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Cholesterol gallstones: Pathophysiology, risk factors, diagnostic approaches, and contemporary management strategies-an updated review

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***Abstract*---Background:** Cholesterol gallstone disease is one of the most prevalent gastrointestinal disorders worldwide and represents a major cause of healthcare utilization and surgical intervention. Although many individuals remain asymptomatic, symptomatic disease can result in recurrent biliary pain and serious complications. Increasing evidence indicates that cholesterol gallstone formation is a

multifactorial process involving genetic susceptibility, metabolic abnormalities, altered cholesterol homeostasis, gallbladder dysmotility, bile supersaturation, inflammatory responses, intestinal cholesterol absorption, and gut microbiota alterations. **Aim:** This review aimed to provide a comprehensive overview of the pathogenesis, risk factors, current therapeutic approaches, and emerging preventive strategies for cholesterol gallstone disease. **Methods:** A narrative review of contemporary literature was conducted, focusing on the molecular, metabolic, and clinical mechanisms underlying cholesterol gallstone formation, current management options, and potential future therapeutic targets. **Results:** Cholesterol gallstones account for more than 80% of gallstone cases and arise from complex interactions between systemic metabolic dysfunction and local gallbladder abnormalities. Laparoscopic cholecystectomy remains the standard treatment for symptomatic disease, while ursodeoxycholic acid provides benefit in carefully selected patients. Lifestyle modification, including weight management, healthy dietary patterns, and regular physical activity, plays a critical role in primary prevention. **Conclusion:** Cholesterol gallstone disease should be regarded as a systemic metabolic disorder rather than solely a biliary condition. Future management should integrate individualized treatment with evidence-based preventive strategies targeting the underlying metabolic and molecular mechanisms responsible for gallstone formation.

***Keywords*---**Cholesterol gallstones, Cholelithiasis, Cholesterol metabolism, Gallbladder motility, Bile acids, Ursodeoxycholic acid, Insulin resistance, Gut microbiota, Prevention, Cholecystectomy.

Introduction

Cholesterol gallstone disease represents one of the most prevalent gastrointestinal disorders worldwide and continues to impose a substantial clinical and economic burden on healthcare systems. In Western countries, its prevalence reaches approximately 15% of the adult population, making it one of the leading causes of hospital admission for gastrointestinal diseases across Europe [1, 2]. Beyond its high prevalence, gallstone disease is associated with considerable healthcare expenditure, particularly when gallstones become symptomatic or lead to complications requiring hospitalization, endoscopic intervention, or surgical management [1, 3, 4]. Consequently, cholesterol gallstones remain an important public health concern because of their impact on patient morbidity, healthcare resource utilization, and overall quality of life. Although gallstone disease has traditionally been regarded as a disorder affecting adults, increasing evidence indicates that its incidence is steadily rising among pediatric and adolescent populations. While childhood cholelithiasis remains less common than adult disease, its growing frequency has become a matter of clinical concern [5, 6]. Epidemiological studies from several countries have demonstrated a marked increase in pediatric cholecystectomy rates over recent decades. In England, the incidence of cholecystectomy among children aged 16 years or younger increased

from 0.78 per 100,000 population in 1997 to 2.7 per 100,000 in 2012 [7]. Likewise, a large Canadian population-based retrospective cohort study reported that the crude incidence of cholecystectomy in individuals younger than 18 years increased from 8.8 to 13.0 per 100,000 person-years between 1993 and 2012 [8]. Similar findings have been documented in the United States, where a retrospective investigation spanning nine years until 2012 revealed a remarkable 216% increase in cholecystectomies performed for pediatric non-hemolytic, cholesterol-related gallstone disease [9]. These epidemiological trends strongly suggest that cholesterol gallstones are no longer confined to older populations but are increasingly affecting younger individuals.

