A Comparative Study of Biliary and Serum Lipid Profiles in Patients of Obstructive jaundice due to stones in common bile duct

Authors

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Abstract
The prevalence of cholesterol gallstones and common bile duct (CBD) stones in India is very high depending on dietary patterns and ethnic background. It is now widely accepted that the primary event in the pathogenesis of cholesterol gallstones is an altered lipid metabolism giving rise to a greater proportion of cholesterol relative to other bile lipids secreted from the liver into bile. The association of cholesterol super saturation of bile with cholesterol gallstones paved the way to a physical-chemical basis for gallstone formation. It however, soon became clear that other factors including nucleation of cholesterol crystal, binding together of these crystals with mucin, and hypo motility of the gall bladder played an equally important roles in gallstone formation.

In the present study bile and serum were collected from 46 choledocholithiasis patients during cholecystectomy at the Nalanda Medical College Hospital, Patna, India. Of the 46 bile samples collected, the male to female ratio was 1:6.67 (6men and 40women). Between 19 and 88 years of age, peak incidence of choledocholithiasis was at age 40. Serum and biliary lipid profile was done by enzymatic methods. Serum lipid profile was done for 19 healthy controls of both sexes aged between 25 and 55 years. Results showed significant elevation in both serum high density lipoprotein (HDL) and low density lipoprotein (LDL) when compared with controls. Biliary cholesterol, HDL and LDL was raised significantly when compared with serum samples. There is correlation between serum and biliary lipid profiles in causing common bile duct (cbd) stones in Indian population. Both serum and biliary HDL consistently showed higher values in Indian patients.

Keywords: HDL: High density lipoproteins LDL: Low density lipoproteins CSI: Cholesterol saturation index LI: Lithogenic index.

Introduction
In India, cholesterol choledocholithiasis is a highly prevalent disease, and this is attributed to the dietary habits and ethnic background. Approximately, 20% of subjects with CBD stones become symptomatic and require treatment, which
currently involves the surgical removal of the CBD stones (choledochotomy). About 10% of these patients undergoing choledochotomy have diabetes[1]. It is now widely accepted that the primary event in the pathogenesis of cholesterol gallstones is an altered lipid metabolism because of which there is a relative increase in the cholesterol levels compared to other lipids secreted by the liver into the bile[2]. Alterations in the lipid metabolism may arise as a result of a combination of various factors such as excess dietary cholesterol/fat, obesity, diabetes and genetic causes. Genetic aspects are exemplified by studies conducted in North American Indians[3] and Caucasian family members of affected individuals [4–7].

The association of cholesterol super saturation of bile with cholesterol gallstones paved the way for the physical-chemical basis for gallstone formation. However, it soon became evident that other factors including nucleation of cholesterol crystals, binding together of these crystals with mucin, and hypomotility of the gallbladder also played an equally important role in gallstone and CBD stone formation. Although the molecular events that underlie these processes are far from clear, many loose ends are being tied. One knot recently tied is that an increase in biliary arachidonyl lecithin may lead to increased prostanoid synthesis which may be responsible for increased mucin secretion as well as gallbladder hypomotility [8, 9]. Individuals are predisposed to cholesterol gallstones if their bile has an increased proportion of cholesterol, relative to its two more hydrophilic lipids, bile acids (salts) and phospholipids. This relative proportion of lipids is known as the cholesterol saturation index (CSI) or lithogenic index (LI) and is the major indicator determining whether bile is over-saturated with cholesterol (CSI>1.0) or within desirable levels (CSI<1.0). Cholesterol saturation index of >1.0 is a prerequisite for cholesterol gallstone/CBD stone formation. Thus, cholesterol CBD stones cannot develop if the CSI is <1.0, whereas they are frequently formed if the CSI is >1.0 [10,11].

Cholesterol solubilization in conjugated bile salts system is relatively lower than unconjugated bile salts system with or without added calcium; however co-existing bilirubin minimizes these differences. The pH-dependency of cholesterol and bilirubin solubilization is less in conjugated bile salts system. On the contrary, it is remarkably greater in unconjugated bile salts-calcium system [12].

