Original Article

Association of Serum Bilirubin, Cholesterol, Calcium and Phosphate Levels with Gallstone Type in Patients Undergoing Cholecystectomy: A Cross-Sectional Study

Abhishek Galkate[1], Yashpal Ramole[2], Anuradha Choudhary[3], Vijay Singh Tanwar[1], Archana Ramole[4]

[1] PG Resident, Department of General Surgery, Gandhi Medical College & Hamidia Hospital, Bhopal, MP, India. [2]Professor, Department of General Surgery, Gandhi Medical College & Hamidia Hospital, Bhopal, MP, India. [3]Associate Professor, Department of General Surgery, Gandhi Medical College & Hamidia Hospital, Bhopal, MP, India. [4]Assistant Professor, Department of Pathology, Gandhi Medical College & Hamidia Hospital, Bhopal, MP, India

Abstract

Background: Gallstone disease is a frequent hepatobiliary disorder that is caused by the interplay of metabolic, biochemical and environmental factors. The known roles of cholesterol, bilirubin, calcium and phosphate in gallstone formation are very important. The present study aimed to assess the correlation between the serum biochemical parameters and gallstone characteristics in cholelithiasis patients. **Material and Methods:** It is a cross sectional study (observational) which was carried out for a period of 18 months in the Department of General Surgery, Gandhi Medical College and Hamidia Hospital, Bhopal. The total number of patients included were 90 who were diagnosed with ultrasonography as having cholelithiasis and were operated for cholecystectomy. Serum total bilirubin, total cholesterol, calcium and inorganic phosphate levels were measured preoperatively. Gallstones were grouped into three categories: cholesterol stones, pigment stones or mixed stones according to their morphology. One-way analysis of variance (ANOVA), Pearson's correlation coefficient and the Chi-square test were used to analyze the statistical data, where p value <0.05 was statistically significant. **Results:** The mean age of the study population was 46.48 ± 14.15 years, and females constituted 73.3% of patients. Pigment stones were the most common type (41.1%), followed by mixed stones (34.4%) and cholesterol stones (24.4%). Multiple gallstones were present in 67.8% of patients, while choledocholithiasis was observed in 7.8%. Mean serum bilirubin, calcium, phosphorus, and cholesterol levels were 1.11 ± 0.53 mg/dL, 8.69 ± 1.01 mg/dL, 3.81 ± 1.24 mg/dL, and 158.95 ± 39.01 mg/dL, respectively. Serum bilirubin levels differed significantly among gallstone types and were highest in patients with pigment stones (p <0.001). No significant differences were observed for serum calcium (p = 0.424), phosphorus (p = 0.638), or cholesterol (p = 0.944). No significant correlations were identified among the studied biochemical parameters. **Conclusion:** Gallstone disease was more common in middle-aged females, with pigment stones being the predominant stone type. Serum bilirubin demonstrated a significant association with gallstone type, particularly pigment stones, whereas serum calcium, phosphorus, and cholesterol showed no significant association. These findings suggest a potential role of bilirubin metabolism in pigment stone formation and highlight the multifactorial nature of gallstone pathogenesis.

Keywords: Cholelithiasis; Gallstones; Bilirubin; Cholesterol; Calcium; Phosphate.

Received: 05 May 2026 **Revised: 20 May 2026** **Accepted: 02 June 2026** **Published: 07 June 2026**

Introduction

Gallstones (cholelithiasis) are a common disease of the bile ducts and are a significant cause of morbidity in the gastrointestinal system around the world. It is caused by precipitation of the components of bile inside the gall bladder or the biliary tree, and is known as calculi. The clinical course is variable, from no symptoms to serious complications such as acute cholecystitis, choledocholithiasis, acute pancreatitis and, rarely, gallbladder carcinoma. The incidence of gallstone disease has risen throughout the world, partially due to changing lifestyle, sedentary behavior, obesity and other metabolic diseases. Therefore, it is crucial to know the causes of gallstone formation to prevent and treat this common condition.[1]

