

77838_Auto_EditedC.docx

Name of Journal: *World Journal of Gastrointestinal Surgery*

Manuscript NO: 77838

Manuscript Type: MINIREVIEWS

Research progress on the immune microenvironment of the gallbladder in patients with cholesterol gallstones

Jiao JY *et al.* Cholesterol gallstones

Jing-Yi Jiao, Xiao-Jun Zhu, Chun Zhou, Peng Wang

Abstract

Cholesterol gallstones are very common in hepatobiliary surgery and have been studied to a certain extent by doctors worldwide for decades. However, the mechanism of cholesterol gallstone formation is not fully understood, so there is currently no completely effective drug for the treatment and prevention of cholesterol gallstones. The formation and development of cholesterol gallstones are caused by a variety of genetic and environmental factors, among which genetic susceptibility, intestinal microflora disorders, impaired gallbladder motility, and immune disorders are important pathogenesis of cholesterol gallstones. This review focuses on recent advances in these mechanisms. We also discuss some new targets that may be effective in the treatment and prevention of cholesterol gallstones, which may be hot areas in the future.

Key Words: Microflora; Cholesterol gallstones; Gallbladder; Pathogenesis; Immune disorders

Jiao JY, Zhu XJ, Zhou C, Wang P. Research progress on the immune microenvironment of the gallbladder in patients with cholesterol gallstones. *World J Gastrointest Surg* 2022; In press

Core Tip: Cholesterol gallstone disease is very common. At present, some new progress has been made in the research on the pathogenesis of cholesterol gallstones, and we have also gained a new understanding of this disease. Here, we discuss the latest research progress of genetic susceptibility, intestinal microflora disorders, impaired gallbladder motility, and immune disorders in the formation of cholesterol gallstones and some new drug targets.

INTRODUCTION

Gallstones occur in about 20% of adults in western countries and are one of the most common diseases of hepatobiliary surgery^[1]. In past research studies^[2], we found that more than 90% of gallstones are mainly composed of cholesterol, called cholesterol gallstones.

Normally, mixed micelles are composed of cholesterol, phospholipids (mainly phosphatidylcholine), and bile salts in bile. Under the action of mixed micelles, bile is thermodynamically stable and cholesterol does not precipitate. When the cholesterol molecules in bile exceed the maximum limit that the mixed micelles can accommodate, cholesterol is in a supersaturated state and cholesterol is prone to precipitate^[3]. The relative saturation of cholesterol in bile varies with the concentration of bile salts and phospholipids^[4].

In past studies, we found that risk factors for cholesterol gallstones comprise both unmodifiable and modifiable factors. Non-modifiable factors include age, sex, race, and genetic factors. Modifiable factors include the following: metabolic syndrome features such as diabetes^[5], insulin resistance, and obesity^[6]; dietary habits such as high-calorie and low-fiber diets^[7]; intestinal damage such as colectomy^[8]; Crohn's disease; drug factors such as octreotide^[9], lipid-lowering drugs, and hormones; and impaired gallbladder motility.

More than 20% of patients with cholesterol gallstones develop symptoms, such as biliary colic, during their lifetime and are at risk of developing cholecystitis, gallbladder cancer^[10] and pancreatitis^[11]. To date, surgery is the best way to treat cholesterol gallstones patients when they develop these symptoms or complications, but it comes with heavy economic and social burdens^[12]. Therefore, it is urgent and important to treat and prevent cholesterol gallstones by studying the pathogenesis of gallstones and taking corresponding intervention measures for specific pathogenic links.

In this review, we focus on the important roles of genetic susceptibility, intestinal microflora disorders, and impaired gallbladder motility. We also discuss some

strategies for the treatment and prevention of cholesterol gallstones, which inhibit some of the pathogenic aspects of cholesterol gallstones.

