



THE CORRELATION BETWEEN BIOCHEMICAL PROFILE OF THE PATIENT WITH BIOCHEMICAL PROFILE OF GALLSTONES: A CROSS SECTIONAL PROSPECTIVE STUDY FROM A SOUTH INDIAN TERTIARY HOSPITAL.

KEYWORDS

cholesterol, gallstone, HDL.

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ABSTRACT

Introduction: biochemical characteristics of gallstone depends on the type of gallstone which in turn is influenced by geographical location as prevalence of the disease is different among different population groups .

Aim: to study the correlation between biochemical profile of the patient with biochemical profile of gallstones.

Materials & methods: it is a cross sectional prospective study. Serum biochemical profiling together with chemical analysis of gallstone was done of all 100 patients under study.

Results and conclusion: chemical analysis showed differences in the quantitative composition of various types of gallstones. Significant association between serum cholesterol level and occurrence of cholesterol type stone ,inverse association between serum HDL levels and occurrence of cholesterol type stone was found. Correlation between serum and stone parameters revealed a moderately positive correlation for serum cholesterol and cholesterol content of mixed type of stone.

Introduction:

Differences in the incidence of gallstone formation among different populations are striking (1), Pathogenic factors are likely to be multifactorial and to vary among populations. The overall prevalence of gallstones in south India appears to be lower than in the North. However cholecystectomy once an extremely uncommon surgery in south India has become very frequent [2]. The mechanism of stone formation has been the subject of extensive research for several years, there has been continuous effort by the researchers all over the world to understand the aetiopathogenesis of Gallstone formation by determining the chemical nature of gall stone.

METHODOLOGY:

It was a Cross sectional study done between the period of JANUARY 2015 TO JULY 2016 in department of general surgery, Government Medical College, Kozhikode. Subjects included were the patients hailing from north Kerala admitted in the department wards. Inclusion criteria consisted of all stable patients without any complications like emphysematous gallbladder, gall bladder perforation diagnosed as cholelithiasis in CECT/ USG abdomen >13 years willing to participate in the study were included. Exclusion criteria was Patients who were not willing for study, seriously ill patients and patients with deranged renal functions {Sr creatinine>1.5} were excluded. College ethical committee approval was sought for the study. Data was analyzed using the SPSS-statistical software. Appropriate statistical test was applied for analysis and interpretation.

1. CLINICAL PROFILING:

Diagnosed case of Cholelithiasis meeting the inclusion criteria was taken up for the study and initial assessment was made regarding the identification. Blood was drawn for serum profiling of patients for LFT, FLP, serum calcium and serum phosphate and results were noted down after testing from our biochemistry laboratory, where automatic analyzer was used. Post Cholecystectomy Gallstones were retrieved under aseptic conditions and sent to biochemistry department for analysis.

2. BIOCHEMICAL ANALYSIS:

Gall stone collected was initially divided into 3groups depending on their colour:

1. Pale yellow and whitish stones as cholesterol calculi
2. Black and blackish brown as pigment calculi
3. Brownish yellow or greenish with laminated features as mixed calculi.

The stones were then powdered in a pestle and mortar and dissolved in different solvents depending upon the type of chemical constituent to be analysed.

To determine total cholesterol and total bilirubin, 30mg stone powder was dissolved in 3 ml chloroform in a test tube. The tube was kept in boiling water bath for 2 min. The stone solution thus obtained was used for determination of total cholesterol and total bilirubin using colorimetric method. To determine calcium, oxalate, inorganic phosphate 30 mg stone powder was dissolved in 3 ml IN HCL in graduated 10 ml tube and its final volume was made up to 10 ml with distilled water. The tube was kept in boiling water bath for 1 hr and solution thus obtained was used for determination of calcium by opec kit and inorganic phosphate and oxalate by colorimetric method. To measure fatty acids, the stones were dissolved in ethyl alcohol-solvent ether in (3:1 mixture) and solution analysed by colorimetric method.