Materials and Methods
Bile samples were collected from 46 choledocholithiasis patients during cholecystectomy at the Nalanda Medical College Hospital, Patna, India. Of the 46 samples collected, 6 were from male patients and 40 were from female patients, with the male to female ratio being 1:6.67. The patients were aged between 19 and 88 years, with a peak incidence of choledocholithiasis at 40 years. The total cholesterol levels were estimated by enzymatic colorimetric method[13], total bilirubin levels were estimated by colorimetric method of Accurex Biomedical Pvt. Ltd[14], and triglyceride levels were measured by enzymatic colorimetric method [15]. This study was approved by the Ethics Committee of the Nalanda Medical College, Patna, India.

Results
The triglycerides in serum and bile were not altered in the choledocholithiasis patients, but the serum high density lipoprotein (HDL) and low density lipoprotein (LDL) were increased (p<0.01) when compared with controls (see Table 1). Furthermore, cholesterol (p<0.01), HDL (p<0.01) and LDL (p<0.01) were significantly elevated in the bile when compared with serum. Among male patients, there was a significant increase in the total cholesterol (p<0.05) and HDL levels (p<0.001) in the bile when compared with serum. No alterations were observed in the other components of the bile as well as the serum lipid profile. Female patients in the study were divided into two groups: ≤40 years of age and >40 years of age.
the group ≤40 years of age, HDL (p<0.001) and LDL (p<0.001) levels in the serum, and total cholesterol (p<0.001), HDL (p<0.001) and LDL (p<0.001) levels in the bile were significantly increased (see Table 3).

In female patients aged >40 years, serum HDL (p<0.001), and biliary cholesterol (p<0.001) and HDL (p<0.001) were significantly elevated while biliary LDL (p<0.05) was only marginally increased (see Table 4).

Table 1: Lipid profile of the total population studied

|          | TG     | TC     | HDL   | LDL   |
|----------|--------|--------|-------|-------|
| Controls (Serum) | 110±20 | 165±35 | 45±15 | 90±20 |
| Cases (Serum)     | 123.30±40.5 | 185.89±48.77 | 60.86±22.01* | 108.2±35.09* |
| Cases (Bile)       | 111.5±55.54 | 250.5±57.30* | 101.89±51.23* | 134.34±42.19* |

*- P < 0.001

Table 2: Lipid profile of the male subjects

|          | TG     | TC     | HDL   | LDL   |
|----------|--------|--------|-------|-------|
| Controls (Serum) | 110±20 | 165±35 | 45±15 | 90±20 |
| Cases (Serum)     | 128.33±52.42 | 190.50±53.64 | 54.82±16.87 | 108.33±43.28 |
| Cases (Bile)       | 81.16±22.88 | 225.66±47.42* | 100.16±14.82** | 118.66±21.03 |

*p < 0.05 ** p < 0.001

Table 3: Lipid profile of females ≤40

|          | TG     | TC     | HDL   | LDL   |
|----------|--------|--------|-------|-------|
| Cases (Serum) | 122.76±36.66 | 194.20±59.15* | 64.28±26.15** | 115.58±40.12** |
| Cases (Bile)       | 113±53.61 | 252.36±66.33** | 107.24±31.50** | 146.36±43.82** |

*p < 0.05 ** p < 0.001

Table 4: Lipid profile in females >40 years

|          | TG     | TC     | HDL   | LDL   |
|----------|--------|--------|-------|-------|
| Cases (Serum) | 122±45.31 | 171.8±17.32 | 57.6±15.71** | 96.6±17.54 |
| Cases (Bile)       | 121.13±66.16 | 258.73±43.20** | 93.66±35.09** | 120.60±40.30* |

*p < 0.05 ** p < 0.001

Discussion

Gallstone and CBD stone disease is prevalent in about 10–15% of adults in the developed countries, and is one of the most common and most expensive conditions to treat of all digestive disorders requiring admission to hospital [16, 17]. Of all CBD stones found during choledochotomy, cholesterol stones account for 80–90% [18]. Cholesterol stones are primarily made up of cholesterol crystals (70%) which are held together in an organic matrix of glycoproteins, calcium salts, and bile pigments. They could be present either singly or multiply, in various sizes, shapes (spherical/oval) and surfaces (smooth/rugged) [19]. The etiology of cholesterol stones is considered to be multifactorial, with interaction of genetic and environmental factors [20].