IMMUNE DISORDERS LEAD TO CHOLESTEROL GALLSTONES

Immune disorders play a crucial role in the formation and development of cholesterol gallstones. First, low concentrations of various immunoglobulins including IgA, IgG, and IgM were contained in bile^[13]. Among them, IgM is the most effective Ig in promoting the formation of cholesterol gallstones in supersaturated bile, while IgG is less effective and IgA is the least effective^[14-16]. In addition, the formation of cholesterol gallstones is closely related to mucin (MUC) gel accumulation in human and animal models, and MUC gel accumulation occurs before cholesterol gallstone formation and is an important cause of cholesterol gallstone formation^[17-22]. At the same time, MUC may be positively correlated with the calcification of cholesterol gallstones^[23]. Some MUC genes are expressed in human bile duct epithelial cells such as MUC1, MUC2, MUC3, MUC4, MUC5AC, MUC5B, and MUC6^[24], and the expression of these MUC genes and the production and secretion of MUC are regulated by inflammatory mediators in the immune system^[25-27]. Cholesterol secretion can also be promoted by inflammatory mediators, which promote liver lipid metabolism and secretion, lead to bile cholesterol supersaturation, and promote cholesterol gallstone formation. For example, in mice, the formation of cholesterol gallstones can be promoted by the administration of lipopolysaccharide (LPS) or pro-inflammatory cytokines [interleukin (IL)-1, tumor necrosis factor (TNF)], because these result in elevated serum cholesterol levels and increase the production of 3-hydroxy-3-methylglutarate mono-acyl-coenzyme A reductase (HMG-CoA reductase)^[28-30]. In addition, cholesterol catabolism can be inhibited by LPS, which reduces the production of cholesterol 7 alpha-hydroxylase (CYP7A1), CYP7B1, or CYP27A1 protein, leading to bile supersaturation and cholesterol gallstone formation^[31,32]. Recent studies have found that immune factors can also influence the formation of cholesterol gallstones by influencing the movement of gallbladder contraction. Interstitial Cajal-like cells (ICLCs) are widespread in the

12
gallbladder and bile duct and play a significant role in the regulation of gallbladder contractile motion. The density of ICLCs in the gallbladder is significantly reduced in patients with cholelithiasis, suggesting that decreased gallbladder contraction and cholesterol gallstone formation are closely associated with reduced ICLCs. Ursodeoxycholic acid protects ICLCs in the gallbladder from apoptosis by inhibiting the TNF- α /caspase 8/caspase 3 pathway^[33], thereby protecting the contractile activity of the gallbladder and ultimately inhibiting the formation of cholesterol gallstones. These objective results indicate that immune disorders play a crucial role in the formation and development of cholesterol gallstones.

The role of adaptive immunity in cholesterol gallstone formation was analyzed by giving *Helicobacter pylori* (*H. pylori*)-infected and uninfected homozygous mice, as well as homozygous immunodeficient Rag mice, a lithogenic diet in a former study. Lymphocyte metastasis studies were also performed to determine which cell subsets are responsible for cholesterol gallstone formation^[34]. *H. pylori* usually cause disease by inducing a pro-inflammatory immune response mediated by T-assisted type 1^[35,36]. When fed the lithogenic diet for 2 mo, more cholesterol gallstones were found in non-immunodeficient mice than in Rag mice. There was a statistically significant increase in cholesterol gallstone prevalence in *H. pylori*-infected mice compared with uninfected mice. In addition, T lymphocyte transfer to Rag mice significantly increased the prevalence of cholesterol gallstones, while B lymphocyte transfer did not significantly increase cholesterol gallstones. A detailed description of the association between adaptive immunity and cholesterol gallstone formation was provided in this study, which suggested that T cells are an important link in the formation of cholesterol gallstones in mice (Figure 1).

The vital role of neutrophil external traps (NETs) in cholesterol gallstone formation and development was expounded upon in a recent study^[37]. By fluorescence microscopy, patchy extracellular DNA (ecDNA), large ecDNA aggregates, and strong neutrophil elastase activity were found in both human and porcine cholesterol gallstones. In previous reports, obesity is related to the release of ecDNA into plasma in

mice and humans^[38], and ecDNA in peripheral circulation has contact with the risk of metabolic syndrome^[39], both of which are risk factors for cholesterol gallstones. Upon contact with neutrophils, cholesterol or calcium crystals are ingested by neutrophils. This process of pinocytosis causes the granular enzymes in lysosomes to leak and bind to the DNA in the cytoplasm, ultimately decondensed chromatin and externalizing to form NETs. Cholesterol crystals and calcium crystals in the bile of the gallbladder are aggregated to form cholesterol gallstones by the “glue” role of NETs. Meanwhile, the formation of NETs is dependent on the activity of peptidyl arginine deiminase type 4 and the production of reactive oxygen species. In addition, this study confirmed that the formation and development of cholesterol gallstones can be effectively reduced by the inhibition of NET formation or neutrophils. The results of this study verify that the formation of NETs is the key link in the formation of cholesterol gallstones caused by the accumulation of crystals in bile, and the formation of neutrophils and NETs may be new targets for the prevention and treatment of cholesterol gallstones (Figure 1).