The rising incidence of cholesterol gallstones in childhood and adolescence has been attributed to profound changes in lifestyle and metabolic health. Increasing rates of obesity have emerged as one of the principal contributors to this epidemiological shift, supported by substantial clinical and epidemiological evidence [6, 10–16]. Additional factors, including reduced physical activity, insulin resistance, type 2 diabetes mellitus, and early pregnancy, further contribute to the growing burden of gallstone disease among adolescents and young adults [6]. These observations reinforce the concept that cholesterol gallstone disease is closely linked to contemporary metabolic disorders and lifestyle-related risk factors rather than representing an isolated biliary abnormality. Cholesterol stones account for more than 80% of all gallstones encountered in clinical practice, making them the predominant subtype of cholelithiasis worldwide [17]. Their development is recognized as a multifactorial process involving a complex interaction between local biliary factors and systemic metabolic disturbances. At the local level, alterations in bile composition, cholesterol supersaturation, impaired gallbladder motility, accelerated cholesterol crystal nucleation, and mucin hypersecretion collectively facilitate stone formation [17]. However, increasing evidence indicates that these local mechanisms alone cannot fully explain disease pathogenesis. Instead, systemic metabolic and inflammatory abnormalities significantly influence biliary physiology and contribute to cholesterol crystallization and gallstone growth. Chronic low-grade inflammation has recently emerged as a pivotal component in the pathogenesis of cholesterol gallstone disease. Elevated circulating concentrations of several inflammatory cytokines, including interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-12 (IL-12p70), and interleukin-13 (IL-13), have been associated with an increased susceptibility to gallstone formation [18]. These inflammatory mediators may alter hepatic cholesterol metabolism, bile acid synthesis, gallbladder contractility, and immune regulation within the biliary tract, thereby creating a biological environment favorable for cholesterol precipitation and stone development. Such findings suggest that immune dysregulation and systemic inflammation are integral components of gallstone pathophysiology rather than secondary consequences of the disease.

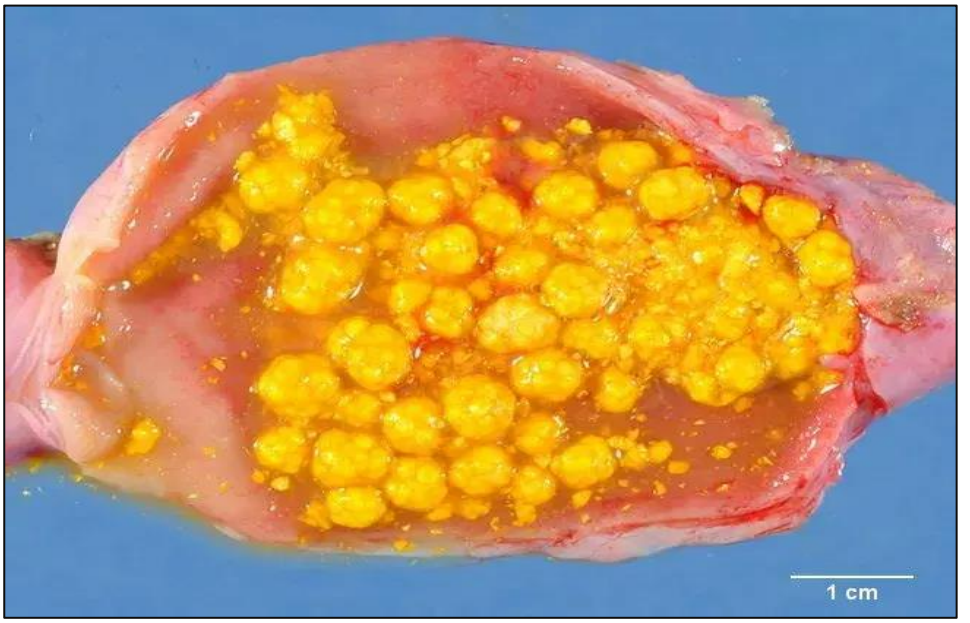

Fig. 1: Cholesterol gallstones

An expanding body of epidemiological evidence further supports the notion that cholesterol gallstone disease is closely associated with several chronic systemic disorders. Multiple large-scale studies have demonstrated significant associations between gallstone disease and an increased risk of incident ischemic heart disease, cardiovascular mortality, all-cause mortality, and disease-specific mortality, even after adjustment for established cardiovascular risk factors such as obesity, smoking, physical inactivity, diabetes mellitus, hypertension, and hypercholesterolemia [19–22]. These observations imply that gallstone disease shares common pathogenic mechanisms with cardiovascular disorders and may serve as a clinical marker of broader metabolic dysfunction. The relationship between cholesterol gallstones and systemic metabolic disease extends beyond cardiovascular pathology. Gallstone disease has consistently been linked with insulin resistance, obesity, type 2 diabetes mellitus, non-alcoholic fatty liver disease, and the metabolic syndrome, reflecting shared abnormalities in lipid metabolism, glucose homeostasis, chronic inflammation, and hepatic function [23–32]. These metabolic disorders collectively promote cholesterol supersaturation of bile while simultaneously impairing gallbladder emptying, thereby establishing conditions favorable for gallstone formation. Consequently, cholesterol gallstones should be viewed as part of a broader spectrum of metabolic disease rather than as an isolated hepatobiliary condition. Emerging evidence has also identified significant associations between cholesterol gallstone disease and several extra-biliary malignancies. Epidemiological investigations have demonstrated increased risks of tumors involving the liver, stomach, colon, and other organs among individuals with gallstones, suggesting that common inflammatory, metabolic, and molecular pathways may contribute to both gallstone formation and carcinogenesis [33–37]. Collectively, these findings support the contemporary concept that cholesterol gallstone disease represents the clinical manifestation of complex systemic metabolic and non-metabolic

abnormalities, involving intricate interactions among genetic susceptibility, inflammation, metabolic dysregulation, environmental exposures, and biliary physiology [38]. This evolving understanding has transformed cholesterol gallstone disease from a localized biliary disorder into a multisystem condition with important implications for prevention, risk assessment, and comprehensive clinical management.

