STUDY OF SERUM AND BILE FROM PATIENTS OF CHOLELITHIASIS IN NORTH-EASTERN INDIA

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ABSTRACT

Biochemical analysis of serum and bile from patients with cholelithiasis hailing from north-eastern India was done to provide pointers for lithogenicity. In comparison to controls, patients with cholelithiasis showed significant increase in serum phospholipid levels and lecithin cholesterol acyl transferase activity was significantly decreased. Enzymes like gamma glutamyl transferase, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase showed a small though significant increase as compared to controls, suggesting evidence of subclinical hepatic dysfunction and probable evidence of relative bile stasis. Levels of cholesterol, lecithin and lecithin cholesterol acyl transferase activity were seen to be increased in gall bladder bile as compared to hepatic duct bile.

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KEYWORDS : Cholelithiasis; Bile; Biochemistry.

Introduction

Cholelithiasis in India shows a definite geographical predilection. Published data suggest a very high incidence in the states of Jammu & Kashmir, Punjab, Haryana, Uttar Pradesh and Bihar. Isolated reports from Southern states reveal a very low occurrence [1]. However, no data is available for the country as a whole. The etiology of gall stone formation appears to be complex. Hepatic synthesis and excretion of bile acids, cholesterol, phospholipids and alteration during gall bladder storage possibly play a role in this process. Stasis, infection, inflammation, obstruction are local factors which may modify lithogenicity. Certain risk factors like heredity, age, sex, diet, parity and drug intake are known to contribute to gall stone formation [2-4].

In a hospital located in north-eastern India we found cholelithiasis to be a major problem. This study was undertaken to analyse abnormalities of serum and bile from patients of cholelithiasis hailing from north-eastern

states of India.

Material and Methods

The study was carried out during the period June 1992 to December 1993 on 78 consecutive patients of cholelithiasis hailing from a geographical area comprising the districts of Golaghat, Jorhat, Sibsagar and Dibrugarh of Assam and the adjoining areas of Nagaland and Arunachal Pradesh. Examination of serum and bile was done in 50 cases. The controls were 25 healthy individuals hailing from non lithogenic areas of the country with no history of cholelithiasis and no evidence of haemolytic disorder or hepatobiliary disease.

Sera were collected from patients a day prior to surgery. Total cholesterol, alkaline phosphatase (AP), serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum gamma glutamyl transferase (GGT) were estimated using a commercially available reagent kit (Ames) and autoanalyser (RA 50, Miles India). Results of AST, ALT, GGT and AP were

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expressed in IU/L. Ester cholesterol was determined by precipitating free cholesterol in the serum by digitonin [5]. After centrifugation ester cholesterol in the supernatant was estimated. To determine lecithin cholesterol acyl transferase (LCAT) activity fresh serum was tested for free and esterified cholesterol. The serum was then incubated for 5 hours at 37°C and ester cholesterol redetermined. The difference in the ester concentration divided by 5 gives the LCAT activity of the serum (in mg/hr) [5].

The procedure followed for phospholipid determination was as described by Connerty et al [6]. Results were expressed as serum phospholipid phosphorous (mg/dl). Serum phospholipid phosphorous when multiplied by 25 gave serum lecithin levels. Statistical analysis was done by the Student's t test.

The samples of bile from gall bladder and CBD were collected using sterile precautions. They were plated on freshly prepared blood agar and cystine lactose electrolyte deficient (CLED) media, incubated both aerobically and anaerobically at 37°C for 48 hours. Biliary AP, bilirubin, cholesterol, LCAT activity and phospholipids were determined as previously described. In only 50 cases biochemical parameters of corresponding samples of serum and bile could be analysed.

Gall stones obtained in 54 cases were washed in saline, dried on filter paper, weighed and powdered. Ten milligrams of the powder was tested for total bilirubin, cholesterol and calcium and percentage composition determined [14]. Calcium was detected using a commercially available kit (Ames).

All 78 excised gall bladders were subjected to histopathology.

Results

The male : female ratio was 2 : 5. The preoperative serum levels of cholesterol, LCAT activity, serum phospholipid phosphorous, GGT, AP, AST and ALT as compared with normal controls are shown in Table 1. The serum lecithin : cholesterol ratio in patients of cholelithiasis was seen to be 3 : 4

which was the reverse of that seen in normal controls (4 : 3).

TABLE 1
Results of serum analysis

Test	Preoperative (n=50)	Controls (n=25)	p value
Total cholesterol (mg/dl)			
Mean	200	136	< 0.01
(SD)	32.33	11	
Ester cholesterol (mg/dl)			
Mean	94.14	78.78	< 0.01
(SD)	44.36	10.28	
LCAT (mg/hr)			
Mean	1.68	5.78	< 0.10
(SD)	0.56	2.73	
Serum phospholipid phosphorous (mg/dl)			
Mean	6.49	8.14	< 0.01
(SD)	1.8	1.05	
GGT (IU/L)			
Mean	37.96	12.33	< 0.01
(SD)	15.72	3.13	
Alkaline phosphatase (IU/L)			
Mean	177.36	65.0	< 0.01
(SD)	46.62	36.2	
AST (IU/L)			
Mean	16.22	9.24	< 0.05
(SD)	6.64	6.64	
ALT (IU/L)			
Mean	16.91	13.12	< 0.10
(SD)	8.27	7.72	

Levels of serum enzymatic activity (AST, ALT, GGT and AP) show a small but significant increase in the patient group as compared to healthy controls.

