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Phospholipid and Cholesterol Concentration in the Bile of Cholelithiasis Patient in SRHF Mizoram, India.

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ABSTRACT

Background: The purpose of the study was to estimate quantitatively the phospholipids and cholesterol concentration of the gallbladder and hepatic bile and find out the possible role of their variation in the pathogenesis of cholelithiasis. **Methods:** The study comprised of 100 patients with cholelithiasis who were operated in the department of General Surgery in the State Referral Hospital, Falkawn (SRHF), Mizoram, India, during the period of June 2016 to May 2018. A group of 20 subjects undergoing surgery for conditions other than hepatobilliary diseases constituted the control group. **Results:** The value of total cholesterol and phospholipids obtained from the gallbladder bile of study was 226mg% and was 608mg% respectively and the ratio being 2.69. The value of total cholesterol and phospholipids obtained from hepatic bile of study was 121mg% and was 197mg% respectively and the ratio being 2.45, whereas the total cholesterol and phospholipids value obtained from the gallbladder bile of control group was 259mg% and 1077mg% respectively and the ratio being 4.16. **Conclusion:** The biochemical study shows that the phospholipids to cholesterol ratio are reduced in gallbladder and hepatic bile of patients with cholelithiasis as compared with normal subjects. This reduced ratio is found to be mainly due to decreased level of phospholipids rather than increased level of cholesterol in the bile of gallstone.

Keywords: Phospholipids, Cholesterol, Cholelithiasis, Gallbladder Bile, Hepatic Bile.

INTRODUCTION

The biochemical alterations in bile are considered as one of the most important factors in the aetiopathogenesis of gallstones. A significant decrease in phospholipid/cholesterol ratio in the gallbladder and hepatic bile was noticed in patients of cholelithiasis as compared to normal subjects. The reduction in the phospholipids/cholesterol ratio is mainly due to a decrease in the phospholipids values rather than any rise in the cholesterol levels.[1] They considered alteration in the phospholipids level to be more important than those of other lipids in the formation of gallstones.[2]

The three major constituents of bile, making over 90% of the dry weight are bile salt, phospholipids and cholesterol. The common primary (cholates and chenodeoxycholates) and secondary (deoxycholaes and lithocholates) biles salts are water-soluble detergents-like molecules that form small aggregegates in aqueous solutions called micelles. Lecithin, the predominant phospholipids in bile, is insoluble in aqueous systems but swells to form liquid crystals. Cholesterol, totally insoluble in aqueous systems, is incorporated into the lecithin liquid crystal, and bile salts chop up these insoluble liquid crystals into small aggregates called mixed micelles. The capacity of the mixed micelles to solubilize cholesterol is related to the relative amounts of both lecithin and bile salts.[3]

Gallstones are classified into Cholesterol, black pigment and brown pigment stones. Cholesterol stones are the most common and contain 51-99% pure cholesterol. Black pigment stones are largely composed of an insoluble bilirubin pigment polymer mixed with calcium phosphate and carbonate. Brown pigment stones contain calcium bilirubinate, calcium palmitate and calcium stearate, as well as cholesterol. Brown pigment stones are rare in the gallbladder-they form in the bile duct and are related to bile stasis and infected bile.[4] Cholesterol and Phospholipids synthesized in the liver are the principal lipids found in bile. The synthesis of phospholipids and cholesterol by the liver is in part regulated be bile acids. The color of the bile is due to the presence of pigment bilirubin diglucuronide which is the metabolic product from the breakdown of haemoglobin, and is present in bile in concentration 100 times greater than in plasma. Gallstones form as a result of solids setting out of solution. The major organic solutes in bile are bilirubin, bile salts, phospholipids and cholesterol. In western countries, about 80% of gallstones are

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cholesterol stones and about 15-20% are black pigment stones. Brown pigment stones account for only a small percentage. Both types of pigment stones are more common in Asia. Asian countries like Japan, black stones account for a much higher percentage of gallstone than in the western hemisphere.