Gallstones formation occurs by multifactorial process with complex interactions between metabolic, genetic and environmental factors. The key roles in stone formation include changes to the composition of bile, hypomotility of the gallbladder, and stone forming crystals. Increased bile saturation with cholesterol encourages cholesterol crystallization, and the pigment stones are formed by the increased levels of bilirubin and calcium salts in the bile. With time the crystals build up and increase in size, which can cause clinical problems in the form of gallstones.[2]

Chemically, gallstones are categorized into cholesterol stones,

Address for correspondence: Dr. Abhishek Galkate, PG Resident, Department of General Surgery, Gandhi Medical College & Hamidia Hospital, Bhopal, MP, India.
E-mail: drabhishekgalkate@gmail.com

DOI: 10.21276/amit.2026.v13.i2.721

How to cite this article: Galkate A, Ramole Y, Choudhary A, Tanwar VS, Ramole A. Association of Serum Bilirubin, Cholesterol, Calcium, and Phosphate Levels with Gallstone Type in Patients Undergoing Cholecystectomy: A Cross-Sectional Study. Acta Med Int. 2026;13(2):553-559.

pigment stones and mixed stones. The most common type of cholesterol stones is caused by supersaturation of bile with cholesterol and often coincidences with obesity, metabolic syndrome. Stones often develop during the pregnancy. Dietary factors are also a common cause of cholesterol stones. Most pigment stones consist of calcium bilirubinate, and are typically linked to hemolytic diseases, biliary infections and hepatic dysfunction. Mixed stones are a large fraction of gallstones seen in practice, and present a wide spectrum of cholesterol, bilirubin and calcium salts.[3]

Cholesterol, bilirubin, calcium, and phosphate are important biochemical factors that have been implicated in the process of gallstone formation. Increased serum cholesterol level can lead to cholesterol supersaturation of bile while high level of bilirubin promotes the development of calcium bilirubinate, a main component of pigment stones. Calcium and phosphate are involved in the precipitation of insoluble mineral complexes that provide a nidus for stone formation by the initiation of crystallisation. Therefore, changes in these biochemical parameters can be indicative of the metabolic changes a factor of gall stone disease and can help us understand its etiopathogenesis.[4]

The role played by each biochemical in the occurrence of gallstones is not fully understood, although some studies have looked at the individual components. The relationship between the levels of serum total cholesterol, serum total bilirubin, calcium and phosphate in patients with cholelithiasis is not completely understood. Thus, the present study was conducted to compare the correlation of these serum biochemical parameters in gallstone disease patients and to see the possible role of these serum biochemical parameters in cholelithiasis pathogenesis.[5]

Aim: To evaluate the relationship between serum total cholesterol, total bilirubin, calcium, and inorganic phosphate levels in patients with gallstone disease and their association with age and sex.

Objectives

1. To estimate serum total cholesterol, total bilirubin, serum calcium, serum inorganic phosphate levels in patients with gallstone disease.
2. To assess the association of these biochemical parameters with age and sex among patients with gallstone disease.
3. To determine the correlation among serum total cholesterol, total bilirubin, calcium, and inorganic phosphate levels in patients with gallstone disease.

Materials and Methods

Study Design and Setting: It is a cross sectional observational study which was conducted in the department of General Surgery at Gandhi medical college and Hamidia hospital, Bhopal over 18 months. Patients with gallstone disease, who were to undergo cholecystectomy, were included in the study.

Study Population: The patients included in this study were those who were diagnosed to have cholelithiasis in ultrasonogram and were given informed written consent for cholecystectomy. The study population was selected to evaluate serum biochemical parameter and to assess the gallstone characteristics.

Sample Size

The sample size was calculated using the formula:

$n = (Z^2 \times p \times q) / d^2$

where Z = 1.96 at a 95% confidence level, p = 6% (estimated prevalence of gallstone disease), q = 94%, and d = 5%.

