acute bleeding. In a recent large meta-analysis by Bai *et al*[14], it was noted that higher Child Pugh scores result in a higher rate of encephalopathy post TIPS. The Child Pugh score is a reflection of the extent of liver disease and one of the major determinants of this score is the presence of ascites. It is therefore inherent that patients with ascites have higher Child Pugh scores which correlates with higher risk for hepatic encephalopathy. Encephalopathy is a common complication of TIPS placement and has a known incidence of 54.9%. This compares to an incidence of 38.1% in controls undergoing large volume paracentesis[15]. Fortunately, as in our experience, encephalopathy is usually responsive to medical therapy. Pre-existing hepatic encephalopathy is a relative contraindication to TIPS as it may precipitate uncontrollable encephalopathy. One of our patients had grade 1 pre-existing hepatic encephalopathy. One other point of discussion is that the elderly may be more prone to encephalopathy than younger patients. This was confirmed in the meta-analysis by Bai *et al*[14]. It is speculated this may be due to lower cerebral reserve in the elderly with a higher susceptibility to the toxic effect of metabolites such as ammonia[16].

Two patients (Patient No. 14 and No. 15) required shunt revision within 3 wk due to continued gastrointestinal bleeding. Two patients (Patient No. 2 and No. 19) required revision due to shunt closure that occurred within 3 wk with resulting recurrent ascites. Patient No. 2 who had an uncovered TIPS required subsequent shunt revision 2 additional times within the 41 mo after initial shunt placement. One other patient (Patient No. 10) had shunt restenosis at 10 mo requiring revision with a covered stent. Another patient (Patient No. 8) had shunt closure at 20 mo requiring a parallel TIPS. Shunt stenosis or obstruction occurs in 70% of patients at 1 year with uncovered TIPS[17]. This event resulting in recurrent ascites or bleeding may be reduced with the placement of newly developed ePTFE-Covered stent grafts, as we used in our last 15 patients[18]. Long term patency rates have dramatically improved with shunt patency's of 90%, 84%, and 74% at 1, 2 and 3 years respectively[19]. In fact as of December 2004 covered stents have been FDA approved for TIPS[15]. This may eventually result in a possible improvement in morbidity and mortality[19,20].

Patients who are in advanced stages of cirrhosis [Child's class C (> 9) or MELD > 15] should also be cautiously approached due to the higher risk of hepatic failure and potential risk of uncontrollable encephalopathy[21]. It has been suggested in the literature that the use of the MELD scoring system is a better predictor of mortality than the older Child's Pugh classification system[21-25]. In conclusion, TIPS placement can be performed successfully in the elderly who are deemed unsuitable for liver transplantation. TIPS should be performed after careful consideration of alternatives and appropriate patient selection. This procedure appears to offer control of medically refractory ascites, hepatic hydrothorax, and portal hypertensive bleeding in the elderly population who are otherwise often excluded from receiving the TIPS procedure. However, there is a predisposition to hepatic encephalopathy, but this typically responds to medical therapy. The major limitation of our study is that it is retrospective over a 14-year period. Therefore, further study is encouraged.

COMMENTS

Background

Cirrhosis of the liver is the 10th leading cause of death in the United States. Portal hypertension is the major physiologic manifestation of cirrhosis. Tran-

sjugular intrahepatic porto-systemic shunt (TIPS) is a relatively new technique that has revolutionized the treatment of complications from portal hypertension due to cirrhosis. This includes variceal bleeding and massive ascites refractory to standard medical therapy. These patients are now typically offered TIPS and/or liver transplantation with TIPS often being a "bridge" to transplantation. Unfortunately the elderly population is only rarely offered liver transplantation due to age criteria and higher risk. The only potential option for these patients is therefore TIPS. Up until now, the published experience of TIPS in the elderly however is quite limited. This is despite the elderly representing a growing population with cirrhosis and having a mortality of 50% with 1 year of diagnosis.

Research frontiers

Important areas of research in the field are identifying appropriate criteria for offering TIPS to patients based on model for end-stage liver disease (MELD) score and child-Pugh Class and for knowing which risk factors result in complications such as hepatic encephalopathy. However, research in the elderly is limited.

Innovations and breakthroughs

A major innovation has been to risk stratify patients using MELD score. This has improved outcomes and reduced mortality. Another major advance has been the use of covered stents, which have improved patency rates. Portal vein localization techniques have also been helpful in improving technical success rates and reducing complications. In the present study we discussed and incorporated these advancements in the elderly population for TIPS.

Applications

This study shows that TIPS can be safely performed in the elderly with acceptable outcomes for palliation of complications of portal hypertension.

Terminology

TIPS: Is the acronym for transjugular intrahepatic portosystemic shunt. This is an artificially created connection within the liver between the inflow of the portal vein and outflow of the hepatic vein. It is useful in relieving complications of portal hypertension including (1) gastrointestinal bleeding due to varices; and (2) ascites/hepatic hydrothorax. It is typically performed by interventional radiologists using a percutaneous transjugular intrahepatic approach with image guidance. Covered stents: A type of artificial tube which is inserted into the communication between the portal vein and the hepatic vein. Previously bare metal stents were utilized in TIPS. Now a days covered stents are utilized which are lined with vascular graft material. This has resulted in significant improvement in patency for TIPS. MELD score: This is an acronym for model for end stage liver disease. It was initially developed to predict poor survival after TIPS. It was later found to be useful in determining the prognosis of chronic liver disease and in prioritizing patients needing a liver transplant. MELD score is calculated by the United Network for Organ Sharing modification of the original formula: MELD score = 9.6 × $\log_e$ (creatinine mg/dL) + 3.8 × $\log_e$ (bilirubin mg/dL) + 11.2 × $\log_e$ (INR) + 6.4. Child-Pugh score: sometimes called the Child-Turcotte-Pugh score is also used to assess the severity and prognosis of chronic liver disease typically cirrhosis. It has similar usefullness as the MELD score.

