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Original Article

Biochemistry Section

Risk Factors and the Biochemical Evaluation of Biliary Calculi in Rural Kolar, Karnataka, India: A Rural Perspective of an Urban Disease

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ABSTRACT

Objective: To estimate the biochemical parameters which are responsible for the causation of biliary calculi, with risk factor correlation in Kolar district, Karnataka.

Materials and Methods: Clinically diagnosed and post-operatively collected gall stones were analyzed to find their chemical composition, such as cholesterol, triglycerides and bilirubin and to evaluate the risk factors which were responsible for the causation of biliary calculi.

Out of 4256 surgical admissions in our institute, gall stones were removed from fifty patients who presented with acute abdomen, who were confirmed to have calculus cholecystitis on sonography and these were selected for this study. A detailed history was taken from the patients to analyze the risk factors. The stones were analyzed for their composition; the serum of the patients was collected for analysis of fasting lipid and other serum parameters. Statistical analysis was done by using the SPSS package to find out the descriptive parameters.

Results: The highest incidence was seen in patients in the age group of 41-50 years. The female to male ratio was 2.57:1. Among the risk factors, hyperlipidaemia was observed in 64% of the cases, 40% had a sedentary life style, 30% had a history of high fatty diet intake and 12% were on OCP's. The biochemical analysis of the stones showed 68% patients to have mixed stones, with bilirubin being the major constituent of these stones. Bile culture was positive in 68% of the patients, with E.coli being the most common type of organism observed. Chronic cholecystitis was the most common histopathological finding.

Conclusion: Only few studies have been done, which have considered the clinical, epidemiological and the biochemical analysis of gall stone in the south Indian urban population. This study adds to the knowledge of the gall stone risk factors in the rural parts of south India. However, more studies with respect to the pathogenesis of gall stones with more number of patients has to be done, to further conclude the gall stone analysis and the risk factors.

Key Words: Cholelithiasis, Gall stone biochemical analysis, Serum lipid profile, Bile culture

INTRODUCTION

Gall stones disease (GD) is a very common gastro-intestinal disorder which is present commonly in the western world[1,2]. Mixed and pigment stones are common in northern India [3]. Epidemiological studies have shown that demographic characteristics and social customs did not contribute to the pigment gall stone formation[4]. Studies from northern India have looked into the dietary factors which predispose to cholesterol gall stones [5], while such information is not available from southern India. Although this disease has a low mortality rate, its economic and health impact is significant due to its high morbidity [6]. The major elements which are involved in the formation of gall stones are cholesterol, bile pigments, calcium, hepatic bile composition, biliary glycoprotein, infection, age, sex, genetics, oestrogen, dietary factors, geographical prevalence, and cirrhosis of the liver [7]. Now -a -days, the prevalence of gall stones is highly seen in south India, especially in the rural population. Most of the gall stone patients are asymptomatic and thus the present study describes an extensive outlook into the biochemical analysis of the stones, their incidence, age and sex distribution, the risk factors, the type of stones, the lipid profile, their histopathological diagnosis and bile culture.

MATERIAL AND METHODS

This study was conducted at RL Jalappa hospital and research centre a tertiary care rural hospital between October 2007 and April 2009. This is a prospective study which was done on biliary calculi that were removed either laproscopically or by open surgery. Out of 4256 surgical admissions in our hospital, 50 patients were included in this study. The patients were in the age group of 10 to 80 years, of both sexes, who presented with acute abdomen, with a high degree of suspicion of biliary calculi. The confirmation of cholelithiasis and its complications was done by ultrasonography. Patients with intra-hepatic calculi and patients who were not undergoing either open or laproscopic cholecystectomy with or without choledocolithotomy, were excluded from this study.

A detailed history was elicited, with particular attention to the hepatobiliary system, to assess the various risk factors. Each patient was examined physically to assess the general condition and the vital data was recorded. Per abdominal examination was done according to the standard protocol and the findings were documented. Based on the severity of the signs and symptoms and the USG reports, the treatment modality was decided. Patients who presented with acute pain abdomen, guarding, rigidity and

obstructive symptoms with cholelithiasis were managed with intravenous fluids; nil by mouth and Ryle's tube aspiration. After taking the patients' consent, they were taken up for surgery.