There are various steps involved in the formation of cholesterol stones and each of these steps is influenced by genetic and/or environmental factors [20]. The first step in the formation of gallstones and CBD stone is the secretion of bile supersaturated with cholesterol by the liver. The second step in gallstone and CBD stone formation is crystallization. The precipitation of cholesterol crystals initiates the formation of gallstones. When the gallbladder bile becomes abnormally supersaturated with cholesterol, nucleation, flocculation and precipitation of cholesterol crystals occur, leading to the initiation of gallstone formation. The excessive presence of promoters of crystallization and relative deficiency of inhibitors of crystallization are also important in the initiation of nucleation and crystal formation. The promoters and inhibitors are mostly proteins such as mucous glycoproteins. The growth of the crystals to macroscopic stones is further facilitated by the gallbladder mucus [21]. Patients with
cholesterol gallstones may have defects resulting in the production of abnormally supersaturated bile because of an increase in the secretory rate of biliary cholesterol or decrease in the secretory rate of biliary bile salts, lecithin and phospholipids. Changes in the concentration of one of the key promoters of crystallization, mucus glycoprotein, are mediated by mucosal prostaglandins (PGs). Aspirin and nonsteroidal anti-inflammatory drugs prevent microcrystal and gallstone formation by decreasing PG synthesis, especially in obese people on weight-reduction diets. Gallbladder motor dysfunction and stasis also contribute to gallstone formation. Intrahepatic cholestasis is associated with dyslipidemia, which might also contribute to the pathogenesis of the disease.

The major risk factors for cholesterol gallstone/CBD stone disease are age, female gender and parity. However, there are several other risk factors involved too, such as postpartum, estrogen-replacement therapy, oral-contraceptive use, and rapid weight loss. The risk of cholesterol gallstone disease increases with age, obesity, type 2 diabetes, dyslipidemia (hypertriglyceridermia and low HDL levels), hyperinsulinemia, and sedentary lifestyle, similar to atherosclerosis.

The results of our study carried out in an Indian Population of Patna (Bihar) clearly indicate a higher incidence of cholesterol gallstones in this population with females comprising of 85% of the total patients affected. The incidence of cholesterol gallstones, although less in the male population, was probably related to sedentary lifestyle and consumption of high calorie diet, particularly rich in animal fats, refined sugars, and alcohol and poor in vegetable fats and fibers, all of which are significant risk factors for gallstone formation. Diets rich in saturated fatty acids also increase the risk of gallstone formation whereas diets with substituted mono or poly unsaturated fatty acids reduce the risk. A high intake of cis-unsaturated fats has been demonstrated to reduce the risk for gallstone disease in men. In the West, consumption of a high calorie diet is more common and is clearly an important factor in the formation of cholesterol gallstones. This wave has gradually spread even to the East Asian countries, with dietary habits becoming unhealthier. India also has not been spared, as seen in our study.

Cholesterol saturation of the bile is one of the prerequisites for the formation of cholesterol gallstones, and as already discussed, the CSI is an important determinant for this. Women with gallstones have a higher CSI of the bile than women without gallstones with the same level of cholesterol in the blood. In this study, patients >40 years had high cholesterol and normal triglycerides whereas both these components were elevated in gallstone patients in an Indian study.

Conclusion
The present study clearly demonstrates a correlation of serum and biliary lipid profiles with common bile duct and gall bladder stones in Indian population. But unlike in other studies, both serum and biliary HDL were consistently higher in this study population. Females over 40 years of age, especially those with a CSI>1 in the bile are the most susceptible to developing gallstones. Although the incidence of gallstones is less among men, those with poor dietary habits are the ones who are more prone to developing gallstones. Considering that genetic and environmental factors have a major role in the formation of cholesterol gallstones/CBD stones, it would be prudent for susceptible individuals and families to take adequate preventive measures, mainly in terms of watching their diet, and keeping a check on their cholesterol levels.

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