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Bile Metabolism and Cholelithiasis (A comprehensive review for the monthly publication Clinics of North America)

Stephanie Lambou-Gianoukos
University of Massachusetts Medical School

Stephanie Lambou-Gianoukos, Class of 2008
Department of Gastroenterology

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Stephanie Lambou-Gianoukos, MPH, MSIV and Dr. Stephen J. Heller, MD
Lahey Clinic, Burlington, MA

Background: Autopsies of Egyptian and Chinese mummies have demonstrated the existence of gallstones for at least 3,500 years. Today, gallstones occur commonly, especially in the West and in westernized societies. Gallstone complications include biliary colic, acute cholecystitis, choledocholithiasis, cholangitis, gallstone pancreatitis, and gallstone ileus. Gallstone disease exacts a considerable amount of financial and social burden worldwide. Gallstones lead to frequent physician visits and hospitalizations. In 2000, more than 750,000 outpatient visits in the United States were due to gallstones. In the same year, gallstone disease (defined as cholelithiasis with acute cholecystitis) was the most common inpatient diagnosis among gastrointestinal disorders, with more than 250,000 hospitalizations, a median inpatient charge of $11,584 (Russo, 2004) and an estimated annual cost of almost $6.5 billion. Notably, cholecystectomy is the most common elective abdominal operation performed in the United States, with more than 700,000 performed annually.

Prevalence of gallstones: It has been estimated that in the United States approximately 20 million persons harbor gallstones. Gallstones, cholecystectomies, and gallbladder disease are more prevalent in women than in men at all ages (Everhart, 1999). The prevalence of gallstones varies widely in different countries and among different ethnic groups living in the same country. The highest rates occur among American Indians (Everhart, 1998), especially the Pima Indians of North America, Scandinavians, and Mexican-American women (Maurer, 1989, Everhart, 1999). The lowest rates are seen among African Americans. Although rarer in the non-westernized world, the prevalence of cholelithiasis increased in African (Adedeji, 1986) and Asian countries (Su, 1992) during the 20th century.   For example, the prevalence of gallstones in Tokyo has more than doubled since the 1940s; a shift from pigment to cholesterol gallstones has also been observed. It has been theorized that this increase is due to nutritional and environmental changes, i.e. the westernization of the diet (increased consumption of imported food, decreased fiber and protein intake, increased fat intake) and the decreased rate of chronic biliary infections. The type of gallstone also varies among populations. For example, cholesterol stones (found primarily in the gallbladder) are more prevalent in developed countries, whereas pigment gallstones (found primarily in the bile ducts) are more common in developing countries of Africa and Asia.

Composition of gallstones: All gallstones consist of poorly soluble components of bile that precipitate on a 3-dimensional matrix of mucins and proteins. Precipitants include cholesterol,

calcium bilirubinates, and calcium salts of phosphate, carbonate or palmitate. The matrix consists of large, polymeric mucin glycoproteins and small, amphipathic polypeptides. Based on their composition, gallstones are categorized as cholesterol, black pigment, and brown pigment, with each category having a unique structural, epidemiologic and risk factor profile. The pathogenesis of each type of stone is defined based on the physical-chemical properties of each stone and their differences result mainly from changes in the lipid and lipopigment composition of gallbladder bile.

Bile metabolism: In order to understand the pathogenesis of gallstones, one must be familiar with the role of bile, bile acids and the importance of enterohepatic circulation. Bile is formed in hepatic lobules and is an isotonic fluid whose electrolyte composition resembles that of blood plasma. It is then secreted into a complex network of canaliculi, small bile ductules, and larger bile ducts. Bile acids are carried from the liver through these ducts to the gallbladder, where they are stored for future use. The composition of bile in the gallbladder differs from that of hepatic bile because water and inorganic anions (chloride, bicarbonate) are reabsorbed across the gallbladder epithelium. The solute composition of bile in the gallbladder includes approximately 80% bile acids, 16% phospholipids (mostly lecithin), 4% unesterified cholesterol, and other compounds (conjugated bilirubin, proteins, electrolytes, mucus, rarely drugs and their metabolites). In lithogenic states, the percentage of unesterified cholesterol can reach 8-10% of the total solute composition.