The institutional ethical committee approval was taken. The gall stones were sent for biochemical analysis and the bile was sent for culture and sensitivity. The pattern of presentation of the biliary duct calculi, the various risk factors which were involved in their formation and the biochemical constituents of these calculi were studied with interest. The type of stone which was formed was correlated with the serum biochemical profile of the patient. The data was analyzed by using the SPSS package.

The stones were powdered by using a mortar and pestle and were dissolved in various solvents, depending upon the various chemical constituents which had to be analyzed.

Thirty milligrams of stone powder was dissolved in 3 ml of chloroform in a test tube. The test tube was kept in boiling water for 2 minutes and the solution was used to determine total cholesterol and total bilirubin.

Thirty milligrams of stone powder was dissolved in 3 ml of 1 NHCL in a graduated 10 ml test tube and its final volume was made up to 10 ml with distilled water. The test tube was kept in boiling water for 1 hour and the solution was used to determine total triglycerides.

The stone content of the triglycerides and total cholesterol was determined by an enzymic colorimetric method of Bayer's Diagnostics India. The total bilirubin levels were estimated by using the commercial kit method of Bayer's Diagnostics India, based on bichromatic methods.

The fasting serum lipid profile estimation was done. Total cholesterol was estimated by using CHOD-PAP, triglycerides were estimated by GPO-PAP, HDLc was estimated by HDL-C plus second generation, HDL Cholesterol, no pretreatment using Roche-Hitachi autoanalyser kits. LDL-C was calculated by using Friedwald's formula after taking into consideration its limitation, LDLc = TC-{HDLc + (TG/5)}. VLDL was calculated by TG/5.

The liver function tests were done using serum. Total bilirubin was assessed by the Diphylline method, unconjugated bilirubin by direct measure, conjugated bilirubin by a spectrophotometric method, total protein by Biuret's end point, albumin by the Bromocresol green method, AST by an enzymatic colorimetric method (IFCC2002), ALT by UV with p5p (IFCC 2002) and ALP by PNPP. AMP buffer and GGT were estimated by the G – glutamyl – p – nitro anilide (IFCC 2002) method.

RESULTS

During the period from October 2007 to April 2009, the total number of surgical admissions in R. L. Jalappa hospital, Tamaka, Kolar, South India was 4256, of which 50 patients presented with symptomatic biliary calculi. Therefore, the incidence of symptomatic biliary calculi with respect to surgical admissions in this series was 11.75 per 1000. The youngest patient was 20 years old and the oldest patient was 80 years old. The mean age was 45.88 ± 14.83. The bulk of the disease presented in the age group of 41-50 years. Females outnumbered males in the ratio of 2.57:1.

Biliary calculi can present themselves in young age groups to the centenarian groups. Cholelithiasis had a peak incidence in the middle age group of 41–50 years. No age group was exempted from the disease process.

Chronic cholecystitis was found histopathologically in 35 (70%) cases. One case had adenomatous hyperplasia. Acute and chronic changes were seen in 5 cases. Acute cholelithiasis was observed in 7 patients, of which one patient had empyema. Two patients with empyematous cholelithiasis had gangrenous changes intra-operatively, as well as histopathologically. The histopathological findings showed 70% (n=35) patients to be having chronic cholecystitis.

Post-operatively, the biochemical analysis of the biliary calculi was done in all the 50 cases to estimate cholesterol and bilirubin. The incidence and the findings are given in [Table/Fig-1].