Together, these findings suggest that immune dysfunction is also an important link in the formation and development of cholesterol gallstones. Targeting immune disorders ¹ in the pathogenesis of cholesterol gallstones ¹ will be a new hotspot in the treatment and prevention of cholesterol gallstones in the future.

ROLE OF INTESTINAL FLORA DYSREGULATION IN CHOLESTEROL GALLSTONES

Bacteria are present ¹⁴ in the bile, cholesterol gallstones, and even gallbladder tissue of patients with cholesterol gallstones^[1]; however, the role of these bacteria in cholesterol gallstone formation is not fully understood. A lower incidence of cholesterol gallstones in germ-free mice was found in one of the earliest studies^[40]. Another study showed that mice infected with enterohepatic *H. pylori* had an increased risk of cholesterol gallstones^[41]. A recent study ³ comparing the biliary microbiota of lithiasis and non-lithiasis groups found that the *Alcaligenaceae* reached higher relative abundance in lithiasis samples^[42]. In this family, *Alcaligenes recti* ³ are reportedly involved in the

metabolism of various bile acids. These findings suggest that cholesterol gallstone formation appears to be related to intestinal microbiome dysregulation. The abundance and diversity of intestinal flora decreased, the number of *Firmicutes* decreased, and the ratio of *Firmicutes* to *Bacteroidetes* decreased in mice with gallstones^[43]. In addition, the intestinal bacteria phylum *Proteobacteria* were significantly increased, while *Faecalibacterium*, *Lachnospira*, and *Roseburia* were significantly decreased^[44]. The number of Gram-positive fecal anaerobes in the cecum was increased in patients with gallstones compared with those without gallstones, and 7 α -dehydroxylation activity was also increased, which seemed to explain the increased concentration of hydrophobic secondary bile acid deoxycholic acid in patients with gallstones^[45].

Enrichment of *Desulfovibrionales* has been found in patients with metabolic syndrome and obesity associated with cholesterol gallstones^[46], but the specific link between the bacteria and cholesterol gallstones has not been clarified. A recent study found that the abundance of *Desulfovibrionales* in the feces of cholesterol gallstone patients and cholesterol gallstone-susceptible mice was significantly higher than that in the non-gallstone population, and that the transplantation of intestinal flora from cholesterol gallstone patients into cholesterol gallstone-resistant mice resulted in a statistically significant increase in cholesterol gallstone prevalence^[47]. The production of secondary bile acids will be promoted by a large number of *Desulfovibrionales* rich in the cecum, and the hydrophobicity of bile acids will therefore increase, resulting in increased absorption of intestinal cholesterol and easy to cause cholesterol gallstones. In addition, the intestinal lipid absorption process is regulated by CD36. The expression of CD36 can be induced by *Desulfovibrionales*; thus, the intestinal lipid absorption is enhanced, which may also lead to the formation of cholesterol gallstones^[48]. On the other hand, hydrogen sulfide, a metabolite of *Desulfovibrionales*, can induce farnesoid X receptor and inhibit the expression of CYP7A1. The expression of cholesterol transporter ATP-binding cassette transporter G5/G8 (ABCG5/ABCG8) in the mouse liver was also induced by *Desulfovibrionales*, which promoted cholesterol secretion in the biliary tract. This study shows that cholesterol gallstone formation is promoted by intestinal *Desulfovibrionales*,

which influences bile acid and cholesterol metabolism, further supporting the important role of intestinal microbiome imbalance in cholesterol gallstone formation.

GENETIC SUSCEPTIBILITY TO CHOLESTEROL GALLSTONES

In addition to these two mechanisms, there are other factors that contribute to the formation of cholesterol gallstones, such as genetic factors and gallbladder dyskinesia^[49]. Indigenous populations in North and South America are reported to be at highest risk of gallstones in the world. Prevalence rates are lower in Asian populations and lowest in African populations^[1]. A study of 43141 twins with gallstone disease in Sweden showed that about 25% of gallstones were caused by a genetic susceptibility^[50]. These objective results suggest that gallstones risk and genetic susceptibility are inextricably linked.