Gall bladder bile show a increase in concentration of cholesterol, phospholipids, bilirubin and LCAT activity as compared to hepatic duct bile (Table 2).

Morphological typing of the stones revealed most to be of the cholesterol type. The mean percentage composition of gall stones is shown in Table 3. There were no pure pigment stones or calcium carbonate stones. Bile culture grew *E. coli* in 6 out of 71 cases. Histopathological examination of excised

TABLE 2
Results of bile analysis (n=50)

	Bilirubin (mg/dl)	Cholesterol (mg/dl)	LCAT (mg/hr)	PL (mg/dl)	ALP (IU/lt)
Hepatic bile					
Mean	13.45	224	5.37	2.55	206
(SD)	7.0	100	2.6	1.5	120
Gall bladder bile					
Mean	50.5	712	26.5	19.75	140
(SD)	18.0	125	5.5	8.5	90

The data shows the concentration of various biliary solutes on storage within the gall bladder (PL = Phospholipid).

TABLE 3
Mean percentage composition of gall stones

Type	No. of cases	Mean % Concentration		
		Cholesterol	Calcium	Bilirubin
Cholesterol	51	95.01	1.27	3.71
Mixed	3	60.85	9.13	30.03

gall bladders showed evidence of chronic cholecystitis in 74 (94.8%) and carcinoma gall bladder in 3 (3.8%) cases. One case had a carcinoma of the common bile duct (1.2%).

Discussion

Cholelithiasis is a common disease with incidence as high as 15-20% in the general population [1]. In India the pattern of distribution is uneven. The southern states show a significantly low incidence as compared to the western and northern parts. No prior study has reported the incidence in north-eastern regions of India. Atma Prakash in his study from north-western India has reported a male to female ratio of 1 : 2.2 [7]. The present study shows a male : female ratio of 2 : 5.

Admirand and Small [8] were the first to analyse the physicochemical basis of cholesterol gallstone formation in man. They examined bile salts, lecithin and cholesterol content of both lithogenic and nonlithogenic populations and plotted it in triangular coordinates to achieve a complete separation of normal and abnormal bile. Coyne et al [9] proposed that since bile is an aqueous medium and that cholesterol is insoluble in water, bile saturated with cholesterol is un-

stable and may precipitate, resulting in gall stone formation. Bile acids are amphipathic and possess both hydrophilic and hydrophobic portions and form simple micelles in solution. Incorporation of lecithin into bile acid micelles enhances the ability of the micelle to solubilise cholesterol. Lecithin allows water to penetrate into the mixed micelle causing it to swell, and also solubilises cholesterol in equimolar amounts. Bile saturated with cholesterol is thermodynamically unstable and has potential in cholesterolithogenesis. Small and Rapo [10] showed that cholesterol saturated bile originates in the liver and postulated that hepatocytes of the cholelithogenic population secrete more cholesterol into both bile and circulation. In this study, cholesterol levels of patients of cholelithiasis have been demonstrated to be higher compared to their normal controls. This is similar to the findings of Wani et al [11] and Ramachandran et al [12].

The gall bladder plays an important role in gall stone formation. Progressive concentration, constant temperature, occasional mixing and abundance of mucus favours stone formation [13]. This is concordant with our study where there was a relative increase in

the gall bladder phospholipid and cholesterol concentration as compared to bile from the hepatic duct (Table 2).

Decreased LCAT activity is one mechanism for the decreased level of cholesterol esters seen in patients of liver disease [5]. In this study, LCAT activity of serum in the patient group was significantly less. Decreased LCAT activity of serum has also been reported in cholelithiasis by Ramachandran et al [12].

Stott et al [14] studied serum cholesterol lecithin ratios and found that patients with gall stone disease had a reversal of serum lecithin cholesterol ratios and suggested it prognosticates stone formation. In this study we also have found a reversal of cholesterol lecithin ratios and this could prove to be a valuable prognostic marker along with serum levels of LCAT activity.

Ramachandran [12] also found AP activity of serum lower in normals (8.3 KAU) compared to patients (10 KAU). AP activity was significantly raised in this study suggesting relative bile stasis (Table 1). Significant increases in the serum enzyme activity of AST, ALT and GGT could possibly be a pointer towards subclinical hepatocellular dysfunction.

Dube et al [15] examined gall stones and found cholesterol stones to be 20%, pigment stones to be 5% and mixed stones to be 75%. In this study, 51 cases had cholesterol stones while only three cases were of the mixed type. No pure pigment stones were detected. Ananthakrishnan et al [16] found 75% stones to be of the mixed infective type, 12% to be pure cholesterol stones, and pigment stones to be about 5%.

Culture of bile from the gall bladder and the common bile duct grew three positive cultures for *E. coli*. Hence, infection as a primary cause of cholelithiasis is unlikely as also observed by Dube et al [15].

Thus this study on patients with cholelithiasis from north-eastern India, shows significantly raised serum cholesterol, reversed cholesterol lecithin ratio, lowered

LCAT activity and small yet significant rise in serum AP, AST, ALT and GGT. Gall bladder altered hepatic bile by increasing the concentration of cholesterol and phospholipid. Cholesterol stones were the most common.

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