Peer review

The article describes the feasibility of application of TIPS in elderly cirrhotic people who are generally denied this kind of procedure. The message deserves reporting as the results, complications and follow up seem comparable to those reported in younger adult individuals with end stage cirrhosis. It is of educational value and of clinical relevance as it highlights another opportunity to prevent/treat some life-threatening complications of cirrhosis at an advanced age as well. Criteria for patient selection, the specific diseases requiring TIPS, the way of performing TIPS, and follow up were appropriately considered and described.

REFERENCES

1 **Karsan HA**, Rojter SE, Saab S. Primary prevention of cirrhosis. Public health strategies that can make a difference. *Postgrad Med* 2004; **115**: 25-30

2 **Grinberg AR**, de la Serna F, Silva M, Finkielman JD, Magariños E, Festa HM, Lorenzino GA, Cazenave CA. [Use of transjugular intrahepatic portosystemic shunt (TIPS) in an 84 year-old patient during acute variceal bleeding]. *Medicina* (B Aires) 1998; **58**: 295-297

3 **Schmucker DL**. Aging and the liver: an update. *J Gerontol A Biol Sci Med Sci* 1998; **53**: B315-B320

4 **Freeman RB**, Wiesner RH, Harper A, McDiarmid SV, Lake J, Edwards E, Merion R, Wolfe R, Turcotte J, Teperman L. The new liver allocation system: moving toward evidence-based transplantation policy. *Liver Transpl* 2002; **8**: 851-858

5 **Fontaine AB**, Verschyl A, Hoffer E, Borsa J, Dowd M. Use of CT-guided marking of the portal vein in creation of 150 transjugular intrahepatic portosystemic shunts. *J Vasc Interv Radiol* **1997; 8**: 1073-1077

6 **Colombato L**. The role of transjugular intrahepatic portosystemic shunt (TIPS) in the management of portal hypertension. *J Clin Gastroenterol* 2007; **41** Suppl 3: S344-S351

7 **Heyman MB**, LaBerge JM, Somberg KA, Rosenthal P, Mudge C, Ring EJ, Snyder JD. Transjugular intrahepatic portosystemic shunts (TIPS) in children. *J Pediatr* 1997; **131**: 914-919

8 **Moore KP**, Wong F, Gines P, Bernardi M, Ochs A, Salerno F, Angeli P, Porayko M, Moreau R, Garcia-Tsao G, Jimenez W, Planas R, Arroyo V. The management of ascites in cirrhosis: report on the consensus conference of the International Ascites Club. *Hepatology* 2003; **38**: 258-266

9 **Rössle M**, Gerbes AL. TIPS for the treatment of refractory ascites, hepatorenal syndrome and hepatic hydrothorax: a critical update. *Gut* 2010; **59**: 988-1000

10 **Strauss RM**, Martin LG, Kaufman SL, Boyer TD. Transjugular intrahepatic portal systemic shunt for the management of symptomatic cirrhotic hydrothorax. *Am J Gastroenterol* 1994; **89**: 1520-1522

11 **Gordon FD**, Anastopoulos HT, Crenshaw W, Gilchrist B, McEniff N, Falchuk KR, LoCicero J, Lewis WD, Jenkins RL, Trey C. The successful treatment of symptomatic, refractory hepatic hydrothorax with transjugular intrahepatic portosystemic shunt. *Hepatology* 1997; **25**: 1366-1369

12 **Siegerstetter V**, Deibert P, Ochs A, Olschewski M, Blum HE, Rössle M. Treatment of refractory hepatic hydrothorax with transjugular intrahepatic portosystemic shunt: long-term results in 40 patients. *Eur J Gastroenterol Hepatol* 2001; **13**: 529-534

13 **García-Pagán JC**, Caca K, Bureau C, Laleman W, Appenrodt B, Luca A, Abraldes JG, Nevens F, Vinel JP, Mössner J, Bosch J. Early use of TIPS in patients with cirrhosis and variceal bleeding. *N Engl J Med* 2010; **362**: 2370-2379

14 **Bai M**, Qi X, Yang Z, Yin Z, Nie Y, Yuan S, Wu K, Han G, Fan D. Predictors of hepatic encephalopathy after transjugular intrahepatic portosystemic shunt in cirrhotic patients: a systematic review. *J Gastroenterol Hepatol* 2011; **26**: 943-951

15 **Boyer TD**, Haskal ZJ. American Association for the Study of Liver Diseases Practice Guidelines: the role of transjugular intrahepatic portosystemic shunt creation in the management of portal hypertension. *J Vasc Interv Radiol* 2005; **16**: 615-629

16 **Hassoun Z**, Deschênes M, Lafortune M, Dufresne MP, Perreault P, Lepanto L, Gianfelice D, Bui B, Pomier-Layrargues G. Relationship between pre-TIPS liver perfusion by the portal vein and the incidence of post-TIPS chronic hepatic encephalopathy. *Am J Gastroenterol* 2001; **96**: 1205-1209

17 **Arroyo V**, Ginès P, Gerbes AL, Dudley FJ, Gentilini P, Laffi G, Reynolds TB, Ring-Larsen H, Schölmerich J. Definition and diagnostic criteria of refractory ascites and hepatorenal syndrome in cirrhosis. International Ascites Club. *Hepatology* 1996; **23**: 164-176

18 **Ferral H**, Patel NH. Selection criteria for patients undergoing transjugular intrahepatic portosystemic shunt procedures: current status. *J Vasc Interv Radiol* 2005; **16**: 449-455

19 **Rössle M**, Siegerstetter V, Euringer W, Olschewski M, Kromeier J, Kurz K, Langer M. The use of a polytetrafluoroethylene-covered stent graft for transjugular intrahepatic portosystemic shunt (TIPS): Long-term follow-up of 100 patients. *Acta Radiol* 2006; **47**: 660-666