Pathogenic Clues for Adequate Management of Gallstone Disease

A comprehensive understanding of the pathogenic mechanisms underlying cholesterol gallstone disease is fundamental for developing effective preventive and therapeutic strategies. Rather than arising from a single pathological process, cholesterol gallstone formation results from the interaction of multiple genetic, metabolic, environmental, and biliary factors that collectively disturb cholesterol homeostasis and promote stone formation. The heterogeneous nature of these mechanisms explains the considerable variability in disease presentation, progression, and response to treatment among affected individuals. Consequently, contemporary management of cholesterol gallstone disease increasingly emphasizes identifying the specific pathogenic factors contributing to disease development in each patient rather than relying solely on symptomatic treatment. The formation of cholesterol gallstones begins with a series of interconnected abnormalities involving both the gallbladder and hepatic bile. One of the principal pathogenic events is impaired gallbladder motility, which reduces effective emptying of the gallbladder and prolongs bile stasis. This stagnant environment facilitates cholesterol crystal nucleation and promotes progressive stone growth. Simultaneously, excessive secretion and accumulation of mucin gel within the gallbladder lumen create a favorable matrix that traps cholesterol crystals and accelerates their aggregation into macroscopic gallstones. These events are further amplified by persistent local immune-mediated inflammation, which alters gallbladder function, stimulates mucin production, and perpetuates the inflammatory milieu required for continued stone development. Another essential pathogenic mechanism involves the rapid phase transition of cholesterol from supersaturated hepatic bile into insoluble solid crystals, resulting in progressive cholesterol precipitation and gallstone formation [17, 39]. These interrelated abnormalities collectively represent the fundamental biological processes responsible for cholesterol cholelithiasis.

Beyond these local biliary mechanisms, numerous additional pathogenic factors contribute to disease initiation and progression. Alterations in hepatic cholesterol metabolism frequently result in excessive cholesterol secretion into bile, thereby increasing biliary cholesterol saturation beyond its capacity for solubilization. Enhanced intestinal absorption of both dietary and biliary cholesterol further augments the hepatic cholesterol pool, exacerbating cholesterol supersaturation within bile. Furthermore, delayed intestinal transit prolongs contact between luminal cholesterol and the intestinal mucosa, promoting increased cholesterol absorption and facilitating enterohepatic recycling. Recent investigations have also highlighted the importance of alterations in the intestinal microbiota. Quantitative, qualitative, and spatial changes in gut microbial communities influence bile acid metabolism, cholesterol absorption, immune regulation, and intestinal permeability, thereby contributing to the pathogenesis of cholesterol

gallstones through multiple complementary mechanisms [17, 40]. These observations reinforce the concept that cholesterol gallstone disease should be regarded as a systemic metabolic disorder rather than merely a localized disease of the gallbladder. Genetic susceptibility constitutes another major determinant of cholesterol gallstone formation. Numerous epidemiological studies have demonstrated a strong association between a positive family history of cholelithiasis and an increased risk of disease, suggesting an important hereditary component [41, 42]. Likewise, marked differences in disease prevalence among specific ethnic populations further support the contribution of inherited genetic factors to gallstone susceptibility [43, 44]. Advances in molecular genetics have subsequently identified multiple gene polymorphisms associated with altered cholesterol metabolism, biliary lipid secretion, bile acid synthesis, inflammatory regulation, and gallbladder function, thereby providing additional evidence for a substantial genetic contribution to disease development [45–61].

