Chapter

Pathophysiology of Gallstones

Mela Ram Attri, Irshad Ahmad Kumar, Ferkhand Mohi Ud Din, Ashiq Hussain Raina and Aakarshit Attri

Abstract

Gallstones are the stones developing in the gallbladder. Evolution of pathophysiology changes the trends of treatment of a disease. Laparoscopic revolution was only because of gallstones diseases. The shifting of food habits increased the incidence of diseases in developing countries. There are mainly three types of stones Cholesterol, pigment and brown stones. The pathophysiology of which is different for each type. Cholesterol stones being most common owing to the risk factors being prevalent in the developing and developed societies. Pigment stones being most common in blood disorder patients while brown stones are most common in common bile duct and are infected ones.

Keywords: gallstone, pathogenesis, cholesterol, pigment stones, diet, genes

1. Introduction

The gallstones (**Figure 1**) are hard, pebble-like pieces of material, usually made of cholesterol or bilirubin, that develop in the gallbladder [1]. These stones are formed due to various disorders. Five defects primarily play critical role in pathogenesis of cholesterol stones Viz lithogenes and genetic factors; hepatic hypersecretion of cholesterol; rapid phase transition of cholesterol in bile; impaired gallbladder motility; intestinal factors involving absorption of cholesterol, slow intestinal motility and altered gut microbiota.

Gallstones affect about 15% of United States population. About 10–20% of the United States population will get gallstones at some point in their life time and females are more affected than men [2].

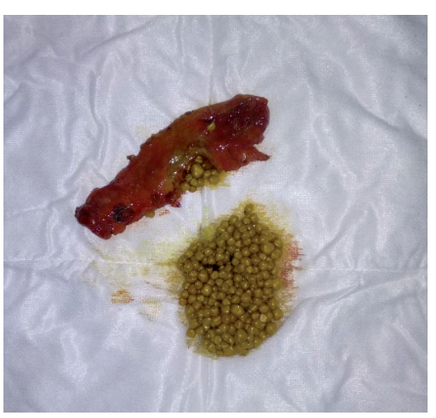

Figure 1.
Cholesterol gallstones and gallbladder after cholecystectomy.

Gallstones can cause biliary colic, cholecystitis, pancreatitis, empyema Gallbladder, perforation of gallbladder, cholangitis, bile duct obstruction [3], and cancers (gallbladder [4], colorectal [5, 6], pancreatic [7]). In 85% of patients with gallbladder cancer, gall stones are present [8]. Gallbladder cancer is a very fatal cancer with average survival of 6 months [9] and 5-year survival rate of 5% [8]. This bad prognosis is because of silent features. An increased mortality from cardiovascular disease and cancer was found to be associated with gallstones by an 18 year national study [10]. Asymptomatic cholelithiasis are present in 80% of cases and most of these are incidentally found when the patients are under investigation for other ailments [11]. In general, 10–25% of asymptomatic cases may develop symptoms during the patient's lifetime [12]. The estimated cost for gallbladder diseases was 6.5 billion dollar annually in United States only in 2002 [13].

There are three types of gallstones; first and most common type is cholesterol stone. Black and brown pigmented stones are the other two types of gallstones. The prevalence of various types of gallstones in the western world is: 75% of gallstones are cholesterol stones, 20% are black pigment stones, and 5% or less are brown pigment stones. Cholesterol and black pigment stones are formed exclusively in gallbladder in a sterile medium, while as brown pigment stones are formed everywhere in the biliary tree owing to the anaerobic bacterial infection. Stasis of bile is an essential component in the formation of gallstone or bile duct stone formation [14]. In underdeveloped countries parasitic infection of biliary tree is associated with brown pigment stones [15].

Physical-chemical origin is the basis of pathogenesis of sterile stones (cholesterol and black pigment stones) [16]. There is an alteration in lipid and lipo-pigment composition which results in the formation of stones. In cholesterol and black pigment stones the major component is cholesterol monohydrate crystals and calcium hydrogen bilirubinate respectively. During the long stay in gallbladder, the black pigment gets degraded and polymerized by free radicals and helps in the formation of black pigment stones while as in the brown pigment stones the main mechanism is infectious where the enzymatic hydrolysis of biliary lipids by anaerobic bacterial enzymes produces supersaturated long chain fatty and deconjugated bile acids [14, 17].

2. Risk factors

Gallstone formation occurs as a result of interaction between genetic and environmental factors in which few are unmodifiable and unaltered as age and genetic makeup. The traditional risk factors for gallstones are four "F" female, fertile, fat, forty and some have added fifth "F" for fair skin [18].