The calculated sample size was 86.66, which was rounded off to 90 patients.

Inclusion Criteria

1. Patients with ultrasonographically confirmed gallstone disease.
2. Patients undergoing cholecystectomy for cholelithiasis.
3. Patients willing to provide informed written consent.

Exclusion Criteria

1. Acalculous cholecystitis.
2. Obstructive jaundice.
3. Chronic liver disease.
4. Chronic kidney disease.
5. Patients unwilling to undergo cholecystectomy.
6. Patients unwilling to participate or provide informed consent.

Data Collection

Demographic and clinical data, including age, sex, presenting symptoms, clinical findings, and imaging results, were recorded using a structured proforma. Ultrasonography was performed in all patients to confirm cholelithiasis and assess associated findings.

Preoperative venous blood samples were collected under aseptic conditions for estimation of:

- Serum total cholesterol
- Serum total bilirubin
- Serum calcium
- Serum inorganic phosphate

Surgical Procedure and Gallstone Assessment

All patients underwent laparoscopic or open cholecystectomy as clinically indicated. Retrieved gallstones were examined and classified as cholesterol, pigment, or mixed stones based on their gross morphological characteristics. The number of stones and associated operative findings were documented.

Statistical Analysis

Data were entered into Microsoft Excel and analysed using SPSS version 26. Continuous variables were expressed as mean ± standard deviation, while categorical variables were expressed as frequencies and percentages.

Comparison of mean biochemical parameters among different gallstone types was performed using one-way analysis of variance (ANOVA). Pearson's correlation coefficient was used to assess correlations between continuous variables. Associations between categorical variables were evaluated using the Chi-square test. A p-value <0.05 was considered statistically significant.

Results

A total of 90 patients with cholelithiasis who underwent cholecystectomy were included in the study.

Demographic Characteristics

The age of the patients ranged from 17 to 93 years, with a mean age of 46.48 ± 14.15 years. The most frequently affected age group was 41–50 years (22.2%), followed by 31–40 years (20.0%) and 51–60 years (18.9%).

Table 1: Age distribution of patients

Age group (years)	Number of patients	Percentage (%)
<20	1	1.1%
21–30	14	15.6%
31–40	18	20.0%
41–50	20	22.2%
51–60	17	18.9%
61–70	11	12.2%
>70	9	10.0%
Total	90	100%

Table 2: Sex distribution of patients

Sex	Number	Percentage
Female	66	73.3%
Male	24	26.7%
Total	90	100%

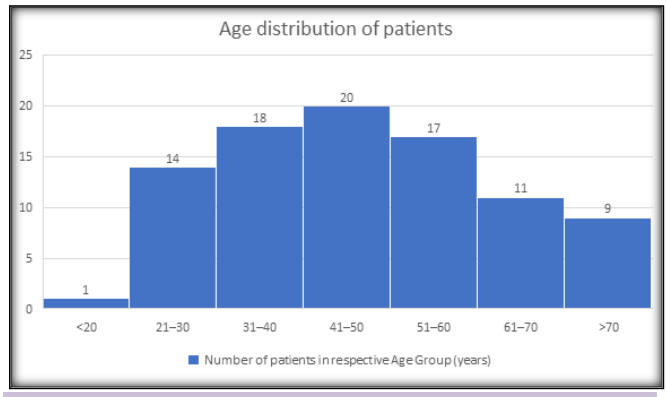

Figure 1: Age distribution of patients

Of the 90 patients, 66 (73.3%) were female and 24 (26.7%) were male, yielding a male-to-female ratio of 1:2.75.