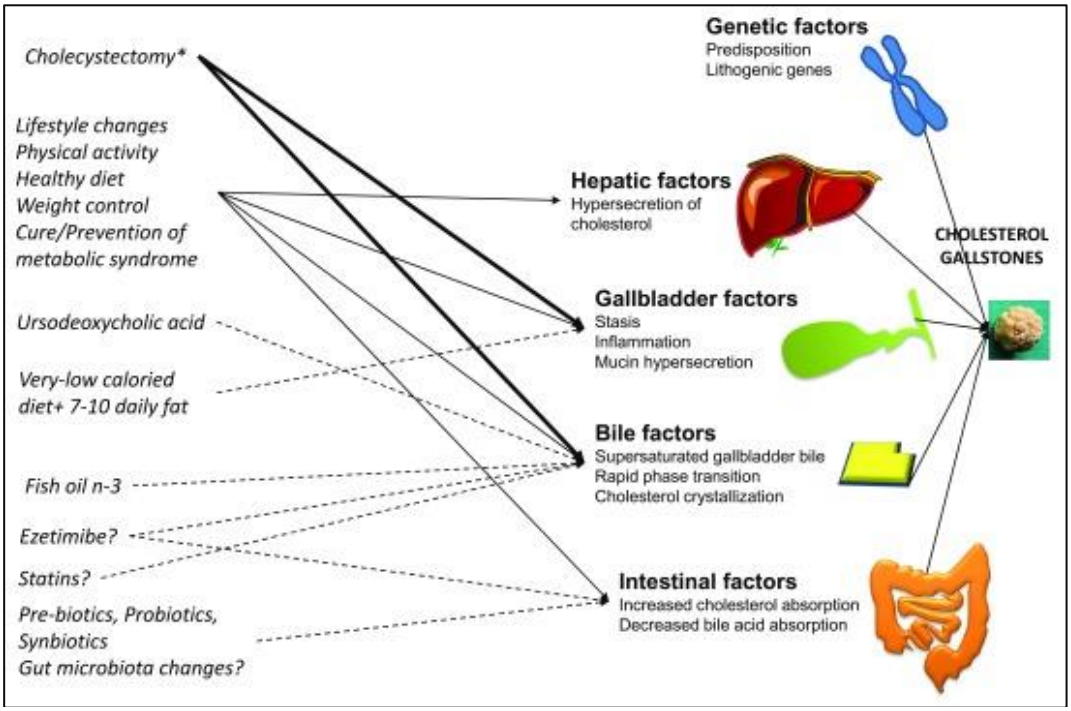

Fig. 2: Pathogenic Factors Affecting Gallstones

Nevertheless, genetic predisposition alone is insufficient to explain the occurrence of cholesterol gallstones. Twin studies have demonstrated that inherited genetic factors account for only approximately 25% to 30% of symptomatic gallstone cases, indicating that environmental influences play an equally important, if not greater, role in determining disease expression [62, 63]. Genetic variants therefore increase individual susceptibility but require interaction with external risk factors before clinically significant gallstone disease develops. This concept has important implications for prevention, as many environmental determinants remain modifiable throughout life. Lifestyle factors are among the most influential environmental contributors to cholesterol gallstone disease. Dietary habits

characterized by excessive caloric intake, high consumption of saturated fats and refined carbohydrates, and insufficient dietary fiber promote obesity, insulin resistance, and cholesterol supersaturation of bile. Similarly, reduced physical activity contributes to impaired lipid metabolism, diminished insulin sensitivity, and decreased gallbladder contractility, thereby increasing the likelihood of cholesterol crystal formation [58, 64–66]. These findings emphasize that lifestyle modification represents an essential component of primary prevention and long-term disease management. Increasing attention has also focused on the contribution of environmental pollutants to gallstone pathogenesis. Exposure to heavy metals and agricultural pesticides has been associated with disturbances in hepatic lipid metabolism, oxidative stress, chronic inflammation, and biliary function, all of which may facilitate cholesterol gallstone formation [67–70]. Importantly, many of these environmental factors appear to exert their biological effects through epigenetic mechanisms that alter gene expression without changing the underlying DNA sequence [17, 71]. Such observations illustrate the dynamic interaction between inherited susceptibility and environmental exposures in determining disease risk.

At the molecular level, cholesterol gallstone formation reflects profound disruption of hepatic cholesterol homeostasis. Cholesterol stones originate from supersaturated bile in which cholesterol exceeds the solubilizing capacity of bile salts and phospholipids contained within mixed micelles and vesicles. Under these conditions, cholesterol precipitates as solid crystals that progressively aggregate within the gallbladder to form mature gallstones [40]. This process involves abnormalities in cholesterol synthesis, absorption, transport, secretion, and enterohepatic circulation, highlighting the complexity of cholesterol metabolism in gallstone disease. Several molecular regulators play central roles in these metabolic disturbances. Among the most important are the ATP-binding cassette transporters ABCG5 and ABCG8, which mediate cholesterol transport from hepatocytes into bile and from enterocytes back into the intestinal lumen. Functional alterations in these transport proteins increase biliary cholesterol secretion, thereby promoting cholesterol supersaturation and crystallization [72]. Hormonal regulation also substantially influences gallstone formation. Estrogen stimulates hepatic cholesterol secretion and reduces bile acid synthesis, insulin resistance alters lipid metabolism and biliary composition, while thyroid hormones modulate cholesterol synthesis, transport, and biliary excretion [23, 30, 73–75]. In addition, several nuclear receptors regulate genes responsible for cholesterol metabolism, bile acid synthesis, lipid transport, and inflammatory signaling, making them important determinants of gallstone susceptibility [23, 76–78]. Finally, disturbances in the enterohepatic circulation of cholesterol and bile acids further impair cholesterol homeostasis, enhancing biliary cholesterol saturation and accelerating gallstone formation [79–81].