Cholesterol is secreted into bile as cholesterol-phospholipids vesicles. Cholesterol is held in solution by micelles, a conjugated bile salts, phospholipids-cholesterol complex, as well as by the cholesterol-phosholipids vesicles. The presence of vesicles and micelles in the same aqueos compartment allows the movement of lipids between the two. Vesicular maturation occurs when vesicular lipids are incorporated in micelles. Vesicular phospholipids are incorporated into micelles more readily than vesicular cholesterol. Therefore, vesicles may become enriched in cholesterol, become unstable and nucleate cholesterol crystals. About one third of biliary cholesterol is transported in micelles, but the cholesterol-phospholipids vesicles carry the majority of biliary cholesterol.[5]

MATERIALS AND METHODS

The study was carried out in the department of General Surgery in collaboration with the Department of Biochemistry, State Referral Hospital of Mizoram Institute of Medical Education and Research, Falkawn, Mizoram, India, during the period of June 2016 to May 2018. A total of 100 patients with cholelithiasis who were operated in the Department of General Surgery were included in the study group. A group of 20 subjects undergoing surgery for conditions other than hepatobilliary diseases constituted the control group. All the patients in both the group were subjected to detailed history regarding name, age, sex, religion, address, parity, family history and clinical examinations. A pre-designed proforma was used to record the details of the patients who were included in the study. A bile sample was collected from the common hepatic duct during operation by fine needle aspiration (insulin syringe) after occluding the cystic duct, and gallbladder bile was collected from the removed gallbladder specimen. In control group, the bile was collected from gallbladder and common hepatic duct during operation by fine needle aspiration(insulin syringe). The following estimations were done on the same day of the operation.

Total cholesterol was measured by the enzymatic end point calorimetry method as described by Allain CC et al., 1974. About 5-10 times dilution is necessary in case of bile. An enzymatic method is described for total bile cholesterol by use of a single aqueous reagent. Cholesterol esterase hydrolyzes cholesterol ester. Free cholesterol is oxidized by the cholesterol oxidase to cholesterol 4-en-3-one and hydrogen peroxide. Hydrogen peroxide formed reacts with 4-amino antipyrine and phenol in the presence of peroxidase to produce pink colored quinoneimine dye. The intensity of the color produced is proportional to the cholesterol concentration. In the manual assay of total cholesterol by the present method, 10microlitre of the diluted bile is incubated with 1.0ml of reagent at 37oC for 5 minutes or at room temperature for 15 minutes. The absorbance on a photocalorimeter with green filter or on a spectrophotometer at 505nm of the test and standard is measured against a reagent blank.

The determination of phospholipids was done by Fiske and Subbarow, 1925 as modified by Youngburg and Youngburg, 1930. The lipids are extracted in to an ethanol-ether mixture and digested with sulphuric acid and hydrogen peroxide as introduced by Youngburg and Youngburg (1930). The phosphorus, now present as phosphate, is then determined calorimetrically by a method for inorganic phosphorus.

RESULTS

Hundred cases of cholelithiasis were studied. The study was conducted in the Department of General Surgery and Biochemistry, State Referral Hospital, Falkawn, Mizoram, India, during the period from June 2016 to May 2018. The value of total cholesterol obtained from the gallbladder bile of study group ranged from 98mg% to 360mg% with a mean value of 226, the value of phospholipids obtained from the gallbladder bile of study group ranged from 212mg% to 1038mg% with a mean value of 608, whereas the mean value of cholesterol and phospholipids in the gallbladder bile of normal group was 259mg% and 1077mg% respectively as shown in the [Table 1]. The ratio of Phospholipids/Cholesterol in the gallbladder in the study group was 2.69 whereas in the normal group was 4.16.

Table 1: The total cholesterol and phospholipids level of gallbladder bile.

Groups	No. of cases	Phospholipids	Cholesterol	Ratio
Normal	20	1077mg%	259mg%	4.16
Study	100	608mg%	226mg%	2.69

The value of total cholesterol obtained from the hepatic bile study group ranged from 78mg% to 252mg% with a mean value of 121mg%. The value of phospholipid obtained from the hepatic bile of study group ranged from 191mg% to 595mg% with a mean value of 297mg%, whereas the mean value of cholesterol and phospholipids in the hepatic bile of normal group was 105mg% and 351mg% respectively as shown in [Table 2. The ratio of Phospholipids/Cholesterol in the gallbladder in the study group was 2.45 whereas in the normal group was 3.34.

Table 2: The total cholesterol and phospholipids level of hepatic bile.