Various intrinsic and extrinsic risk factors for the causation of different types of gallstones are genetic factors, advanced age, female gender, parity, ethnicity, rapid weight loss, different medications (oestrogen replacement therapy, oral contraceptive pills), total parenteral nutrition [19], obesity, westernized diet, Type 2 diabetes mellitus, metabolic syndrome [20], dyslipidaemia, hyperinsulinemia [21], increased enterohepatic circulation of bilirubin [22], defective gall bladder motility [23] as shown in **Table 1**.

2.1 Age and genetics

Age is an important factor for the stone formation, with increase in age enzyme 7 α hydroxylase (Limiting enzyme for bile acid synthesis) activity decreases which increases the saturation of cholesterol in bile and hence increasing the chances of stone formation [24].

Risk factors for gallstone diseases	
Intrinsic factor	**Extrinsic factor**
Age	Lipid profile
Gender	Diet
Ethnicity	Obesity
Defective gallbladder motility	Weight loss
	Physical activity
Genetics	Diseases
	Medication

Table 1.
Risk factors for gallbladder stone diseases.

Various genes are linked with increased susceptibility of gallstones like Lith genes identified in mouse models [25]. Two variants of ATP binding Cassette transporters ABCG5-R50C and ABCG8-D19H [26]; 3 variants of Farnesoid X Receptor gene (FXR) (rs 35724, rs 11110358, rs 11110386) [27]; polymorphism of apolipoprotein E4 allele [28]; mucin genes [29]; fibroblast growth factor receptor 4 (FGFR4) [30], polymorphism in CCK 1-R gene [31] are linked with cholelithiasis. The genetic factors are responsible for 25–30% of symptomatic gallstones as inferred from twin studies [32]. Gene expressions are affected by environmental factors and gene-environmental interactions through epigenetic mechanisms [33] also involving fat storage and insulin resistance [34]. These factors primarily include micro RNAs [35] Viz. 114 miRNA [36], miR-122 [37].

2.2 Gender and ethnicity

Females are having a higher risk factors due to various reasons for cholelithiasis such as higher oestrogen levels naturally [38], multiparity [39] and ingestion of oral contraceptives containing oestrogens [38]. Females also tend to undergo cholecystectomy procedure more than males [40].

Ethnicity plays an important non-modifiable role in cholelithiasis as few ethnic groups are having higher incidence of having gall stones as in North American Indians (73% among women more than 30 years old within Prima Tribe) [14], American Indians (men 29.5%, women 64.1%) [41]. Prevalence of Gallstone and Gallbladder Cancer are co-related and effects certain indigenous population like South America & North India, especially younger population [42].

Risk of deaths from gallbladder cancer and other malignancies has been observed to be seven times more in tribal members with gallstones [43].

2.3 Lipid profile and diet

The co-relation between lipids and cholesterol stone formation is complex, multi factorial and is dependent on other factors also as few studies are in favour and few showing inverse relationship [14]. Few studies show high LDL [44], high cholesterol [45], low HDL [46] are associated with higher gall stone formation which seems to be evident but other studies show there are having inverse relationship with gallstone formation like low HDL, high cholesterol and high LDL [14].

Cholesterol gallstones are associated with western diet [47]. Multiple studies have shown high carbohydrate intake couples with high glycemic load [48], chronic

hypernutrition [49] fibre depleted diet [50] are associated with higher risk for cholelithiasis in process which are associated with decreased risk are intake of in saturated fat [51], coffee [52], fibre [53], fish oil [54], calcium [55], ascorbic acid [56], fresh fruits and vegetables [57] and nuts [51].

2.4 Obesity and weight loss

Obesity is itself risk factor for cholelithiasis. It has been shown that an obese women (BMI ≥ 30 kg/m^2) has two fold and morbidly obese women (BMI ≥ 45 kg/m^2) has seven fold higher risk for cholelithiasis as compared to lean women (BMI < 25 kg/m^2) [58].

Rapid weight loss i.e., >1.5 kg/week and or loss of body weight >25% [59] are risk factors for cholelithiasis which usually occur after bariatric surgery. Weight loss has also been found to reduce risk of gallstones except when rapid loss occurs.

2.5 Physical activity

Physical activity decreases risk for cholelithiasis [60] as it improves hepatobiliary funtions, gut motility [61], increasing HDL [62], improves insulin release and plasma triglyceride levels [63]. An endurance exercise (cycling and running) 5 times per week for 30 minutes daily decreases risk by 34% for cholelithiasis [64] or 2–3 h/week of recreational exercise decrease risk by 20% for cholecystectomy [60]. While as reduced physical activity increases risk for cholelithiasis [64].