Sex-related differences play a fundamental role in the epidemiology and pathogenesis of cholesterol gallstone disease. Women consistently exhibit a substantially higher prevalence of gallstones than men, largely because of the physiological and pathological effects of estrogen on cholesterol metabolism and biliary lipid composition [73]. Estrogen promotes a lithogenic state by simultaneously enhancing hepatic cholesterol synthesis and suppressing bile acid (BA) synthesis, thereby increasing cholesterol saturation within bile. Since bile

acids constitute the principal catabolic products of cholesterol metabolism in humans and are essential for maintaining cholesterol solubility, any reduction in their synthesis predisposes to cholesterol crystallization and subsequent gallstone formation. The molecular mechanisms underlying these hormonal effects involve activation and upregulation of estrogen receptor-1 (ERα) together with G protein-coupled receptor 30 (GPR30), both of which regulate hepatic cholesterol metabolism and biliary lipid secretion [82, 83]. Clinical evidence further supports these biological observations, as a recent meta-analysis demonstrated a significant positive association between exposure to exogenous estrogen, including hormone replacement therapy and oral contraceptive use, and an increased risk of cholelithiasis [74]. These findings emphasize the importance of hormonal regulation as a major determinant of gallstone susceptibility and help explain the marked sex disparity observed in the incidence of cholesterol gallstones. In addition to hormonal influences, cholesterol gallstone disease is strongly associated with several interconnected metabolic disorders that collectively promote a lithogenic environment. Insulin resistance, obesity, hypertriglyceridemia, type 2 diabetes mellitus, and metabolic syndrome represent the principal systemic conditions linked to cholesterol gallstone formation [30, 44, 84–90]. These disorders share common pathophysiological pathways characterized by altered lipid metabolism, chronic low-grade inflammation, impaired glucose homeostasis, and hepatic metabolic dysfunction. Consequently, cholesterol gallstone disease is increasingly recognized as one manifestation of a broader metabolic syndrome rather than an isolated disorder confined to the hepatobiliary system.

Among these metabolic abnormalities, insulin resistance occupies a central role because of its widespread effects on cholesterol metabolism and biliary physiology. Insulin resistance stimulates hepatic cholesterol biosynthesis through activation of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol synthesis, thereby increasing the hepatic cholesterol pool available for biliary secretion [91]. Simultaneously, insulin resistance alters the expression of the ATP-binding cassette transporters ABCG5 and ABCG8, which regulate cholesterol transport from hepatocytes into bile. Enhanced transporter activity increases biliary cholesterol secretion and contributes directly to cholesterol supersaturation, thereby facilitating cholesterol crystal nucleation and gallstone formation. Moreover, insulin resistance disrupts the function of the hepatic transcription factor forkhead box protein O1 (FOXO1), an important regulator of cholesterol homeostasis and high-density lipoprotein (HDL)-mediated reverse cholesterol transport to the liver [94, 98]. Impaired FOXO1 activity compromises hepatic cholesterol handling and further aggravates disturbances in lipid metabolism associated with gallstone disease. Another important molecular regulator affected by insulin resistance is the bile acid-sensitive nuclear receptor farnesoid X receptor (FXR), which serves as a key regulator of cholesterol metabolism, bile acid synthesis, and enterohepatic circulation [23, 76, 77]. Under physiological conditions, FXR maintains cholesterol homeostasis by coordinating bile acid production and regulating the expression of genes involved in cholesterol transport. Activation of FXR also stimulates hepatic expression of the ABCG5 and ABCG8 transporters through interaction with another nuclear receptor, liver X receptor (LXR), thereby integrating cholesterol secretion with bile acid metabolism [78]. Appropriate FXR

activity therefore protects against cholesterol gallstone formation by maintaining balanced cholesterol and bile acid homeostasis. Conversely, impaired FXR signaling contributes to cholesterol supersaturation of bile and increases susceptibility to gallstone formation. This mechanistic understanding has stimulated interest in pharmacological agents capable of modulating FXR activity. One promising compound is 6-α-ethyl-ursodeoxycholic acid (6EUDCA), which has demonstrated potential therapeutic value through regulation of FXR-mediated pathways and restoration of biliary cholesterol balance [99]. Similarly, experimental evidence from animal models suggests that piperine, a naturally occurring alkaloid with cholesterol-lowering properties, may inhibit cholesterol gallstone formation by suppressing the expression of ABCG5, ABCG8, and LXR, thereby reducing biliary cholesterol secretion [100]. Although these findings require further clinical validation, they highlight novel molecular targets for future preventive and therapeutic interventions.