Lipid composition in the biliary tract ⁴ is regulated by complex ATP-binding cassette (ABC) transporters on the hepatocyte canalicular membrane. The transport of bile salts into the biliary tract is carried out by the ABC transporter ABCB11^[51]. The transport of phosphatidylcholine into the biliary tract is carried out by the ABC transporter ABCB4^[52]. The transport of cholesterol into the biliary tract is carried out by the ABC transporters ABCG5 and ABCG8^[53].

Mutations and variants of ABCB4 inhibit the secretion of phospholipids from the liver to the bile ducts, resulting in a decrease or deficiency of phospholipids in bile and the formation of cholesterol gallstones, known as low phospholipid-associated cholelithiasis. A recent study compared the chemical composition of fresh gallbladder bile between ABCB4 knockout and wild-type mice and found cholesterol supersaturation and the presence of cholesterol crystals in gallbladder bile in the former but not in the latter. The results of this study demonstrate the critical role of ABCB4 in phospholipid transport and the important role of ABCB4 mutations in the formation of cholesterol gallstones^[54]. A strong association between gallstone disease and ABCG8 was shown in a genome-wide association study (GWAS) involving 280 patients with gallstones and 360 controls in 2007^[55]. ABCG8 is responsible for transporting cholesterol

into the biliary tract and intestinal lumen, and its association with cholesterol gallstones is attributed to a familiar variant that causes guanine at position 55 to become cytosine, resulting in the replacement of aspartic acid, the amino acid residue at position 19 of the transporter, by histidine (ABCG8D19H, RS11887534). ABCG8D19H constitutes a functional acquisition mutation, which increases the transport activity of ABCG8 by three fold, increases the hepatic cholesterol discharge into the biliary tract, increases the absolute cholesterol saturation in bile, and ultimately leads to the occurrence of cholesterol gallstones^[55-57].

In 2016, four new gallstones susceptibility loci, namely SULT2A1, TM4SF4, GCKR, and CYP7A1, were identified in a large GWAS (there were 8720 gallstones patients and 55152 people who did not have gallstones in the discovery set, and 6489 gallstones patients and 62797 people who did not have gallstones in the validation set), and the association between ABCG8 and gallstones were confirmed^[58]. The metabolism of cholesterol into bile acid in the liver is mainly regulated by cholesterol CYP7A1, and its reduced function may lead to the formation and development of cholesterol gallstones by reducing the catabolism of cholesterol into bile acid^[59]. The transport of cholesterol from the intestinal lumen into intestinal cells and from bile into liver cells is in the charge of ¹³ Niemann-Pick C1-like protein 1 (NPC1L1). Reduced activity of the NPC1L1 gene leads to reduced uptake of cholesterol from the lumen to intestinal cells and from bile to liver cells, resulting in increased cholesterol content in the biliary tract, increased absolute cholesterol saturation in the biliary tract, and increased risk of cholesterol gallstone formation^[60].

According to a 2019 study, six new gallstone-related or highly related variants were associated with blood cholesterol levels (HNF4A, HNF1A, FUT2, FADS2, MARCH 8, and JMJD1C)^[61]. However, the association between these variants and cholesterol gallstone formation and development is unclear. In the future, GWASs will find more new cholesterol-gallstones related variants, and further studies are needed to determine the molecular basis behind these variants^[62].