Results:

Of the total 100 subjects in the study population , Males were 32 and Females were 68. Mean age of the study population is 44 yr. Mean Total Cholesterol of the study population was 157.72 mg/dl with SD of 24.14. Mean LDL of the study population was 88.03 mg/dl with SD of 11.20. Mean HDL of the study population was 54.14 mg/dl with SD of 12.92 . Mean TG in the study population was 142.13 mg/dl with SD of 27.13. Mean total bilirubin was 0.11 with SD of 0.31. Mean serum calcium of the study population was 8.894 mg/dl. Mean serum Phosphorous of the study population was 3.353 mg/dl. Mean Stone Cholesterol levels was 648.42 with SD of 156.03. Mean Stone Fatty Acid levels was 21.94 with SD of 6.46. Mean Stone Bilirubin levels was 3.64 with SD of 2.10. Mean Stone Calcium levels was 17.51 with SD of 6.55. Mean Stone Phosphate was 14.93 with SD of 2.89. Mean Stone Oxalate was 7.52 with SD of 1.44.

Table1: DEPENDING ON STONE TYPE MEAN VALUES OF SERUM PARAMETRES AND AGE:

Mean values	STONES	N	MEAN AGE	BMI	SERUM CHOLESTEROL	SERUM LDL	SERUM HDL	SERUM TG	SERUM CAL	SERUM PHOS	SERUM CHLORI
GROUP 1	CHOLESTEROL STONES	20	49.2	33	191.3	101.2	33.65	175.7	9.55	3.38	105.5
GROUP 2	MIXED STONES	57	43.64	28	152.3	87.45	56.15	144.9	8.78	3.32	107.6
GROUP 3	PIGMENT STONES	23	40.39	24	142.4	78.04	66.95	106.7	8.59	3.39	99

Table2: DEPENDING ON STONE TYPE MEAN VALUES OF STONE PARAMETRES

Mean values	STONES	STON CHOL	STON TG	STON FA	STONE BIL	STON CAL	STON PHO	STON OXA
GROUP 1	CHOLESTEROL STONES	838.6	64.95	30.75	0.41	6.65	19	9.52
GROUP 2	MIXED STONES	676.9	49.05	20.31	3.54	18.7	15.1	7.58
GROUP 3	PIGMENT STONES	412.4	27.13	18.3	6.7	23.9	11.2	5.65

DISCUSSION

The Gallstones collected from patients were divided into 3 groups based on their colour: cholesterol calculi, mixed calculi and pigment calculi. Out of the 100 stones collected, 57 were mixed calculi, 23 were pigment calculi and 20 were cholesterol calculi.

1.Serum parameters:

According to the type of stone the mean values with SD were as shown in the table3.

Table3: showing comparison between serum parameters and type of Gallstone. (Data are mean ± SD) mg/dl

Sl. No	Serum Parameters	Overall mean	Cholesterol Gallstones (GROU P 1) n=20	Mixed Gallstones (GROU P 2) n=57	Pigment Gallstones (GROU P 3) n=23	p value by t-test between the Groups		
						Gp1 vs. GP 2	GP 2 vs. GP 3	GP 1 vs. GP 3
1	Cholesterol	157.72±24.14	191.3±5.63	152.3±2.03	142±13.65	>0.05	>0.05	<0.05
2	LDL	88.03±11.2	101.2±7.69	87.45±9.07	78.04±6.51	>0.05	>0.05	>0.05
3	HDL	54.14±12.92	33.65±2.20	56.15±7.61	66.95±5.98	<0.05	>0.05	<0.01
4	TG	142.13±27.13	175.7±13.01	144.9±16.72	106±6.78	>0.05	>0.05	>0.05
5	Calcium	8.894±0.72	9.55±0.69	8.78±0.66	8.59±0.63	>0.05	>0.05	>0.05
6	Phosphate	3.53±0.61	3.38±0.63	3.32±0.59	3.39±0.65	>0.05	>0.05	>0.05
7	Chloride	103.2±6.2	105.5±5.91	107.6±6.69	99.9±3.69	>0.05	>0.05	>0.05