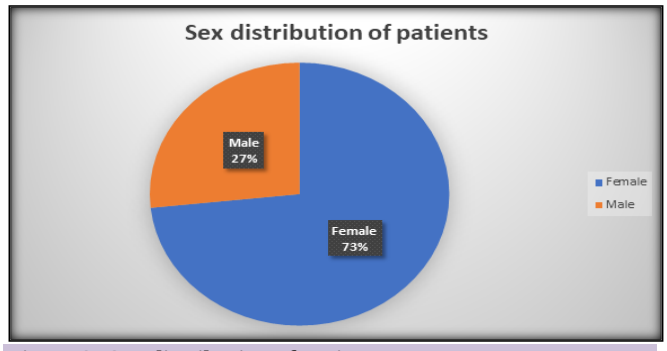

Figure 2: Sex distribution of patients

Gallstone Characteristics: Pigment stones were the most common type, observed in 37 patients (41.1%), followed by mixed stones in 31 (34.4%) and cholesterol stones in 22 (24.4%).

Table 3: Distribution of gallstone types

Type of stone	Number	Percentage
Pigment	37	41.1%
Mixed	31	34.4%
Cholesterol	22	24.4%
Total	90	100%

Table 4: Number of gallstones

Number of stones	Number	Percentage
Single	29	32.2%
Multiple	61	67.8%
Total	90	100%

Table 5- Presence of CBD stones

CBD Stone	Number	Percentage
Present	7	7.8%
Absent	83	92.2%
Total	90	100%

Table 6- Mean serum biochemical parameters according to type of gallstone

Parameter	Cholesterol Stone	Mixed Stone	Pigment Stone
Bilirubin	0.62	0.89	1.58

Calcium	8.89	8.52	8.72
Phosphorus	3.59	3.84	3.91
Cholesterol	161.13	157.40	158.94

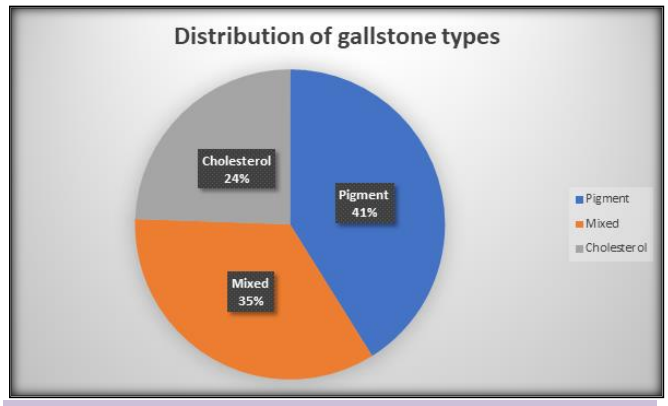

Figure 3: Distribution of gallstone types

Multiple gallstones were present in 61 patients (67.8%), while 29 patients (32.2%) had a solitary stone.

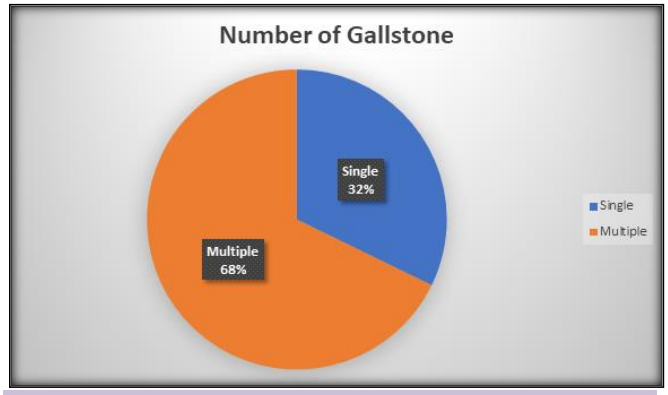

Figure 4: Number of gallstones

Associated choledocholithiasis was identified in 7 patients (7.8%).