The small intestine also plays a critical role in maintaining systemic cholesterol homeostasis and significantly influences the risk of cholesterol gallstone formation. Intestinal cholesterol metabolism regulates both the absorption of dietary cholesterol and the reabsorption of biliary cholesterol during the enterohepatic circulation of bile acids [101]. The efficiency of these processes varies considerably among individuals because of genetic, metabolic, and environmental influences [102, 103]. Cholesterol transport across the intestinal epithelium depends on the coordinated activity of two opposing transport systems. Cholesterol uptake from the intestinal lumen into enterocytes is mediated primarily by the Niemann-Pick C1-like 1 (NPC1L1) transporter, whereas cholesterol efflux from enterocytes back into the intestinal lumen occurs through the ATP-binding cassette transporters ABCG5 and ABCG8 [101]. The balance between these influx and efflux mechanisms ultimately determines the quantity of cholesterol entering the systemic circulation and subsequently reaching the liver. Genetic variations affecting these transport proteins have provided important insights into the relationship between cholesterol metabolism, cardiovascular disease, and gallstone formation. A large observational study demonstrated that specific polymorphisms in the ABCG5 and ABCG8 genes associated with lower circulating low-density lipoprotein (LDL) cholesterol concentrations confer protection against myocardial infarction while simultaneously increasing the risk of symptomatic cholesterol gallstone disease [106]. These findings suggest that enhanced biliary cholesterol secretion, although beneficial for reducing plasma LDL cholesterol, paradoxically increases cholesterol saturation within bile and promotes gallstone formation. One of the best-characterized variants is the D19H (rs11887534) polymorphism in the ABCG8 gene, which enhances cholesterol transport into bile and thereby increases susceptibility to cholesterol cholelithiasis [45]. Interestingly, despite carrying this polymorphism, patients with gallstones exhibit significantly reduced intestinal cholesterol absorption together with increased or unchanged rates of endogenous cholesterol synthesis [107, 108]. This distinctive metabolic phenotype may precede clinical gallstone formation and could potentially serve as an early marker of disease susceptibility in high-risk populations [107].

Insulin resistance further influences intestinal cholesterol metabolism independently of obesity. Experimental and clinical studies have demonstrated

that insulin resistance reduces intestinal cholesterol absorption while simultaneously stimulating endogenous hepatic cholesterol synthesis [109, 110]. This metabolic adaptation enlarges the hepatic cholesterol pool available for biliary secretion, thereby promoting cholesterol supersaturation and facilitating gallstone formation. These observations further reinforce the concept that cholesterol gallstone disease results from complex interactions among endocrine regulation, hepatic lipid metabolism, intestinal cholesterol handling, genetic susceptibility, and systemic metabolic dysfunction. A detailed understanding of these interconnected mechanisms provides valuable opportunities for developing individualized preventive strategies and targeted therapies aimed at correcting the underlying metabolic disturbances responsible for cholesterol gallstone formation. Experimental investigations have substantially expanded the current understanding of cholesterol gallstone pathogenesis by identifying novel therapeutic targets capable of interrupting key metabolic pathways responsible for cholesterol crystallization. One of the most promising targets is the intestinal cholesterol transporter Niemann-Pick C1-like 1 (NPC1L1), which mediates the absorption of dietary and biliary cholesterol by enterocytes. Animal studies have demonstrated that pharmacological inhibition of NPC1L1 with ezetimibe effectively prevents cholesterol gallstone formation by reducing intestinal cholesterol absorption and limiting the amount of cholesterol delivered to the liver through the enterohepatic circulation [79–81]. As hepatic cholesterol availability decreases, biliary cholesterol secretion is reduced, leading to lower cholesterol saturation within bile and diminished crystal nucleation. These findings indicate that intestinal cholesterol absorption is a critical determinant of biliary cholesterol homeostasis and support the concept that cholesterol gallstone disease may be prevented through targeted modulation of intestinal lipid transport.