20 **Angermayr B**, Cejna M, Koenig F, Karnel F, Hackl F, Gangl A, Peck-Radosavljevic M. Survival in patients undergoing transjugular intrahepatic portosystemic shunt: ePTFE-covered stentgrafts versus bare stents. *Hepatology* 2003; **38**: 1043-1050
21 **Feyssa E**, Ortiz J, Grewal K, Azhar A, Parsikia A, Tufail K, Hashemi N, Brady P, Araya V. MELD score less than 15 predicts prolonged survival after transjugular intrahepatic portosystemic shunt for refractory ascites after liver transplantation. *Transplantation* 2011; **91**: 786-792
22 **Ferral H**, Gamboa P, Postoak DW, Albernaz VS, Young CR, Speeg KV, McMahan CA. Survival after elective transjugular intrahepatic portosystemic shunt creation: prediction with model for end-stage liver disease score. *Radiology* 2004; **231**: 231-236
23 **Angermayr B**, Cejna M, Karnel F, Gschwantler M, Koenig F, Pidlich J, Mendel H, Pichler L, Wichlas M, Kreil A, Schmid M, Ferlitsch A, Lipinski E, Brunner H, Lammer J, Ferenci P, Gangl A, Peck-Radosavljevic M. Child-Pugh versus MELD score in predicting survival in patients undergoing transjugular intrahepatic portosystemic shunt. *Gut* 2003; **52**: 879-885
24 **Forman LM**, Lucey MR. Predicting the prognosis of chronic liver disease: an evolution from child to MELD. Mayo End-stage Liver Disease. *Hepatology* 2001; **33**: 473-475
25 **Malinchoc M**, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology* 2000; **31**: 864-871

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BRIEF ARTICLE

Evaluation of adherence to oral antiviral hepatitis B treatment using structured questionnaires

Leesa Giang, Christian P Selinger, Alice Unah Lee

Leesa Giang, Christian P Selinger, Alice Unah Lee, Department of Gastroenterology and Liver Services, Hospital Road, Concord NSW 2139, Australia

Author contributions: Giang L and Lee AU designed the study; Giang L collected the data; all authors analysed and interpreted the data; Giang L wrote the draft manuscript; Selinger CP and Lee AU critically reviewed the manuscript; all authors approved the final version.

Correspondence to: Alice Unah Lee, Professor, Department of Gastroenterology and Liver Services, Hospital Road, Concord NSW 2139, Australia. alice.lee@sswahs.nsw.gov.au
Telephone: +61-2-96766111 Fax: +61-2-97676767
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Abstract

AIM: To assess adherence rates to nucleos(t)ide analogues (NUCs) therapy in patients with chronic hepatitis B virus infection and determine factors associated with adherence.

METHODS: The questionnaire study was conducted in the liver clinics at Concord Repatriation General Hospital. All patients who were currently taking one or more NUCs were asked to complete a structured, self-administered 32-item questionnaire. Adherence was measured using visual analogue scales. The patient's treating clinician was also asked to assess their patient's adherence *via* a structured questionnaire.

RESULTS: A total of 80 patients completed the questionnaire. Sixty six percent of the patients (n = 49) reported optimal adherence whilst 25 (33.8%) graded their adherence to NUCs as suboptimal. Thirty four (43%) patients reported to have omitted taking their NUCs sometime in the past. Recent non-adherence was uncommon. Amongst the patients who reported skipping medications, the most common reason cited was "forgetfulness" (n = 27, 56.25%). Other common reasons included: ran out of medications (n = 5, 10.42%), being too busy (n = 4, 8.33%) and due to a change in daily routine (n = 5, 10.42%). Patients who reported low adherence to other prescription pills were also more likely to miss taking NUCs (P = 0.04). Patients who were under the care of a language-discordant clinician were also more likely to report suboptimal adherence to NUCs (P = 0.04).

CONCLUSION: Adherence rates were much less than that expected by the physician and has potential adverse affect on long term outcome. Communication and education appear central and strategies need to be implemented to improve ongoing adherence.

Key words: Patient compliance; Patient adherence; Antiviral agents; Hepatitis B; Chronic

Peer reviewers: Dr. Joseph Ahn, Department of Medicine, Loyola University Health System, Room 007, Building 54, 2160 S First Avenue, Maywood, IL 60153, United States; Frank Tacke, Professor, Department of Medicine III, Pauwels Str. 30, Aachen 52074, Germany

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INTRODUCTION

Poor adherence to therapy is a complex challenge for physicians treating patients with chronic diseases. In clinical practice, adherence rates averages 50%, falling most dramatically after the first 6 mo of treatment[1]. Adherence has been extensively studied in other chronic medical conditions such as asthma, hypertension, and human

immunodeficiency virus (HIV)/acquired immunodeficiency syndrome. However, little data is available in patients receiving oral antiviral therapy for chronic hepatitis B virus (CHB) infections.

Hepatitis B infection is a major global health problem with an estimated 2 billion people infected worldwide and 350 million suffering from CHB. Many individuals will eventually attain a non-replicative state, up to 40% of the people will develop complications such as cirrhosis, liver failure and hepatocellular carcinoma[2,3]. Recent developments in antiviral therapy may prevent, reverse or delay disease progression and thus ultimately improve survival[4]. There are currently five approved nucleos(t)ide analogues (NUCs) for the treatment of CHB in Australia including: lamivudine, entecavir monohydrate, telbivudine, adefovir dipivoxil and tenofovir disoproxil fumarate. However, these treatments are rarely curative, with viral suppression and not eradication remaining the virological goal of therapy. As such, patients require long term, potentially lifelong therapy in order to derive continued clinical benefit[2,4].

Guidelines on CHB therapy emphasise the need for optimal adherence, with risk of resistant viral strains emerging if the virus has a drug free holiday[3]. For example, antiviral resistance has been reported in up to 70% patients after 4 years on Lamivudine, 29% after 5 years of Adefovir dipivoxil and 1% after 4 years of Entecavir monohydrate[5-7]. The number of dose omissions that may lead to this is variable but any omission poses a potential risk of viral replication breakthrough. Although, the data for CHB are lacking, it is evident from the HIV literature that near-perfect adherence (> 95% adherence rates) is needed to achieve a non detectable viral load and avoid emergence of resistant strains[8,9]. Hence, in the clinical setting of CHB therapy, the goal of adherence remains 100%.