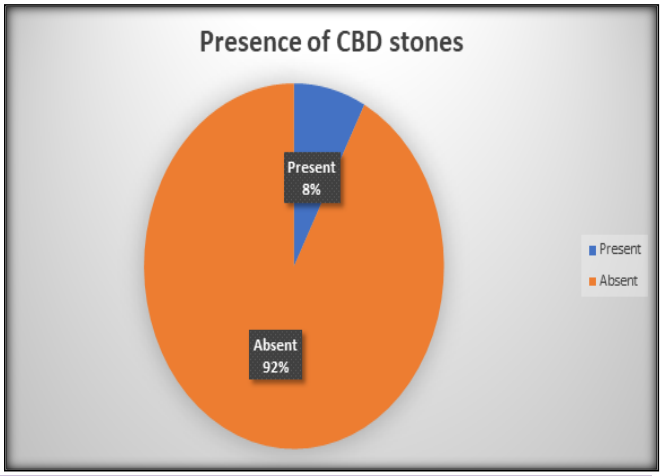

Figure 5: Presence of CBD stones

Biochemical Parameters According to Stone Type: The mean serum biochemical parameters according to gallstone type are shown in Table 6. Patients with pigment stones demonstrated higher mean serum bilirubin levels (1.58 mg/dL) compared with those with mixed (0.89 mg/dL) and cholesterol stones (0.62 mg/dL). Mean serum calcium, phosphorus, and cholesterol levels were comparable across the three groups.

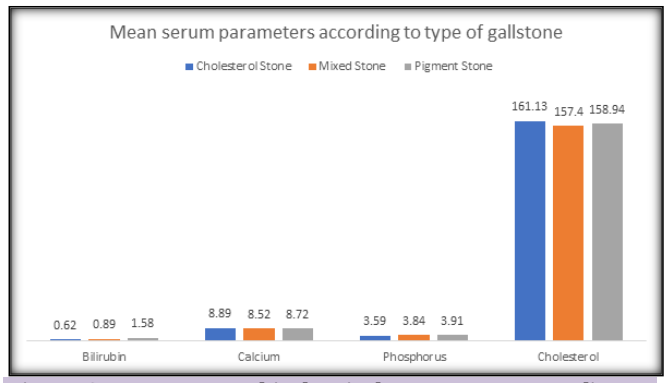

Figure 6: Mean serum biochemical parameters according to type of gallstone

Overall Biochemical Profile: The overall mean serum bilirubin level was 1.11 ± 0.53 mg/dL (range: 0.47–2.9 mg/dL), serum calcium was 8.69 ± 1.01 mg/dL (range: 5.99–11.13 mg/dL), serum phosphorus was 3.81 ± 1.24 mg/dL (range: 1.1–7.1 mg/dL), and serum cholesterol was 158.95 ± 39.01 mg/dL (range: 65.9–265 mg/dL).

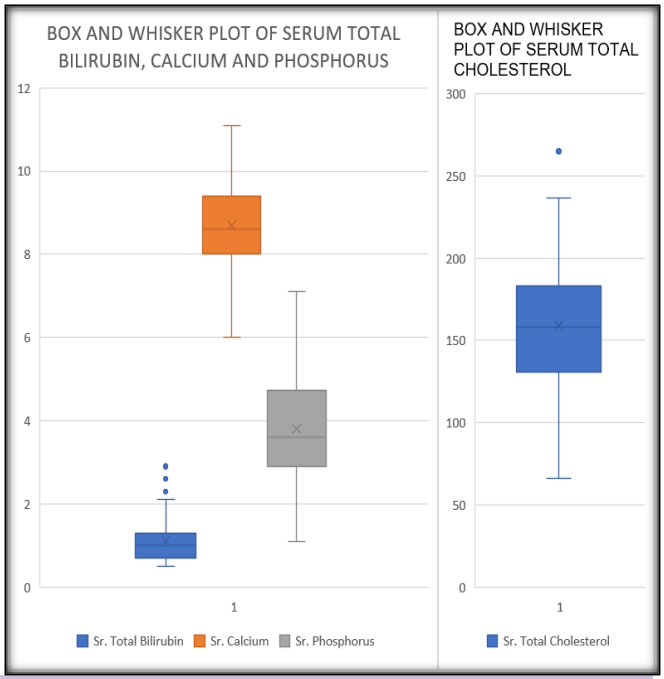

Figure 7: Box and whisker plot of serum total bilirubin, calcium, phosphorus and total cholesterol