Additional evidence from experimental models has identified osteopontin (OPN), a multifunctional cytokine and extracellular matrix-associated glycoprotein, as another important regulator of cholesterol metabolism. Osteopontin is widely expressed in numerous tissues and biological fluids and participates in inflammatory, metabolic, and tissue remodeling processes. Studies utilizing OPN-deficient (OPN−/−) mice fed lithogenic diets have demonstrated a marked reduction in cholesterol gallstone formation compared with wild-type controls [111]. This protective effect appears to result from suppression of NPC1L1 expression within the small intestine, thereby reducing intestinal cholesterol absorption and decreasing hepatic cholesterol delivery. These observations further emphasize the importance of intestinal cholesterol handling in gallstone pathogenesis and suggest that modulation of osteopontin signaling may represent a future therapeutic strategy for preventing cholesterol cholelithiasis. Growing evidence also implicates the intestinal microbiota as a major contributor to cholesterol gallstone disease. Advances in metagenomic sequencing have revealed substantial alterations in gut microbial composition among patients with gallstones compared with healthy individuals. Specifically, patients with cholesterol gallstones demonstrate enrichment of bacteria belonging to the phylum Proteobacteria together with depletion of beneficial genera including *Faecalibacterium*, *Lachnospira*, and *Roseburia* [112]. These microbial alterations are believed to influence cholesterol metabolism, bile acid transformation,

intestinal permeability, and mucosal immune responses, thereby contributing to a pro-lithogenic environment.

Changes in bacterial metabolism of bile acids appear to play an especially important role in this process. The cecal microbiota of patients with gallstones contains increased numbers of Gram-positive anaerobic bacteria possessing enhanced 7α-dehydroxylation activity, an enzymatic process responsible for converting primary bile acids into the secondary bile acid deoxycholate [113]. Deoxycholate is considerably more hydrophobic and lithogenic than primary bile acids, promoting cholesterol supersaturation and crystal formation within bile. Increased concentrations of secondary bile acids may also result from enrichment of the bacterial genus *Oscillospira*, which has been negatively associated with primary bile acid synthesis [114]. Conversely, reduced abundance of the phylum *Bacteroidetes* has been observed in individuals with cholesterol gallstones, suggesting disruption of the normal microbial balance governing bile acid metabolism. Experimental studies further support these observations, demonstrating that mice receiving lithogenic diets develop gallstones alongside significant reductions in microbial richness and alpha diversity, decreased abundance of the phylum *Firmicutes*, and a lower *Firmicutes*-to-*Bacteroidetes* ratio [115]. Collectively, these findings suggest that intestinal dysbiosis contributes significantly to cholesterol gallstone formation through complex interactions involving bile acid metabolism, cholesterol absorption, and immune regulation. Gallbladder motor dysfunction remains one of the central pathogenic mechanisms underlying cholesterol gallstone disease. Numerous clinical investigations have demonstrated that impaired gallbladder contractility represents a major independent risk factor for cholesterol stone formation [44, 116–119]. Patients predisposed to gallstones frequently exhibit increased fasting gallbladder volumes, impaired postprandial contraction, and delayed gallbladder emptying following meal ingestion. These abnormalities prolong bile residence time within the gallbladder lumen, creating favorable conditions for cholesterol supersaturation, crystal nucleation, and progressive stone growth [120–122]. Gallbladder stasis also contributes to disease recurrence following successful extracorporeal shock-wave lithotripsy, oral bile acid dissolution therapy, or combined treatment modalities, emphasizing its importance in both primary and recurrent gallstone disease [123, 124].

Importantly, defective gallbladder motility often precedes the clinical development of gallstones rather than occurring solely as a consequence of stone formation. Approximately one-third of patients demonstrate impaired gallbladder motor function even before detectable gallstones are present, indicating that dysmotility represents an early pathogenic event [120, 125–132]. Experimental studies have shown that these functional abnormalities arise, at least partially, from the toxic effects of unesterified cholesterol accumulating within the plasma membranes of gallbladder smooth muscle cells [133–135]. Cholesterol infiltration into the muscular layer disrupts membrane fluidity and impairs excitation-contraction coupling through inhibition of action potential generation and calcium ion influx [136]. Simultaneously, cholesterol accumulation reduces the density of cholecystokinin-1 receptors (CCK-1R) expressed on smooth muscle cells [137] and disrupts intracellular signal transduction by preventing effective G-protein activation following receptor stimulation [122, 138–143]. Additional pathological

changes include smooth muscle cell proliferation together with chronic inflammatory infiltration of the gallbladder mucosa and lamina propria, both of which further compromise contractile function [119, 144]. Beyond smooth muscle dysfunction, abnormalities involving the intrinsic neural network of the gallbladder further contribute to impaired motility. Histopathological studies have documented significant reductions in neuronal density, enteric glial cells, mast cells, and interstitial cells of Cajal within gallbladder tissue obtained from patients with cholesterol gallstones [145]. Because these specialized cellular components coordinate gallbladder contractility and neuromuscular transmission, their depletion leads not only to impaired postprandial contraction but also to defective relaxation during fasting, thereby disrupting the normal physiological cycle of gallbladder emptying and refilling [146, 147].