	Bilirubin	Cholesterol	Both
Cholelithiasis	36	32	28
Choledocholithiasis	02	02	02
Cholelithiasis with complications	08	08	08

[Table/Fig-1]: Number and biochemical findings of the biliary calculi in different clinical conditions (n= 50)
Chisquare value = 0.24, df =2, p-value 0.05 and not significant

Mixed stones were observed in 56% (n=28) of the cases. Pigment stones were present in 72% (n=36) of the cases, out of which one case showed primary bile duct stone, while pure cholesterol stones were found in 8% of the cases. The cholesterol stones were not associated with any major complications which were often noticed with cholelithiasis, such as choledocolithiasis, acute appendicitis, acute pancreatitis or empyematous cholelithiasis, but the cholesterol stones were seen in 64% (n=32).

A high intake of a fatty diet (FD), a sedentary life style (SLS), hyperlipidaemia (HL) and the intake of OCPs were considered as the risk factors in our study.

Thirty percent of the patients had a history of the intake of a fatty diet. Forty percent of the cases led sedentary life styles. Hyperlipidaemia was present in 64% of the cases. Twelve percent of the cases had history of OCP intake. Among 15 patients who had history of the intake of fatty diet, the post-operative analysis of the calculi revealed 12 mixed stones, 2 pigment stones and one pure cholesterol stone. Of the 20 cases who had led sedentary lifestyles, 9 had mixed stones and one had a pure cholesterol stone. In hyperlipidaemic patients, the stone analysis revealed 2 patients with mixed stones and 7 with pigment stones. Four patients with hyperlipidaemia had pure cholesterol stones. Among six patients with the intake of OCPs, 3 had mixed stones, one had a pigment stone and 2 had pure cholesterol stones. Among the 50 patients who were included in this study, 10 did not had any risk factors.

Considering the individual risk factors such as high intake of fatty diet, sedentary life style and hyperlipidaemia, three cases each with history of intake of fatty diet and OCPs and five with hyperlipidaemias respectively, were observed to have mixed stones on biochemical analysis. One patient with hyperlipidaemia and another with history of OCP intake had pigment stones. Cholesterol stones were present in one hyperlipidaemic patient.

On considering the multiple risk factors, one patient was found to have history of the intake of fatty diet with sedentary life style and the analysis revealed a mixed stone. Among eight hyperlipidaemic patients with sedentary life styles, 4 had mixed stones

and four had pigment stones. Two hyperlipidaemic patients with history of the intake of OCPs had pure cholesterol stones. Among four hyperlipidaemic patients with history of intake of fatty diet and sedentary life style, one had mixed stone, 2 had pigment stones, and one had a pure cholesterol stone. Among the remaining 10 patients with none of the risk factors, 6 patients had mixed stones, while 4 had pigment stones.

Serum Lipid Profile

Among the 50 patients, 36 had altered fasting lipid profile, of which 32 had hyperlipidaemia (HL) with elevated total cholesterol (TC) and triglycerides (TG) with low HDL [Table/Fig-2]. Twenty one among them had mixed stones (MS), 7 had pigment stones (PS), and 4 had pure cholesterol stones (CS). The other 4 patients had low HDL levels, of which 3 had mixed stones and 1 had a pigment stone. Among the 14 patients with normal lipid profile, 10 had mixed variety and 4 had pigment stones [Table/Fig-3].

Parameter	Mean (mg/dl) ± SD	SEM
Total Cholesterol (TC)	176.37 ± 49.73	8.06
Triglycerides (TG)	192.60 ± 85.01	13.79
HDL	33.58 ± 06.50	1.05
TC/HC	5.56 ± 1.22	0.19
LDL	110.81 ± 34.72	5.63
VLDL (TG/5)	38.57 ± 16.97	2.75

[Table/Fig-2]: Serum lipid profile with mean values, standard deviation (SD) and standard error of mean (SEM)

Serum Lipid Profile	MS	PS	CS	No.	(%) age
HL + HTG + Low HDL	21	7	4	32	64
NL + NTG + Low HDL	3	1	–	4	8
NFLP	10	4	–	14	28
Total	34	12	4	50	100

[Table/Fig-3]: Serum Lipid Profile Vs Biochemical analysis of stones (n=50)
HL: Hyperlipidemia, HTG: High Triglyceridemia, NFLP: Normal fasting lipid profile. Chi square (χ^2) = 0.25, df = 2, p>0.05.