Groups	No. of cases	Phospholipids	Cholesterol	Ratio
Normal	20	351mg%	105mg%	3.34
Study	100	297mg%	121mg%	2.45

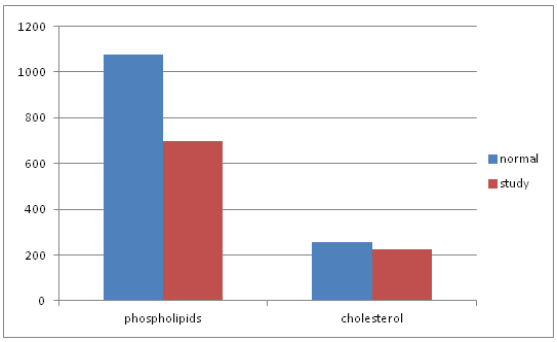

Figure 1: Phospholipids and cholesterol concentration in the gallbladder bile samples in the normal and cholelithiasis patients.

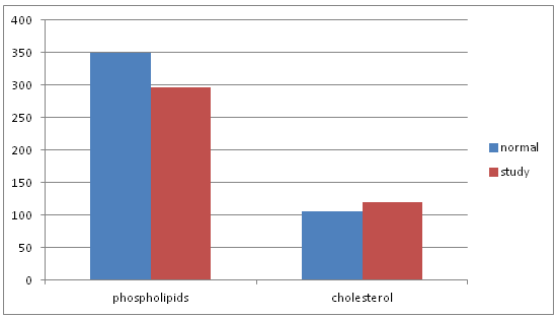

Figure 2: Phospholipids and cholesterol concentration in the hepatic bile samples in the normal and cholelithiasis patients.

DISCUSSION

Phospholipids and cholesterol have been quantitatively estimated from the gallbladder bile of patients with cholelithiasis and in normal subject. Direct samples of pure bile were obtained at operation. The phospholipids/cholesterol ratio in the gallbladder bile of normal group was 4.16. This ratio was reduced to 2.69 in those patients with cholelithiasis. The phospholipids/cholesterol ratio in the hepatic bile of normal group was 3.34, this ratio was reduced to 2.45 in those patients with cholelithiasis. The reduction in phospholipids/ cholesterol ratio was mainly due to a decreased in the phospholipids values rather than any rise in the cholesterol level as reported by Tompkins RK et al (1970). Lewis KO (1973) considers alteration in phospholipids level to be more important than those of other lipids in the aetiopathogenesis of gallstones. This would seem to be borne out by our findings. Normal gallbladder has a remarkable concentrating power and approximately 90% water is removed in this process (Dam H et al, 1966).[6] It was found that the concentrating power of gallbladder suffered as the disease progress. Changes in the wall of the gallbladder similar to that of chronic cholecystitis (as reported by Udupa KN et al, 1968)[7] may affect the concentrating power of the gallbladder resulting in reduced levels of its constituents. The role of phospholipids as a cholesterol solubilizing factor was indicated by Neiderheiser DH et al (1966) and later by Tompkins RK (1967).[8] The significant decrease in the phospholipids/cholesterol ratio in patients with cholelithiasis in gallbladder bile points to the relative decrease of phospholipids as a factor of importance in the precipitation of cholesterol. The study made by different authors was comparable as in the study made by Talwar BL et al (1978).[9] The reduction in the phospholipids – a cholesterol solubilizing factor, in patients with cholelithiasis as compared to normal suggests its importance in the aetiopathogenesis of gallstone.

Kanwar R.S et.al (1996),[10] estimated Cholesterol and phospholipids in serum and bile from hepatic duct and gallbladder of twenty five patients of gallstone with functioning gallbladder (Group-I) and an equal number of patients having diseases other than of hepatobiliary system acting as control (Group-II). Group-I patients showed high serum cholesterol and low serum phospholipid levels as compared to those of Group-II. Cholesterol levels in hepatic duct and gallbladder bile were higher in Group-I than in Group-II whereas the phospholipid levels in the bile of Group-I were lower than in Group-II. The phospholipid: cholesterol ratios in hepatic duct and gallbladder bile of Group-I were 2.76 and 3.03 respectively as compared to 5.62 and 5.92 in Group-II. This study corresponds to our findings.

CONCLUSION

This study shows that the phospholipids to cholesterol ratio are reduced in both gallbladder and hepatic bile of patients with cholelithiasis as compare with normal subjects. This reduced ratio is found to be mainly due to decreased level of phospholipids rather than increased level of cholesterol in the bile of gallstone.

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