CHOLESTEROL GALLSTONE FORMATION BY IMPAIRED GALLBLADDER MOTILITY

Whatever mechanism causes cholesterol gallstones to form, these processes are slow. Cholesterol gallstones cannot form if the gallbladder is completely emptied several times a day. Therefore, the total or partial extension of bile storage due to impaired gallbladder movement seems to be another important condition for cholesterol gallstone formation. Insufficient gallbladder motility contributes to cholesterol gallstone formation and is impaired under many risk factors for cholesterol gallstone formation, such as pregnant women, obese patients, and their rapid weight loss, diabetes mellitus, and patients receiving total parenteral nutrition^[63]. A recent study showed that 78 of 959 patients (8%)² who underwent laparoscopic Roux-en-Y gastric bypass (RYGB) or sleeve gastrectomy² developed symptomatic gallstone disease within 24 mo^[64]. In patients without gallstones before RYGB surgery, ursodeoxycholic acid treatment reduced the occurrence of symptomatic gallstone disease compared with placebo^[65]. On an empty stomach, bile drained from the liver is stored in the gallbladder. After eating, bile is discharged by the gallbladder into the duodenum and small intestine. The motor function of the smooth muscle of the gallbladder is mainly regulated by cholecystokinin (CCK), a key gastrointestinal hormone. The release of CCK is mainly caused by the stimulation of dietary lipids and proteins. Insufficient gallbladder contraction during fasting is caused by reduced gallbladder stimulation. Patients using the somatostatin analog octreotide may develop cholesterol gallstones because postprandial CCK release and gallbladder contraction was inhibited by octreotide^[9]. Injection of CCK in patients receiving total parenteral nutrition, or the addition of dietary fat to promote the release of CCK in the gastrointestinal tract of people who lose weight quickly, enhances the ability of their gallbladder to contract and prevents the formation of cholesterol gallstones^[66,67]. Mice with reduced CCK or damaged CCK-1 receptor genes had slower small bowel movement^[68,69], suggesting that CCK not only promotes contraction of gallbladder smooth muscle but also speeds up intestinal transport through a CCK-1 receptor signaling cascade. Loss of the CCK-1 receptor gene in mice led to reduced

gallbladder contraction and reduced intestinal transport, which in turn led to cholestasis and increased intestinal cholesterol absorption, ultimately increasing the risk of gallstone formation^[69]. In addition, ICLCs are widespread in the gallbladder and bile duct and play a significant role in the regulation of gallbladder contractile motion^[70,71]. Previous studies have found that the density of ICLCs in the gallbladder is significantly reduced in patients with cholesterol gallstones, suggesting that decreased gallbladder contraction and cholesterol gallstone formation are closely associated with reduced ICLCs^[72-74].

CONCLUSION

Cholesterol gallstones are common in hepatobiliary surgery and their incidence is increasing. At present, surgery is the preferred treatment for symptomatic cholesterol gallstones disease, but there is still a lack of primary prevention drugs for cholesterol gallstones. The pathogenesis of cholesterol gallstones is extremely complex. We identified the modifiable factors in the pathogenesis of cholesterol gallstones through research to provide strategies for the prevention of cholesterol gallstones disease in high-risk groups. At the same time, more emphasis should be placed on the prevention of cholesterol gallstones, which seems to be a better option than cholecystectomy.

8%

SIMILARITY INDEX

PRIMARY SOURCES

- 1

Agostino Di Ciaula, David Q.H. Wang, Helen H. Wang, Leonilde Bonfrate, Piero Portincasa. "Targets for Current Pharmacologic Therapy in Cholesterol Gallstone Disease", Gastroenterology Clinics of North America, 2010

Crossref

54 words — 2%
- 2

www.pubfacts.com

Internet

32 words — 1%
- 3

Ru Feng, Tianyu Zhang, Masood ur Rehman Kayani, Zhengting Wang, Yao Shen, Kenn Liu Su, Kouken Bielike, Lei Chen. "Patients with Primary and Secondary Bile Duct Stones Harbor Distinct Biliary Microbial Composition and Metabolic Potential", Frontiers in Cellular and Infection Microbiology, 2022

Crossref

29 words — 1%
- 4

www.digital-world-medical-school.net

Internet

22 words — 1%
- 5

pure.rug.nl

Internet

11 words — < 1%
- 6

www.jucm.com

Internet

11 words — < 1%
- 7

www.ukessays.com

Internet

11 words — < 1%

8	www.vanguardngr.com Internet	11 words — < 1%
9	Patel, Ramesh N.. "ENZYMATIC PREPARATION OF CHIRAL PHARMACEUTICAL INTERMEDIATES BY LIPASES*", Journal of Liposome Research, 2001. Crossref	9 words — < 1%
10	link.springer.com Internet	9 words — < 1%
11	www.karger.com Internet	9 words — < 1%
12	www.science.gov Internet	9 words — < 1%
13	Ko, Cynthia, and Sum Lee. "Obesity and Gallbladder Disease", Handbook of Obesity, 2014. Crossref	8 words — < 1%
14	Portincasa, P.. "Cholesterol gallstone disease", The Lancet, 20060715/21 Crossref	8 words — < 1%
15	matci.jp Internet	8 words — < 1%

EXCLUDE QUOTES OFF
EXCLUDE BIBLIOGRAPHY OFF

EXCLUDE SOURCES OFF
EXCLUDE MATCHES OFF