2.6 Diseases

Various diseases increase the risk for cholelithiasis as these work by different mechanisms like causing abnormal gallbladder motility, malabsorption of bile salts, decreased bile salt synthesis [65], increasing bile cholesterol saturation [66], supersaturation of bile and increased hepatic cholesterol secretion [67]. Different diseases increasing risk for cholelithiasis viz. metabolic syndrome, dyslipidaemia, diabetes (2–3 fold [68]), insulin resistance or hyperinsulinemia [69], chronic hepatitis C virus [66], liver cirrhosis (25–30% increased risk [65] and Chron's disease [70].

2.7 Alcohol and smoking

Both these habits have controversial results from various studies some favouring and others refuting association with cholelithiasis.

Men consuming alcohol 0–20 g per day have higher risk than those who consume 20–60 g per day [24]. But few studies show severe alcohol abuse in itself and also led to chronic cirrhosis (pigment stones), which is an independent risk factor for cholelithiasis [71].

Cigarette smoking is another risk factor for gallstones among woman [72], while as few studies refute these claims show no association with gallstones [73].

2.8 Intestinal absorption of cholesterol

An imbalance between absorption and synthesis of cholesterol i.e., increased biliary cholesterol secretion from high dietary cholesterol and decreased bile acid synthesis and pool, all driving bile supersaturation [22]. High cholesterol diet and high intestinal cholesterol absorption are two independent risk factors for gallstones [74], regulated by many factors like expression of sterol transport protein [75].

2.9 Gut microbiota

In patients with cholelithiasis there is evidence of altered gut microbiota with increment of intestinal bacterial phylum proteobacteria decrement of *Faecalibacterium* spp., *Lechnospira* spp., and *Roseburia* spp. [76], also increased amount of Gam positive anaerobic bacteria [77].

2.10 Gallbladder motility

Defective gallbladder motility is another risk factor for cholesterol stones [78]. About one-third of patients with cholesterol gallstones display enlarged fasting and post prandial residual gallbladder volume with delayed emptying which antedates gallstone formation [79]. Sufficient time for cholesterol nucleation and gallstone growth is provided by dysfunctional gallbladder motility [80]. Various conditions are associated with dysfunctional gallbladder motility like insulin resistance, diabetes, irritable bowel syndrome, liver cirrhosis, and so on [81]. There is increase in lithogenic bile secretion to small intestine directly from liver in fasting motility defect, leading to faster recycling of bile acids and increasing bile acid pool hydrophobicity [82]. This is another predisposing factor for cholesterol crystallization and cholelithiasis [83].

3. Pathogenesis of gallstones

3.1 Cholesterol gallstones

Various defects occur simultaneously for the nucleation and crystallization of cholesterol monohydrate viz unphysiological supersaturation with cholesterol, accelerated nucleation and gallbladder hypomotility [16]. The mucin glycoprotein hypersecretion follows and lead to the stone formation [84].

Excessive secretion of cholesterol into bile leads to cholesterol supersaturation [85] owing to multiple biochemical defects either from increased input (de novo synthesis, lipoprotein uptake) or decreased disposition (de novo bile salt and cholesteryl ester synthesis) [84]. However single defects in hepatocellular processing of cholesterol are known viz

1. Increased number of apolipoprotein B/E and E receptors (constitutional/oestrogens/rapid weight loss/diet)

2. Increased activity of hydroxy-methyl-glutaryl-CoA reductase (obesity/hypertriglyceridemia/fibric acids)

3. Diminished activity of cholesterol 7α-hydroxylase (constitutional/fibric acids)

4. Diminished activity of Acyl CoA: cholesterol acyltransferase (constitutional/progestogens) [86]

These changes resulting in the increased levels of cholesterol in microsomes [87] and cytosol [88] of hepatocytes in patients with gallstones. Sterol carrier protein 2 concentration also increases simultaneously [89].

At puberty hypersecretion of cholesterol into bile begins [90]. In liver excess cholesterol is delivered to bile by a relay station [91]. Postprandially the cholesterol is completely micellized by bile salts and lecithin during healthy condition [92].