As per the above comparisons serum cholesterol values were significant between cholesterol Gallstone group and pigment group with a p- value <0.05 and serum HDL values were significant for inverse relationship between cholesterol stone group with mixed stone group and cholesterol stone group and pigment stone group with p values of <0.05 and <0.01 respectively. None of the other serum parameters showed any significance between the stone groups. The

present study observe low serum HDL levels and high total cholesterol in patients with Cholelithiasis which is in agreement with other studies by Olokoba AB et al (3), Channa NA et al (4), Virupaksha HS et al (5). Channa NA et al, found that lipids elevation in Cholelithiasis, seems to play a major contributing role in the pathogenesis of Gallstones. Study by Zhao JC et al has described a decrease in HDL in Gallstone patients, and there will be a return to the normal condition after Gallstone removal (6). Other Epidemiologic investigations have also shown that plasma HDL cholesterol levels are inversely correlated with the prevalence of cholesterol Gallstones.[7]

2.STONE PARAMETERS:

Table 4: showing comparison between stone parameters and Gallstone type (data are mean ± SD) mg/gm

Sl. No	Stone Parameters	Overall mean	Cholesterol Gallstones (GROU P 1) n=20	Mixed Gallstones (GROU P 2) n=57	Pigment Gallstones (GROU P 3) n=23	p value by t-test between the Groups		
						Gp1 vs. GP 2	GP 2 vs. GP 3	GP 1 vs. GP 3
1	Cholesterol	648.42±156.03	638.6±97.57	676.9±51.8	412.4±37.23	>0.05	<0.05	<0.05
2	TG	68.9±11.2	64.95±10.11	49.05±8.87	27.13±4.9	>0.05	<0.001	<0.01
3	Fatty Acid	21.94±6.46	30.75±3.19	20.31±4.17	18.3±6.62	<0.05	>0.05	<0.01
4	Bilirubin	3.64±2.1	0.41±0.11	3.54±0.33	6.7±0.67	<0.05	>0.05	<0.01
5	Calcium	17.51±6.55	6.65±0.47	18.7±3.40	23.9±3.05	<0.01	>0.05	<0.01
6	Inorganic Phosphate	14.93±2.89	19±0.59	15.1±1.73	11.2±0.67	<0.05	>0.05	<0.001
7	Oxalate	7.52±1.44	9.52±0.29	7.58±0.87	5.65±0.33	>0.05	>0.05	>0.05

1. *Total cholesterol:* the total cholesterol was significantly higher in cholesterol calculi, compared to pigment stones (p<0.05) and in mixed stones as compared to pigment stones (p<0.05). However, there was an insignificant difference between total cholesterol content of cholesterol stones and mixed stones (p>0.05). These results were in confirmation with the Studies by Pundir CS et al (8), in the region of north Kerala as the staple food is rice which is a good source of carbohydrates this association of cholesterol Gallstone can be explained.

2. *Triglycerides:* The triglyceride content was highest in cholesterol calculi and lowest in pigment calculi. The triglyceride content was significantly higher (p<0.001) in cholesterol calculi and mixed calculi as compared to pigment calculi. However, the difference was insignificant between cholesterol calculi and mixed calculi (p>0.05).

3. *Fatty acid:* The fatty acid content was highest in cholesterol calculi and lowest in mixed calculi. The high content of esterified fatty acids in cholesterol stones might be due to interaction between excessive cholesterol and fatty acids. The difference of fatty acid content was significant between cholesterol calculi and mixed calculi (p<0.05) and between cholesterol calculi and pigment calculi (p<0.01) but insignificant between mixed and pigment calculi (p>0.05).

4. *Total bilirubin:* The total bilirubin concentration was highest in pigment calculi and lowest in cholesterol calculi. It was significantly higher in pigment calculi compared to mixed calculi and cholesterol calculi (p<0.01) and significantly higher in cholesterol calculi as compared to mixed calculi (p<0.05). These observations are in agreement with earlier reports from Udupa KN (9), Bansal SK (10), Kumar D (11) and Pundir CS (8).

5. **Calcium:** The mean calcium content was highest in pigment calculi and lowest in cholesterol calculi. It was significantly higher in pigment calculi and mixed calculi as compared to cholesterol calculi ($p < 0.01$). However, there was an insignificant difference between calcium content of pigment calculi as compared to mixed calculi ($p > 0.05$). The calcium content in various Gallstones was in the following order. Pigment calculi > Mixed calculi > Cholesterol calculi. It is in agreement with report by Pundir CS (8), Udupa KN (9), Bansal SK et al (10) and Verma GR et al (12).