Serum Bilirubin

Among the 50 patients, 9 had icterus with raised serum bilirubin, out of which 6 had pigment stones and 3 had mixed stones. In patients without jaundice, 31 had mixed stones, 6 had pigment stones, and 4 had cholesterol stones. In our study, we observed a significant reduction in the serum total protein and albumin and a marginal elevation of the liver enzymes such as AST, ALT, ALP and GGT. Total bilirubin and direct bilirubin were also marginally elevated in 9 patients [Table/Fig-4].

Parameter	Observed values	Units	Mean ± SD
Total Protein	2.95 – 4.42	gm/dl	3.68 ± 0.36
Albumin	2.23 – 3.34	gm/dl	2.7 ± 0.28
AST	11.2 – 47	u/l	29.1 ± 14.55
ALT	9.6 – 52	u/l	30.8 ± 15.4
ALP	116 – 284	u/l	200.0 ± 100.00
GGT	18 – 56	u/l	37.0 ± 18.5
T. Bilirubin	0.7 – 3.4	mg/dl	2.05 ± 1.03
Direct Bilirubin	0.800 – 1.20	mg/dl	1 ± 0.1

[Table/Fig-4]: Serum liver function tests, observed values, units, mean value with standard deviation (n=50).

Bile Culture

Bile culture versus the type of stones showed that n=34 (68%) had a positive culture and that among them, n = 17 (38%) had mixed stones. Pigment stones (n = 12, 75%) showed positive culture and cholesterol stones were observed in 4 patients, but they did not show positive culture. Micro organisms which were observed in the culture were E.coli (56%), Klebsiella (28%), Enterococcus (12%) and others (4%).

DISCUSSION

Several studies have been done on the gall stone status worldwide, till date. Indian studies showed that the incidence of gall stones was more in northern India as compared to that in southern India. This study was done in a rural part of south India. Many significant findings were observed in our study.

The mean age of presentation was 36.71 ± 11.10 in males and 49.44 ± 14.68 in females. In a Brazilian series study, the age at presentation was 60.2 years [8]. The youngest patient in our study was 20 years old, while the oldest was 80 years old.

Our study showed a female preponderance with 72% females and 28% males, with a ratio of 2.57:1. Our study correlated with the studies conducted by Bockus et al [9].

Cholelithiasis can present from the young age to the centenarian. In our study, cholelithiasis had a peak incidence in the age group of 41-50 years, but no age group was found to be exempted from the disease process. We observed a lower age limit of 20 years as compared to the findings of the Brazilian series [8].

Studies have shown that pain was the most common presenting symptom, as was universally evidenced; we also observed the same symptoms. This pain was either due to the luminal obstruction from an impacted stone, which was characteristically colicky or from an inflammation, which was a burning type of pain [10, 11, 12].

All the 50 cases in our study underwent surgery. Ninety percent of the cases underwent cholecystectomy alone, 8% were treated by cholecystectomy, with common bile duct exploration and T-tube drainage and 2% by cholecystectomy and appendicectomy.

Studies conducted by Gosh SK [13] et al., and Wani [14] et al., reported tenderness in the right hypochondriac region as the most common sign. Even in our study, this finding was consistent, 4 out of 5 patients had tenderness in the right hypochondriac region.

Histopathological studies showed that among 70% of the patients who were suffering from cholecystitis, 14% had histopathologically diagnosed with acute cholecystitis, 10% had acute to chronic cholecystitis, 4% had gangrenous cholecystitis and only 2% had adenomatous hyperplasia.

Biochemical analysis of the stones showed that n=34 (68%) of the cases had mixed stones, n=12 (24%) had pigmented stones and only n=4 (8%) had cholesterol stones. None of the cholesterol stones were associated with complications. Among 68% of the cases with mixed stones, in about 38%, bilirubin was the major constituent and cholesterol in 30% of the cases. Compared to the findings of the studies conducted by Pundir C S et al [15] and Basal S K [16] et al, we observed a high incidence of mixed stones.