Comparison of Biochemical Parameters Among Gallstone Types: One-way ANOVA demonstrated a statistically significant difference in serum bilirubin levels among different gallstone types ($F = 63.79$, $p < 0.001$). No significant differences were observed for serum calcium ($p = 0.424$), serum phosphorus ($p = 0.638$), or serum cholesterol ($p = 0.944$).

Table 7: Mean serum biochemical parameters

Parameter	Mean	Standard Deviation	Range
Serum Bilirubin (mg/dL)	1.11	0.53	0.47 – 2.9
Serum Calcium (mg/dL)	8.69	1.01	5.99 – 11.13
Serum Phosphorus (mg/dL)	3.81	1.24	1.1 – 7.1
Serum Cholesterol (mg/dL)	158.95	39.01	65.9 – 265

Table 8: Comparison of Biochemical Parameters Across Gallstone Types

Parameter	Mean ± SD	F-value	p-value	Significance
Bilirubin	1.11 ± 0.53	63.79	<0.001	Statistically significant
Calcium	8.69 ± 1.01	0.86	0.424	Not significant
Phosphorus	3.81 ± 1.24	0.45	0.638	Not significant
Cholesterol	158.95 ± 39.01	0.05	0.944	Not significant

Correlation Analysis

Pearson correlation analysis revealed a weak positive correlation between serum bilirubin and cholesterol ($r = 0.15$, $p = 0.21$) and between serum calcium and phosphorus ($r = 0.28$, $p = 0.09$). A weak negative correlation was observed between serum bilirubin and calcium ($r = −0.13$, $p = 0.24$). None of these correlations reached statistical significance.

Table 9: Correlation Between Biochemical Parameters

Variables Compared	Correlation Coefficient (r)	p-value	Interpretation
Bilirubin vs Cholesterol	+0.15	0.21	Weak positive
Calcium vs Phosphorus	+0.28	0.09	Mild positive
Bilirubin vs Calcium	−0.13	0.24	Weak negative

Association Between Categorical Variables

No statistically significant association was observed between sex and gallstone type ($\chi^2 = 1.97$, $p = 0.37$) or between number of stones and gallstone type ($\chi^2 = 1.12$, $p = 0.57$).

Table 10: Association Between Categorical Variables

Variables Compared	Chi-square Value	p-value	Significance
Sex vs Stone Type	1.97	0.37	Not significant
Number of Stones vs Stone Type	1.12	0.57	Not significant

Correlation of Age with Biochemical Parameters

Age showed no significant correlation with serum bilirubin ($r = −0.03$, $p = 0.72$), calcium ($r = −0.03$, $p = 0.73$), phosphorus ($r = −0.08$, $p = 0.44$), or cholesterol ($r = −0.01$, $p = 0.89$).

Table 11: Correlation of Age with Biochemical Parameters

Parameter	Correlation (r)	p-value	Interpretation
Bilirubin	-0.03	0.72	Not significant
Calcium	-0.03	0.73	Not significant
Phosphorus	-0.08	0.44	Not significant
Cholesterol	-0.01	0.89	Not significant

Clinical Presentation and Complications

Right upper quadrant abdominal pain was the most common presenting symptom. Other symptoms included nausea, vomiting, and dyspepsia.

The majority of patients had uncomplicated gallstone disease. Observed complications included acute cholecystitis (n = 2), chronic cholecystitis (n = 1), acute pancreatitis (n = 1), recurrent pancreatitis (n = 1), empyema of the gallbladder (n = 1), cholecystoduodenal fistula (n = 1), renal calculi (n = 2), and necrotizing pancreatitis (n = 1).