Bile acids are the end products of cholesterol metabolism. Bile acid synthesis is the major mechanism of bodily excretion of excess cholesterol. Since hepatic synthesis can increase only 4-5 times its normal synthetic rate, this mechanism is not sufficient for the excretion of excess dietary cholesterol. Bile acids are detergent-like molecules that can form micelles (molecular aggregates) in aqueous solutions if they are above a critical concentration of 2mM. As a result, they are able to solubilize hydrophobic molecules, such as cholesterol, or emulsify digested fats in the intestine. Regulation of bile salt synthesis is determined by multiple factors, such as the viability of hepatocytes, the availability of cholesterol (the precursor molecule), and the amount of bile salts returning to the liver via the enterohepatic circulation (feedback inhibition by dihydroxy bile salts). Some genetic factors might contribute as well, but they are poorly understood at this point.

As mentioned above, the ultimate fate of bile acids is secretion into the intestine where they aid in the emulsification of dietary lipids, promote the absorption of fat-soluble vitamins, and allow the fecal excretion of excess cholesterol. A small percentage of bile acids is excreted fecally, while the majority is reabsorbed by the intestine and returned to the liver via the portal venous system. This process whereby bile is secreted from the liver, concentrated in the gallbladder, released into the duodenum and finally reabsorbed in the ileum is termed the enterohepatic circulation.

Cholesterol stones: Cholesterol stones are the most common type of gallstones, representing around 80% of gallstones in developed countries. They are mainly composed of cholesterol monohydrate crystals (>50%), as well as calcium salts, bile pigments, proteins, and fatty acids. Grossly, they are often large (up to 4.5 cm in size) and yellowish-white in color. Microscopically, they appear as long, thin crystals that are bound together by a matrix of mucin glycoproteins. Cholesterol stones are typically found in the gallbladder in a bacterially sterile environment. Cholesterol is essentially insoluble in aqueous solution, such as bile. As a result, cholesterol in bile is transported either in unilamellar bilayered vesicles (cholesterol complexed with phospholipids, mostly lecithin) or in mixed multilamellar micelles (cholesterol complexed with phospholipids and bile acids). The total and relative proportions of cholesterol to phospholipids and bile salts determine the solubility of free cholesterol in bile. When cholesterol concentration exceeds its solubility, cholesterol crystals can precipitate in bile, eventually giving rise to gallstones.

Cholesterol crystal formation requires the presence of one or more of the following factors: a) cholesterol supersaturation, b) accelerated nucleation, and c) gallbladder hypomotility/bile stasis. Many risk factors for the formation of gallstones have been identified and studied. These myriad risk factors underscore the multifactorial genesis of gallstone formation. These risk factors include: age, gender, obesity, weight loss, total parenteral nutrition, genetics (first degree family member), pregnancy, diet, ileal disease (Crohn's disease), hypertriglyceremia, low HDL, diabetes, and drugs (estrogen, progesterone, ceftriaxone, and octreotide).

Pigment stones overview: In the United States, pigment stones comprise around 20% of the total gallstones, but this percentage is much higher in Asian populations. Pigment stones are subclassified into black and brown types, each with unique morphology, pathogenesis and clinical associations. Generally, the prevalence of pigment stones increases with age and is higher in women. As their name implies, pigment stones are formed by the precipitation of bilirubin in bile. This can occur as a consequence of an increase in ionized calcium concentration (as in hyperparathyroidism) or an increase in unbound bilirubinate anions in bile.