Unlike other chronic conditions, the rapid viral replication potential and mutation rates of hepatitis B virus require very high levels of adherence to achieve and maintain virological suppression[10]. Suboptimal adherence risks exacerbating existing liver disease, which can be life-threatening particularly in patients with advanced cirrhosis. Furthermore, it can lead to the development of drug-resistant strains, limiting therapeutic options and additionally poses the public health risks of transmission of drug-resistant viral strains to non-immune individuals in the community, or to those whose previous vaccination are no longer protective[4,11]. Given the global burden of the disease, widespread transmission of drug-resistant strains may have serious and wide-reaching consequences.

Adherence is fundamental in the optimal clinical management of CHB patients. However, a physician's assessment can often lead to over-estimation of adherence and inadequate recognition of poor adherence[12,13]. There is currently no gold standard for measuring adherence, but numerous strategies have been reported in the literature. Medication electronic monitors, pharmacy refill records and monitoring drug/metabolite serum or urine concentrations. These are costly and time-consuming; often making them impractical for use in routine clinical practice. Validated self-report tools may sometimes overestimate adherence but are often used because of their low cost, ease of use and adaptability to a wide range of clinical settings. Most importantly, self-report assessments have been shown to be significantly associated with clinical outcomes in numerous studies[14-16].

Despite the importance of optimal adherence to NUCs amongst CHB patients, current understanding and related literature in this area is remarkably scarce. This study addressed adherence rates and possible factors associated with patient adherence to NUCs.

MATERIALS AND METHODS

Research ethics

The study was approved by the Concord Repatriation General Hospital Research Ethics Committee.

Participants

CHB patients on oral NUCs were recruited from liver clinics at Concord Repatriation General Hospital from May 2010 to October 2010. All patients aged 18 years or older taking one or more NUCs were invited to participate. All participants provided written informed consent.

Study procedures

Participants were asked to fill in a self-administered 32-item questionnaire and administrative staff were available to assist patients as required. Data collected included socio-demographic characteristics, treatment–related factors, disease-related factors and healthcare team-related variables (Table 1). Patients were asked to rate their overall adherence to taking NUCs, other prescription medications (if applicable) and appointments on a visual analogue scale, ranging from 1 to 10. Grade of 1 being poor adherence i.e., meant that they frequently skipped taking their NUCs/other prescription pills/appointments whilst a grade of 10 was excellent adherence where they took their NUCs/took their prescription pills/attended their appointments 100% of the time. Optimal adherence was defined as self-graded adherence greater than 9, whilst 9 or less was classed as suboptimal adherence. Patients with limited English skills were given assistance with the questionnaire by a researcher and verbal translations were available for Chinese speaking patients.

Clinic physicians were blinded to the patients' questionnaire. After the clinic consultation an 8-item questionnaire was completed by the patient's treating physician, collecting data on presence of cirrhosis, the doctor's perception of the patient's understanding of treatment requirements, a prediction of the patient's adherence to NUCs and to appointments based on the physician's impression, whether the topic of adherence was discussed during the consultation and whether the patient had participated in a clinical trial before.

Table 1 Patient characteristics

Socio-demographic	Treatment regimen	Disease condition	Healthcare team
Age	Name of HBV antiviral medication	Duration of HBV infection	Language spoken at the consultation with the doctor
Sex	Dose scheduling instructions	Patient's perception of their general health	Whether the patient understood their doctor
Highest level of education	Dietary instructions	Patient's perception of their disease condition	Whether they had received an education session by health professional about their disease condition and their understanding of the importance of medication adherence
Country of birth	Length of treatment	Complications experienced	
Ethnicity	Number of prescription pills taken per day	Cirrhosis status	
Language spoken at home	Whether patient had skipped taking their medications before		
	The last time they had skipped taking their medications		
	Reasons for failing to take their medications		
	Side effects		
	Patient's perception of the therapeutic benefit of their medication		
	Type of medication packaging		
	Use of memory aid		

Statistical analysis

The data collected was cleaned, coded, entered and analysed using SPSS 18 for Windows (SPSS Inc., Chicago, IL, United States). Descriptive statistics were performed on all available data. The statistical analysis consisted of bivariate analysis using χ^2 tests, assessing the association between adherence to NUCs and various factors. Agreement between the treating physician's estimate and patient's self-report of adherence was assessed by calculating a κ statistic and corresponding 95% CI. A value of $P < 0.05$ in a two-tailed tests was considered to be statistically significant.

RESULTS

A total of 80 patients consented and completed the questionnaires. The ages ranged from 19 to 85 years with a mean of 51.65 ± 13.52 years. The majority of patients were male (n = 52, 65%), from Asian background (n = 75, 93.5%) and born in China (n = 28, 35.9%). Ninety one percent of the patients (n = 72) spoke a language other than English at home. Over half (n = 46, 57.5%) of the patients had completed college/university (Table 2).

Treatment characteristics

A majority (n = 60, 78.9%) of the study respondents had been on NUCs for more than a year whilst 16 (21.1%) had been on therapy for less than 1 year. Prescribed NUCs were entecavir (n = 44, 44.1%), lamivudine (n = 20, 21.5%), tenofovir (n = 23, 24.7%), and adefovir (n = 9, 6.8%). Most patients (n = 61, 79.2%) were on a single NUC whilst 16 (20.8%) received dual therapy. Patients reported being prescribed an average of 1.9 ± 1.15 pills per day (NUCs and other prescription pills). Over a quarter (n = 26, 28.26%) of the patients reported taking their NUCs

Table 2 Socio-demographic characteristics of the study participants and association with adherence to nucleos(t)ide analogues (n = 80) n (%)