Cholesterol molecules are absorbed by gallbladder mucosa efficiently from micelles of supersaturated bile [93] where the activity of acyl coenzyme A: cholesterol acyl transferase esterifies the sterol [87]. But the molecules that remain free are dissolved in plasma and intercellular membranes where they become intercalated with and stiffen the phospholipid molecules [86]. Reverse diffusion when gallbladder bile become unsaturated or esterification is the only means of escape of membrane cholesterol as gallbladder mucosa does not produce cholesterol [92].

The abnormity in diurnal variation of gallbladder bile due to hypersecretion of cholesterol lead to the further trapping of excess free cholesterol molecules into gallbladder mucosa and muscle membranes [94]. There occurs the divergence of hepatic bile into gastrointestinal tract due to impaired motor and mucosal functions of gallbladder [95]. This in turn lead to increased bacterial catabolism and turnover of primary bile salts [92], which ultimately increase the production of secondary bile salts (hydrophobic bile salts) and suppress de novo bile salt synthesis [96].

Rapid recirculation and hydrophobicity are harmful for the stability of bile as these lead to the augmentation of secretion of cholesterol and hydrophobic lecithin molecules, which in turn shorten the time of nucleation of supersaturated bile [91]. The hydrophobicity of bile salt pool also increases secretion of total proteins into bile [97] and also altering the ratio of pro and anti-nucleating activities for cholesterol crystallization [97]. Multiple stimuli trigger mucin glycoprotein hypersecretion like cholesterol in itself [87], 'cytotoxic' filamentous cholesterol crystals [98], prostanoids [99], and hydrophobic bile salts e. g Deoxycholate and lithocholate conjugates [100]. This lead to the development of mucin gel on gallbladder wall and then as a crescent in gallbladder lumen, setting stage for accelerated nucleation. This together with hypomotility lead to its accumulation as biliary sludge, failure of complete evacuation of which lead to gallstone formation [84]. However, the end result may become inevitable by an environmental perturbation that inauspiciously tips the delicate pathophysiological and physical-chemical balance of supersaturated bile towards nucleation and stone growth [86].

3.2 Pigment gallstones

Black stones occur in sterile environment [101] with increased frequency in patients of chronic haemolysis. A shift in ratio of bilirubin diconjugates to the favour of monoconjugates especially monoglucuronides occur due to hypersecretion of bilirubin conjugates in bile [102]. Bile pigment output increases by 10-fold with haemolysis [102] and predominantly becomes monoconjugates that are more hydrolysed by endogenous β glucuronidase [101]. This gives rise to very high levels of unconjugated pigment exceeding biliary solubility [101]. The insoluble acid calcium salt [Ca $(HUCB)_2$] forms at pH values typical of gallbladder bile [103]. Unphysiological supersaturation occurs with an elevation in the ion product of calcium and monoacid species of unconjugated bilirubin [101] facilitating factors like in cholesterol stones are important for stone formation. The prevalence of pigment stones increases with age and is gender dependent; and all haemolytic patients does not develop pigment stones [101]. In experimental studies it was shown that there is no gallbladder motility defect in black pigment stone formation [104]. However, mucin hypersecretion occurs [105] in response to the high levels of unconjugated bilirubin [106] or mucosal cell cytotoxicity of earliest precipitates [107]. Nucleation is initiated at glandular crypts of gallbladder mucosa where mucin gel accumulates first [107]. Biliary supersaturation (owing to defective acidification of hepatic bile [101] with organic salts occur which is indicated by presence of crystalline calcium carbonate and phosphates in black pigment stones [101]. 3–20% of black pigment stones are composed of mucin glycoprotein [108]. Various factors came into play

and through different mechanisms give rise to pigment stone formation in alcoholic cirrhosis, ileal dysfunction and in aging Viz bile salt hyposecretion, defective solubilization of unconjugated bilirubin, impaired calcium ion binding, haemolysis, defective bilirubin conjugation [101], enteric hyperbilirubinobilia [86].

Chronic anaerobic infection with functional stasis of bile ducts is necessary for brown pigment stones. Stasis in bile duct occur due to secondary stones from gallbladder, or due to sphincter of Oddi dysfunction or parasitic infections (mostly underdeveloped countries) [84]. Commencing with stasis of bile and then followed by anaerobic bacterial infection lead to accumulation of both mucin gel and bacterial cytoskeleton in bile ducts. Bacterial enzymes (phospholipase A, β glucuronidase) hydrolyse the biliary lipids that contain amide or ester linkages which results in the nucleation of cholesterol monohydrate crystals A trap is laid down in the form of culture medium for anaerobic bacteria by mucin gel and solid components making their exit difficult. This is a vicious cycle formed between growing stone, stasis of bile and bacterial infection [84, 109].

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