Systemic metabolic disturbances also exert important regulatory effects on gallbladder motor function. Although polymorphisms affecting the CCK-1R gene have been associated with increased susceptibility to gallstone disease in some individuals [148], insulin resistance appears to represent one of the most influential systemic determinants of gallbladder dysmotility. Clinical studies have demonstrated impaired gallbladder emptying even in lean, non-diabetic, gallstone-free individuals with insulin resistance, indicating that metabolic dysfunction alone can alter biliary motility before structural gallbladder disease develops [149]. Similar abnormalities have been reported in women with polycystic ovary syndrome, a disorder frequently characterized by significant insulin resistance [150]. Importantly, treatment with the insulin-sensitizing agent metformin has been shown to improve gallbladder contractility [151], while long-term metformin therapy has been associated with a reduced incidence of gallstone disease among patients with diabetes mellitus [152]. These findings reinforce the close relationship between systemic metabolic regulation and gallbladder physiology. Physiological regulation of gallbladder refilling between meals is equally important for maintaining normal biliary function. During the interdigestive period, acid entering the duodenum stimulates release of vasoactive intestinal peptide (VIP), promoting relaxation of gallbladder smooth muscle and facilitating gallbladder refilling. This process is further regulated by fibroblast growth factor 19 (FGF19), known as FGF15 in mice, which is synthesized by the gallbladder epithelium, cholangiocytes, and terminal ileum [153–155]. Bile acids reaching the ileum activate the nuclear receptor FXR, stimulating FGF19 secretion into the portal circulation. FGF19 subsequently binds to the fibroblast growth factor receptor-4 (FGFR4) together with its co-receptor β-klotho in both the liver and gallbladder, inducing smooth muscle relaxation and coordinating gallbladder refilling between meals [119, 153, 156]. Hydrophobic bile acids additionally activate the G protein-coupled bile acid receptor GPBAR-1 located within gallbladder epithelial and smooth muscle cells, promoting relaxation independently of FGF19 through activation of ATP-sensitive potassium (KATP) channels [156–160].

Disruption of these physiological mechanisms governing interdigestive gallbladder motility may substantially contribute to cholesterol gallstone formation. Under normal conditions, rhythmic fasting contractions reduce gallbladder volume by approximately 20% to 30%, preventing prolonged bile stasis [161, 162]. Patients with cholesterol gallstones exhibit impaired interdigestive motility characterized

by fewer migrating myoelectric complex cycles and abnormal motilin secretion compared with healthy individuals [119, 120, 125, 128, 163]. This defect accelerates hepatic secretion of lithogenic bile into the small intestine, enhances enterohepatic recycling of bile acids, increases the hydrophobicity of the bile acid pool, and ultimately promotes cholesterol crystallization and gallstone growth [164, 165]. Animal studies further suggest that cholecystokinin contributes to regulation of fasting gallbladder function through its influence on small intestinal motility. Cholecystokinin-deficient mice exhibit increased fasting gallbladder volume, prolonged intestinal transit time, and enhanced intestinal cholesterol absorption, providing additional evidence that coordinated gastrointestinal motility plays an essential role in maintaining biliary cholesterol homeostasis and preventing cholesterol gallstone formation [166].

Conclusion

Cholesterol gallstone disease is a complex metabolic disorder resulting from interactions among genetic predisposition, abnormal cholesterol metabolism, gallbladder dysmotility, bile supersaturation, inflammatory pathways, and environmental influences. Although laparoscopic cholecystectomy remains the definitive treatment for symptomatic disease, surgery does not correct the underlying metabolic disturbances responsible for gallstone formation. Increasing knowledge of bile acid signaling, cholesterol transport, nuclear receptor regulation, intestinal microbiota, and inflammatory mechanisms has expanded opportunities for targeted therapeutic intervention. Lifestyle modification, including healthy dietary habits, regular physical activity, and maintenance of normal body weight, remains the cornerstone of primary prevention. Emerging pharmacological approaches, including modulation of cholesterol absorption, bile acid metabolism, gut microbiota, inflammatory pathways, and gallbladder function, may provide effective alternatives or adjuncts to surgery in selected patients. Continued translational and clinical research is essential to develop personalized prevention and treatment strategies that reduce disease incidence, recurrence, complications, and the overall healthcare burden associated with cholesterol gallstone disease.

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