Characteristics	Patients	Optimal adherence	*P* value
Sex			0.28
Male	52 (65)	29 (61.7)	
Female	28 (35)	20 (71.4)	
Age (yr)			0.27
18-29	6 (7.5)	2 (33.3)	
30-39	9 (11.3)	4 (50.0)	
40-49	19 (23.8)	14 (77.8)	
50-59	27 (33.8)	16 (66.7)	
⩾ 60	19 (23.8)	13 (77.2)	
Country of birth			0.39
Korea	9 (11.5)	4 (57.1)	
China	28 (35.9)	16 (59.3)	
Hong Kong	11 (14.1)	9 (81.8)	
Malaysia	6 (7.7)	3 (60.0)	
Singapore	1 (1.3)	1 (100.0)	
Vietnam	9 (11.5)	3 (37.5)	
Australia	3 (3.8)	3 (100.0)	
Indonesia	3 (3.8)	3 (100.0)	
Tonga	1 (1.3)	1 (100.0)	
India	1 (1.3)	1 (100.0)	
Fiji	1 (1.3)	1 (100.0)	
Cyprus	1 (1.3)	1 (100.0)	
Cambodia	2 (2.5)	2 (100.0)	
Taiwan	1 (1.3)	0 (0.00)	
Ethnicity			0.60
Anglo-Celt	1 (1.3)	1 (100.0)	
Middle East	1 (1.3)	1 (100.0)	
Asian	75 (93.8)	44 (63.8)	
Pacific	2 (2.5)	2 (100.0)	
Other	1 (1.3)	1 (100.0)	
Highest level of education completed			0.08
Completed high school	28 (35.4)	22 (81.5)	
Completed college/university	46 (58.2)	23 (56.1)	
Did not complete high school	2 (2.5)	3 (75.0)	
Did not complete college/university	1 (1.3)	0 (0.00)	

Table 3 Treatment and disease related characteristics of the study participants and association with adherence to nucleos(t)ide analogues (*n* = 80) *n* (%)

Characteristics	Patients	Optimal adherence	*P* value
Patients			0.24
Treatment duration > 1 yr	16 (21.1)	8 (53.3)	
Treatment duration < 1 yr	60 (78.9)	39 (69.9)	
Adherence to other prescription pills			0.04
Suboptimal	11 (45.8)	4 (40.0)	
Optimal	13 (54.2)	13 (100.0)	
Follow dose scheduling instructions			0.44
Yes	14 (19.4)	7 (53.8)	
No	58 (80.6)	36 (65.5)	
Side effect			0.25
Yes	11 (13.8)	6 (54.5)	
No	66 (82.5)	42 (68.9)	
Don't know	1 (1.3)	0 (0.00)	
Believe in the therapeutic benefit of their antiviral medications			0.29
Yes	64 (80)	40 (67.8)	
No	2 (2.5)	2 (100.0)	
Don't know	12 (15.0)	6 (50.0)	
Patients' perception of their health			0.32
Excellent	10 (12.5)	8 (80.0)	
Very good	23 (28.8)	11 (52.4)	
Good	32 (40)	22 (73.3)	
Fair	10 (12.5)	6 (66.7)	
Poor	3 (3.8)	1 (33.3)	
Patients' perception of their disease condition			0.64
Severe	2 (2.5)	2 (100.0)	
Moderate	15 (8.8)	11 (73.3)	
Mild	36 (45.0)	21 (61.8)	
Don't know	25 (31.3)	8 (63.6)	
Complications			0.48
Yes	4 (5.1)	2 (50.0)	
No	74 (94.9)	47 (67.1)	
Cirrhosis- patients' perception			0.68
Yes	7 (9)	5 (83.3)	
No	55 (70.5)	34 (65.4)	
Don't know	16 (20.5)	10 (66.7)	
Use of memory aids			0.25
Yes	26 (32.5)	15 (57.7)	
No	54 (67.5)	34 (70.8)	
Patient understands everything the doctor says during the consultation			0.31
Yes	78 (97.5)	47 (65.3)	
No	2 (2.5)	2 (100.0)	
Difference in language spoken at home and at consultation with doctor			0.04
Same	42 (52.5)	30 (76.9)	
Different	38 (47.5)	19 (54.3)	

at anytime of the day irrespective of the dose scheduling instructions. Eleven patients reported side effects including: fatigue, chills, haematuria, dizziness, stomach upsets, hair loss, loss of taste, rash, nocturia, tachycardia, anorexia and general sense of being unwell. Most believed that their treatment plan was not difficult (*n* = 76, 95%) and that their medications helped them (*n* = 64, 82%). Most patients reported that the critical role of compliance had been discussed with the doctor or liver specialist nurse at a previous appointment (*n* = 73, 91.3%). Twenty seven (33.8%) of the patients had participated in a clinical trial in the past.

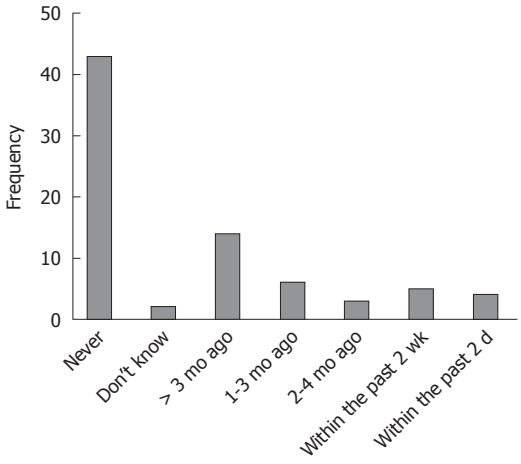

Figure 1 Last time patient skipped taking their nucleos(t)ide analogues.

Disease characteristics

Median duration of CHB was 11 years (1-54 years). Ten (12.8%) patients rated their health in the past year as excellent, 23 (29.5%) as very good, 32 (41%) as good, 10 (12.8%) as fair whilst 3 (3.8%) reported their overall health as poor. Self report on severity of liver disease was as follows: 36 (46%) patients rated it as mild, 15 (19.2%) moderate, and 2 (2.6%) as severe. Twenty five patients (32.1%) were uncertain about the severity of their disease. Seven (9%) reported cirrhosis, 55 (70.5%) did not report cirrhosis whilst 16 (20.5%) patients were uncertain of whether they had cirrhosis. This contrasts to the data from the physician where 11 (13.8%) patients were cirrhotic whilst 69 (86.3%) were non-cirrhotic (Table 3).