A high intake of fatty diet (FD), sedentary life style (SLS), hyperlipidaemia (HL) and intake of oral contraceptives (OCP) were considered as the risk factors in our study. We observed that 30% had history of the intake of fatty diet, 40% followed sedentary life styles, hyperlidaemia was present in 64% of the cases and 12% had a history of the intake of OCP. Among 15 patients who had history of the intake of a fatty diet, the post operative biochemical analysis of the biliary calculi revealed 12 mixed stones, 2 pigment stones and 1 pure cholesterol stone. Of the 20 cases which had sedentary life styles, 13 had mixed stones, 6 had pigment stones and 1 had pure cholesterol stone. In hyperlipidaemic patients, the analysis revealed 21 cases with mixed stones and 7 with pigment stones. Four had pure cholesterol stones. Our study had 6 patients with a history of OCP intake and in them, 3 had mixed stones, 1 had a pigment stone and 2 were observed to be having pure cholesterol stones. Among the 50 patients who were included in our study, 10 patients did not had any of the risk factors.

Among patients with multiple risk factors versus the type of stones, n=12 (24%) had both sedentary life style (SLS) and hyperlipidemia (HL), 8 of these people had mixed stones (MS), 4 had pigment stones (PS), n=7 (14%) had 3 risk factors namely fatty diet (FD), SLS and HL, 4 of these people had mixed stones, 2 had pigment stones and 1 had cholesterol stones (CS). Four patients (n=4) had 2 risk factors, namely FD and HL and all 4 had mixed stones. A similar observation was seen in the study done by Jayanthi V et al [4].

The fasting serum lipid profile was estimated in all the 50 patients. n=36 had an altered lipid profile and n=32 (64%) had increased TG and low HDL (n=21) levels. In these patients, n=21 had mixed stones, n=7 had pigmented stones and n=4 had cholesterol stones. Fourteen patients (28%) had a normal fasting lipid profile, in them; n=10 had mixed stones and n=4 had pigment stones. In this regard, our study correlated with the study conducted by Jorgensen T series [17] and Thijs C [18].

In all the 50 patients (n=50, 100%) serum bilirubin versus the type of stone was studied. Forty one (82%) cases with normal bilirubin levels had 31 with mixed stones, 6 had pigment stones and 4 patients had cholesterol stones. Hyperbilirubinaemia was observed in 9 patients (18%) and among them, 6 had pigment stones and 3 had mixed stones. This clearly showed that a higher incidence of mixed stones was seen in patients with normal bilirubin and that pigment stones were seen in patients with hyperbilirubinaemia.

The bile culture versus the type of stones showed that n = 34 (68%) had a positive culture and that among them, n = 17 (38%) had mixed stones. Pigment stones (n = 12, 75%) showed a positive culture and cholesterol stones were observed in 4 patients but they did not show a positive culture.

Micro organisms which were observed in the culture were E.coli (56%), Klebsiella (28%), Enterococcus (12%) and others (4%). The number of the E. coli positive cases were twice as seen in the study by Stewart et al., who observed E. coli in 27% and Klebsiella in 20% of cases [19].

Thus, we conclude this study with the following findings. The highest age group of the presentation of cholelithiasis was 41 to 50 years, the incidence in females being more common than males. All the patients presented with pain in abdomen and majority of them had tenderness in the right hypochondria. Chronic cholecystitis was the most common mode of presentation and palpable gall bladder was not always malignant; ultra sonogram is the most common imaging modality of choice. Chronic cholecystitis was the most common histopathological diagnosis. Mixed stones were predominant, with high bilirubin content. Life style modification is very much essential in reducing the gall bladder disease, the type of stone correlated with the serum biochemical profile. Majority of the patients had a concomitant positive bile culture, with E. coli being the most common organism cultured.

Further studies with a large number of cases need to be done, to know the other risk factors which cause cholelithiasis, especially in the rural population.

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