6. **Inorganic Phosphate:** The inorganic phosphate content was highest in cholesterol calculi and lowest in pigment calculi. There was no significant difference of inorganic phosphorous content between mixed calculi and pigment calculi ($p > 0.05$). However, there was a significant difference between cholesterol calculi and mixed calculi ($p < 0.05$) and cholesterol calculi and pigment calculi ($p < 0.001$) but no significant difference between mixed and cholesterol calculi ($p > 0.05$). This is in agreement with report from Udupa KN (9) but differed report by Pundir CS (8) where mixed calculi had highest inorganic phosphate content, however, there was no significant difference between mixed and cholesterol calculi.

7. **Oxalate:** The oxalate content in Gallstones was in the following order: Pigment calculi > Mixed calculi > Cholesterol calculi. However, there was no significant difference ($p > 0.05$) between oxalate content of the three calculi.

3. Serum parameters and stone parameters : Correlation coefficients of various components of the serum compared with various stone parameters showed there is moderate positive correlation between serum cholesterol and cholesterol content of the mixed stone [TABLE5].

TABLE5: CORRELATION CO EFFICIENTS OF VARIOUS COMPONENTS OF THE SERUM COMPARED WITH VARIOUS STONE PARAMETERS

COMPONENTS ANALYSED	CHOLESTROL STONES	PIGMENT STONES	MIXED STONES
	SERUM Vs STONE	SERUM Vs STONE	SERUM Vs STONE
1 TOTAL CHOLESTR OL	The value of R is -0.2279. <i>negative correlation</i>	The value of R is -0.2364. <i>Negative correlation.</i>	The value of R is 0.6281. This is a <i>moderate positive correlation</i>
2 TRIGLYCER IDES	The value of R is -0.1612 <i>negative correlation</i>	The value of R is 0.6262. This is a <i>moderate positive correlation</i>	The value of R is 0.0335. <i>weak positive correlation</i>
3 CALCIUM	The value of R is 0.6273. This is a <i>moderate positive correlation</i>	The value of R is 0.6088. This is a <i>moderate positive correlation</i>	The value of R is -0.0086. <i>negative correlation</i>
4 PHOSPORO US	The value of R is 0.1447. <i>weak positive correlation</i>	The value of R is -0.3837 <i>negative correlation</i>	The value of R is 0.0366. <i>Weak positive correlation.</i>

Our study results were on contrary to Khairy et al(13)report of a positive association between high serum cholesterol levels and cholesterol Gallstone development. Similarly, Halldestam et al [14] and Andreotti et al [15] argued that high serum cholesterol levels are positively correlated with Gallstone disease. There was also moderate positive correlation between serum triglyceride and triglyceride content of pigment Gallstone which could not be explained. We even found a moderate positive correlation between serum calcium and calcium content of pigment stone. The relationship between cholesterol, triglycerides, and Gallstone formation is multifactorial and complex and is also dependent on

other individual properties.

CONCLUSION

There is an association of low serum HDL levels and high total cholesterol in patients with Cholelithiasis. Total cholesterol as the major component and total bilirubin, triglycerides, fatty acids, calcium, inorganic phosphate, oxalate as minor components were found in all types of calculi. The cholesterol stones had higher content of total cholesterol, fatty acids, Inorganic phosphate compared to mixed and pigment stones. The mixed stones had higher content of triglycerides than to cholesterol and pigment stones. The pigment stones were richer in total bilirubin, calcium, oxalate, compared to cholesterol and mixed stones. Although total cholesterol was a major component in all 3 types of gallbladder calculi other were different in different gall stones indicating their varied mechanism of formation. Correlation coefficients of various components of the serum compared with stone parameters showed there is moderate positive correlation between serum cholesterol and cholesterol content of the mixed stone, and also between serum triglyceride and triglyceride content of pigment Gallstone. No association was found between serum levels of LD, calcium, phosphate, and chloride with type of gallstone.

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