Adherence rates and reasons for non-adherence

Optimal adherence was reported in 49 (66.2%) whilst 25 (33.8%) graded their adherence to NUCs as suboptimal. This contrasts to the clinician's assessment of only 6 (7.6%) patients with suboptimal adherence. Using the patient's self-report of medication adherence as the referent, the weighted κ statistic describing the concordance between clinician estimation and patient self-report was low, κ = 0.165 (95% CI: 0.12-0.18). Thirty four (43%) patients reported having skipped taking their NUCs. Recent non-adherence was uncommon with the majority of patients having skipped their NUCs over 3 mo prior (Figure 1).

Reasons cited for skipping medication were forgetfulness in taking the medication' (*n* = 27, 56.3%) ran out of medications (*n* = 5, 10.4%), too busy (*n* = 4, 8.3%) or change in daily routine (*n* = 5, 10.4%, Figure 2). Of patients receiving regular medication for other chronic conditions, 11 (45.8%) graded their adherence as suboptimal.

Overall attendance at medical appointments was good at 91.2% (*n* = 73) with 8.8% (*n* = 7) of patients missing appointments. Missed appointments were due to: transportation, other commitments and inconvenient clinic times (Figure 3). Conversely, doctors reported that 22.5% (*n* = 18) of patients had suboptimal attendance to their appointments. Using the patient's self-report on adher-

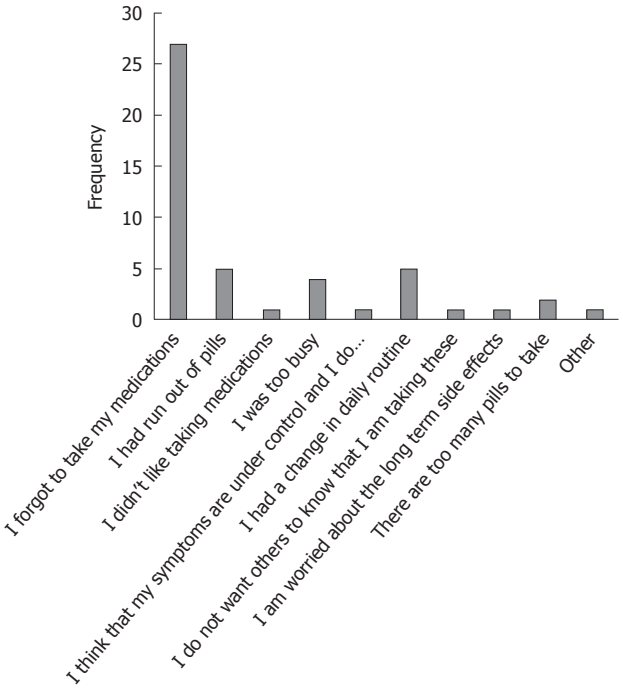

Figure 2 Reasons why patients skipped taking their nucleos(t)ide analogues.

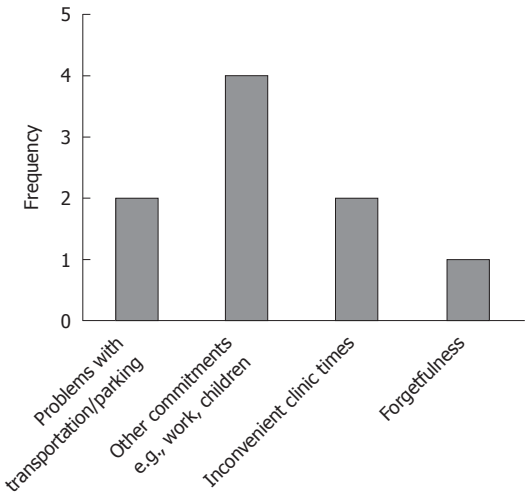

Figure 3 Reasons given on why patients miss appointments.

ence to their appointments as the referent, the weighted κ statistic describing the agreement between clinician opinion and patient self-report was low, κ = 0.130 (95% CI: 0.10-0.16).

Factors associated with suboptimal adherence to NUCs

There was a significant association between the patient's self-reported adherence to other prescription pills and their self-reported adherence to taking NUCs ($P = 0.039$). Patients with poor adherence to their other prescription pills were more likely to skip taking their NUCs. There was a significant association between doctor-patient language discordance and adherence levels ($P = 0.04$). Patients who were under the care of a language-discordant clinician were more likely to report suboptimal adherence to NUCs compared to patients who were under the care of a language-concordant clinician.

DISCUSSION

Whilst the safety, efficacy and therapeutic benefits have been extensively established for CHB NUCs, rates of adherence to therapy and factors that may affect it remain poorly studied. In this questionnaire study, patient self-report of adherence levels were used to determine adherence levels to NUCs. Findings from this study revealed a disappointing 66% optimal adherence rate.

These findings are comparable to the adherence rates to NUCs amongst CHB patients reported by Chotiyaputta *et al*[17]'s retrospective study which evaluated adherence rates based on pharmacy refill records. In their study, 55.3% of patients had good adherence (arbitrarily defined as an adherence rate > 90%). CHB adherence levels are higher than the adherence levels observed in other chronic medical conditions such as inflammatory bowel disease, asthma or hypertension. Possible explanations for the higher adherence rates observed in CHB patients include the simple dosage regimen such as an once daily dosing of a single NUC that is generally well-tolerated with minimal side effects[18]. For many chronic conditions, increased complexity of treatment regimen is associated with lower levels of adherence[19]. Alternatively, it may be that the clinicians, most of whom are gastroenterologists or hepatologists, spend more time counselling patients on the value of adherence to NUCs[19].

Forgetfulness is the most common reason cited for missing their medications. It is probably a little more complex. "Forgetfulness" is the product of both cognitive and motivational factors. Therefore, simply addressing the cognitive aspect of the problem alone, *via* reminders, will not solve the problem. Patients with chronic conditions on long-term therapy often experience drug fatigue, lose motivation and become complacent, with reduced adherence over time[20]. Although, there was no statistical difference in adherence levels observed between new and existing patients in this study. It is important to note that CHB patients on long-term NUCs face similar barriers to those with chronic medical conditions as most will require many years, if not, lifelong administration of NUCs[11]. In these patients, it is essential to consolidate advice and information on the benefits of treatment.