Bilirubin metabolism: One cannot comprehend the pathogenesis of pigment stones without understanding the role of bilirubin and its subsequent metabolism in the liver. Bilirubin is the breakdown product of normal heme catabolism from destroyed erythrocytes. Like cholesterol, it is insoluble in water. In the liver, it is conjugated with glucuronic acid producing diglucuronides (75-80%) and monoglucuronides (20%), which are soluble in water and can be secreted into bile. Normally, the remainder of the bilirubin that reaches the liver (around 3%) is hydrolyzed by beta-glucuronidases and becomes unconjugated. Unconjugated bilirubin and its calcium salts are poorly soluble in water.   In a healthy individual, pigment stones are not formed because the amount of unconjugated, and thus insoluble, bilirubin is not enough to promote stone formation. In abnormal states, though, the excessive amount of unconjugated bilirubin becomes an important factor in pigment gallstone pathogenesis.

Black pigment stones: Black pigment stones are composed primarily of pure calcium bilirubinate, but they also contain calcium carbonate and calcium phosphate (in polymer-like complexes with mucin glycoproteins). They are formed in the gallbladder in a bacterially sterile environment. Black stones are found in people with chronic hemolytic states (i.e. sickle cell disease, hereditary spherocytosis), liver cirrhosis, Gilbert's syndrome, or cystic fibrosis. Patients with ileal disease (i.e. Crohn's disease) or ileal resections are also predisposed to pigment stones.

The pathogenesis of black stones involves two mechanisms: the hypersecretion of bilirubin conjugates and a defect in the acidification of bile. In the presence of chronic hemolysis, the concentration of bilirubin conjugates (especially monoglucuronides) increases ten-fold from the action of the endogenous enzyme beta-glucuronidase. The bilirubin conjugates are then unconjugated, form salts with calcium or phosphate, and eventually precipitate. The inability of an inflamed gallbladder mucosa to acidify bile may be an additional factor in pigment gallstone formation. By increasing the solubility of calcium carbonate, an acidic bile pH promotes the supersaturation of bile with calcium cations and allows the precipitation of calcium salts. To date, no defects in gallbladder motility have been found in patients with black stones (Behar, 1989).

Brown pigment stones: Brown pigment stones are composed of calcium salts of unconjugated bilirubin with varying amounts of cholesterol and protein. They are formed as the result of chronic bacterial infection of the bile and are almost always associated with colonization of bile by enteric organisms. The most common bacteria found in brown stones are *Escherichia coli, Bacteroides,* and *Clostridium*. In populations that are prone to pigment stones, a clear shift to cholesterol gallstones has been observed. This shift has been attributed to the decrease in chronic biliary infections. For example, the percentage of pigment stones in the Japanese population decreased from 60% to 24% since 1940.   Unlike the other two types of stones, they are primarily found in the intrahepatic or extrahepatic bile ducts. Rarely, they are formed in the gallbladder as a consequence of acute cholecystitis.

Brown pigment stones are caused by an excess of unconjugated, insoluble bilirubin in bile that eventually forms stones. The pathogenesis of these stones is thought to involve both stasis in the bile ducts as well as chronic anaerobic infection of bile (Cahalane, 1988). Stasis facilitates the bacterial infection, which in turn promotes the accumulation of both mucin and bacterial cytoskeletons in the bile ducts. The hydrogen ions in the bile are buffered by the mucus, resulting in a less acidic environment where calcium carbonate, phosphate, and bilirubin precipitate easily. The three bacterial compounds that play a key role in brown stone formation are beta-glucuronidase (which produces unconjugated bilirubin), phospholipase A (which produces palmitic and stearic acids), and bile acid hydrolases (which produce unconjugated bile acids). The anionic counterparts of all three products form insoluble complexes with calcium and precipitate to form stones. The enlarging stone causes further ductal obstruction, which promotes more stasis and bacterial infection, thus perpetuating the cycle.

Also, the association of certain parasitic infections and biliary stone formation has been well documented in the literature (ex. *Opisthorchis veverrini, Clonorchis sinensis* – liver flukes prevalent in Thailand and China respectively).   Although the exact mechanism of how parasite infections enhance pigment stone formation is not clearly understood, it is thought that the parasitic worm or egg directly stimulates stone formation. The calcified overcoat of the parasitic egg, for example, may serve as a nidus and may enhance the precipitation of calcium bilirubinate (Sripa, 2004).