This study identified several factors associated with suboptimal adherence. Those reporting suboptimal adherence to other prescription pills were more likely to omit NUCs, possibly partially to increased pill burden. This suggests that patients may have an "universal" non-adherent attitude to all their medications. Identifying at risk patients for increased education and counselling during consultations with clinician or structured individualised or group educational sessions by other health carers should be considered.

Patients cared for by language-discordant physicians

reported more suboptimal adherence. This may be due to language and cultural barriers. Comprehending unfamiliar medical jargon may be challenging even to those who have no language barriers, this becoming much more challenging to those with limited English proficiency[21]. Such barriers may lead to a limited understanding of the rationale for treatment and dosing instructions and compromise the physician-patient relationship, which inevitably affects their adherence[14]. Understanding the cultural aspects of health care delivery and providing appropriate care could also be a significant contributor to improving compliance and medicine adherence.

Poor correlation between physician assessment and patient's self-report of adherence levels was noted. This may be due to patients less reporting non-adherence for fear of disapproval from the physician[22]. Previous studies found that physicians tend to both under-estimate and over-estimate patient's adherence to medications[13,23]. In contrast, this study showed that physicians were more likely to over-estimate adherence, and hence less time may be spent on discussing compliance.

Data collected was cross-sectional and hence, the factors associated with suboptimal adherence cannot be interpreted as predictors of future adherence or used to confer causality between the factors studied and adherence levels. Secondly, although, adherence was assessed as a dichotomous variable, it should be noted that it is essentially a dynamic process that is influenced by multiple factors over time. Future studies should include a longitudinal approach to capture the dynamic nature of adherence. Our sample size and population base was small making extrapolation to other populations difficult as well as limiting reporting of statistically significant results. Furthermore adherence levels may vary in different geographical cohorts. A self-report approach may represent overly optimistic estimates of adherence levels as self-report is often subject to over-estimation due to social desirability and recall bias[16]. A very strict definition of optimal adherence was used and whether this is clinically relevant remains to be determined. Clinical outcome data such viral loads and liver function test levels need to be studied in a longitudinal fashion to draw conclusions about the relationship between adherence levels and treatment outcomes.

This study reports poor rates of optimal medical adherence to NUCs, more than that expected. It has shown that patients who reported low adherence to other prescription pills and those under the care of a language-discordant physician were more likely to report suboptimal adherence to their antiviral treatment. Further understanding the factors that impact patient adherence will assist in the development and subsequent implementation of strategies that may adherence.

COMMENTS

Background

Non-adherence to long-term medication is a common problem in many medical fields. In chronic hepatitis B virus (CHB) non-adherence may give rise to high viral replication and associated progression of liver fibrosis. Most worryingly however non-adherence can lead to viral resistance against nucleos(t)ide analogue (NUC).

Research frontiers

The extent of non-adherence to NUC for CHB remains largely unknown. Furthermore the factors associated with non-adherence have not been fully understood. Ultimately these factors may not only allow for easier identification of patients at risk of non-adherence, they may also provide targets for intervention to improve adherence.

Innovations and breakthroughs

In this study over a third of CHB patients were not adhering optimally to their CHB medication, hence highlighting the importance of measuring adherence. Non-adherence was associated with patient-doctor language discordance. Effective communication about treatment strategies and especially the need for and effect of NUC for CHB may have been suboptimal in these cases. Worryingly physicians overestimated the patients' adherence, therefore missing cases of non-adherence.

Applications

Physicians should not rely solely on their clinical judgement to assess adherence to NUC. Direct questioning or use of simple tools such as visual analogue scales or short validated questionnaires will unmask non-adherence in more patients. Adherence should be discussed with all language discordant patients as we have demonstrated that they are most at risk of non-adherence.

Terminology

Adherence: Extent to which a patient's behaviour matches the agreed treatment plan.

Peer review

This paper is well written and interesting in that there is scarce literature on this subject.

REFERENCES

1 **World Health Organization**. Adherence to long-term therapies: evidence for action. Geneva: World Health Organization, 2003
2 **Australasian Society for HIV Medicine**. B Positive: all you wanted to know about hepatitis B - a guide for primary care providers. Sydney: ASHM and The Cancer Council NSW, 2008
3 **Digestive Health Foundation**. Chronic Hepatitis B (CHB) Recommendations. 2nd ed. Victoria: Gastroenterological Society of Australia, 2009
4 **Cooke GS**, Main J, Thursz MR. Treatment for hepatitis B. *BMJ* 2010; **340**: b5429
5 **Lok AS**, Lai CL, Leung N, Yao GB, Cui ZY, Schiff ER, Dienstag JL, Heathcote EJ, Little NR, Griffiths DA, Gardner SD, Castiglia M. Long-term safety of lamivudine treatment in patients with chronic hepatitis B. *Gastroenterology* 2003; **125**: 1714-1722
6 **Hadziyannis SJ**, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, Marcellin P, Lim SG, Goodman Z, Ma J, Brosgart CL, Borroto-Esoda K, Arterburn S, Chuck SL. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B for up to 5 years. *Gastroenterology* 2006; **131**: 1743-1751
7 **Colonno RJ**, Rose R, Baldick CJ, Levine S, Pokornowski K, Yu CF, Walsh A, Fang J, Hsu M, Mazzucco C, Eggers B, Zhang S, Plym M, Klesczewski K, Tenney DJ. Entecavir resistance is rare in nucleoside naïve patients with hepatitis B. *Hepatology* 2006; **44**: 1656-1665
8 **Paterson DL**, Swindells S, Mohr J, Brester M, Vergis EN, Squier C, Wagener MM, Singh N. Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. *Ann Intern Med* 2000; **133**: 21-30
9 **Low-Beer S**, Yip B, O'Shaughnessy MV, Hogg RS, Montaner JS. Adherence to triple therapy and viral load response. *J Acquir Immune Defic Syndr* 2000; **23**: 360-361
10 **Zoulim F**, Locarnini S. Hepatitis B virus resistance to

nucleos(t)ide analogues. *Gastroenterology* 2009; **137**: 1593-1608. e1-e2
11 **Heathcote EJ**. Treatment of hepatitis B: the next five years. *Clin Med* 2007; **7**: 472-477
12 **Bangsberg DR**, Hecht FM, Clague H, Charlebois ED, Ciccarone D, Chesney M, Moss A. Provider assessment of adherence to HIV antiretroviral therapy. *J Acquir Immune Defic Syndr* 2001; **26**: 435-442
13 **Walshe L**, Saple DG, Mehta SH, Shah B, Bollinger RC, Gupta A. Physician estimate of antiretroviral adherence in India: poor correlation with patient self-report and viral load. *AIDS Patient Care STDS* 2010; **24**: 189-195
14 **Chesney MA**. Factors affecting adherence to antiretroviral therapy. *Clin Infect Dis* 2000; **30** Suppl 2: S171-S176
15 **Chesney MA**, Ickovics JR, Chambers DB, Gifford AL, Neidig J, Zwickl B, Wu AW. Self-reported adherence to antiretroviral medications among participants in HIV clinical trials: the AACTG adherence instruments. Patient Care Committee & amp; Adherence Working Group of the Outcomes Committee of the Adult AIDS Clinical Trials Group (AACTG). *AIDS Care* 2000; **12**: 255-266
16 **O'Donohue W**, Levensky E. Promoting treatment adherence: A practical handbook for healthcare providers. Thousand Oaks, CA: Sage Publications, 2006
17 **Chotiyaputta W**, Peterson C, Ditah FA, Goodwin D, Lok AS. Persistence and adherence to nucleos(t)ide analogue treatment for chronic hepatitis B. *J Hepatol* 2011; **54**: 12-18
18 **Lee M**, Keeffe EB. Study of adherence comes to the treatment of chronic hepatitis B. *J Hepatol* 2011; **54**: 6-8
19 **Stone VE**, Hogan JW, Schuman P, Rompalo AM, Howard AA, Korkontzelou C, Smith DK. Antiretroviral regimen complexity, self-reported adherence, and HIV patients' understanding of their regimens: survey of women in the her study. *J Acquir Immune Defic Syndr* 2001; **28**: 124-131
20 **Stone VE**. Strategies for optimizing adherence to highly active antiretroviral therapy: lessons from research and clinical practice. *Clin Infect Dis* 2001; **33**: 865-872
21 **Houts PS**, Doak CC, Doak LG, Loscalzo MJ. The role of pictures in improving health communication: a review of research on attention, comprehension, recall, and adherence. *Patient Educ Couns* 2006; **61**: 173-190
22 **Weiss JJ**, Bräu N, Stivala A, Swan T, Fishbein D. Review article: adherence to medication for chronic hepatitis C - building on the model of human immunodeficiency virus antiretroviral adherence research. *Aliment Pharmacol Ther* 2009; **30**: 14-27
23 **Miller LG**, Liu H, Hays RD, Golin CE, Beck CK, Asch SM, Ma Y, Kaplan AH, Wenger NS. How well do clinicians estimate patients' adherence to combination antiretroviral therapy? *J Gen Intern Med* 2002; **17**: 1-11

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San Francisco, CA, United States

January 20-21, 2012
AGA Clinical Congress of Gastroenterology and Hepatology: Practice, Evidence and Quality in 2012
Miami, FL, United States

January 27-28, 2012
28th Annual Meeting of the German Association for the Study of the Liver
Hamburg, Germany

January 30-31, 2012
5th International Conference on the Management of Patients with Viral Hepatitis
Paris, France

February 8-10, 2012
Stockholm Liver Week 2012
Stockholm, Sweden

February 16-19, 2012
22nd Conference of the Asian Pacific Association for the Study of the Liver
Taipei, Taiwan, China

March 16 -17, 2012
Hepatitis Single Topic Conference
Atlanta, GA, United States

March 16-17, 2012
ESGE - Workshop on Advanced Endoscopy with Live Demonstrations
Vienna, Austria

March 31-April 1, 2012
27th Annual New Treatments in Chronic Liver Disease
San Diego, CA, United States

April 18-22, 2012
The International Liver Congress by EASL
Barcelona, Spain

April 27-28, 2012
The European Society for Paediatric Gastroenterology, Hepatology and Nutrition
Stockholm, Sweden

May 16-19, 2012
International Liver Transplant Society 18th Annual International Congress 2012
San Francisco, CA, United States

May 19-22, 2012
Digestive Disease Week 2012
San Diego, CA, United States

June 22-23, 2012
EASL Monothematic Conference: Vascular Liver Diseases
Tallin, Estonia

July 1-5, 2012
10th World Congress of the International Hepato-Pancreato-Biliary Association 2012
Paris, France

September 5-8, 2012
International Congress of Pediatric Hepatology, Gastroenterology and Nutrition
Sharm El-Sheikh, Egypt

September 7-9, 2012
Viral Hepatitis Congress 2012
Macclesfield, United Kingdom

September 7-9, 2012
The Viral Hepatitis Congress
Frankfurt, Germany

September 14-16, 2012
The International Liver Cancer Association's 6th Annual Conference
Berlin, Germany

September 20-22, 2012
Prague Hepatology Meeting 2012
Prague, Czech Republic

September 20-22, 2012
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October 18-20, 2012
2nd World Congress on Controversies in the Management of Viral Hepatitis
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November 9-13, 2012
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Ocean International Center,
No. 62 Dongsihuan Zhonglu,

Chaoyang District, Beijing 100025, China
Telephone: +86-10-85381892
Fax: +86-10-85381893
E-mail: wjh@wjgnet.com
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3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

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5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

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Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

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/index.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

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

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).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/1948-5182/g_info_20100107115140.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

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Frontier: http://www.wjgnet.com/1948-5182/g_info_20100315103153.htm

Topic highlight: http://www.wjgnet.com/1948-5182/g_info_20100316080006.htm

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Guidelines: http://www.wjgnet.com/1948-5182/g_info_20100315105107.htm

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