Table 12: Associated Findings & Complications observed included:

Complication	Cases
Acute Cholecystitis	2
Chronic Cholecystitis	1
Acute Pancreatitis	1

Recurrent Pancreatitis	1
Empyema Gallbladder	1
Cholecystoduodenal fistula	1
Renal Calculi	2
Necrotizing Pancreatitis	1

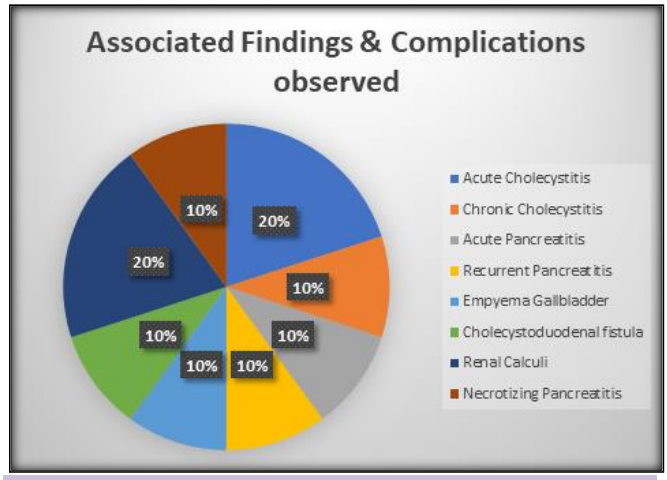

Figure 8: Associated Findings & Complications observed included:

Discussion

The occurrence of gallstone disease continues to be one of the most common hepatobiliary diseases globally, and is a common basis for surgical treatment. A cross-sectional study, involving 90 patients of cholelithiasis undergoing cholecystectomy, was conducted to assess the demographic factors, gallstone profile and serum biochemical parameters.[6]

The mean age of the study population was 46.48 ± 14.15 years, and the highest number of patients were in the age range of 41–50 years. The findings are similar to those of earlier studies that have found that gallstone disease is most common in midlife. Increasing age is known to be a risk factor for cholelithiasis, but such a correlation was not found in the present study with serum bilirubin, calcium, phosphorus or cholesterol concentrations.[7]

The study population was clearly female dominated with females representing 73.3% and a sex ratio being 1:2.75 male:female. Past Indian studies have reported the same. The more common occurrence in women may be attributed to the role of the hormones, especially estrogen and progestins (progesterone) in the metabolism of cholesterol and motility of the gallbladder. There was no significant correlation, however, between sex and the type of stone, implying that, while a female is more likely to become involved, sex is not associated with gallstone composition.[8]

The most prevalent type of gallstones was pigment stones (41.1%), followed by mixed stones (34.4%) and cholesterol stones (24.4%). The distribution is similar to that reported from some Asian populations in which pigment type and mixed stones are more common than pure cholesterol stones. Multiple gallstones were detected in 67.8% of patients, and choledocholithiasis was seen in 7.8% which are all consistent with previously reported literature.[9]

This primary aim of the study was to assess the association between biochemical parameters of serum and the characteristics of the gallstones. The average level of bilirubin in the serum was significantly elevated in the patients with pigment stones as compared to the mixed and cholesterol stones. The association between the type of gallstones and the level of serum bilirubin was highly significant ($p < 0.001$) according to ANOVA. This is biologically plausible because pigment stones are made mainly of calcium bilirubinate, and there is an increase in turnover of this bilirubin and precipitation within the bile.[10]

Levels of serum calcium, phosphorus and cholesterol, however, did not vary significantly with the different types of gallstones. Additionally, it was observed that only weak and non-significant correlations were observed between the parameters analysed. The results indicate that biochemical indicators of the serum alone may not be good indicators of gallstone composition.[11]

However, one important point is that there was no significant correlation between serum cholesterol and cholesterol stones. Serum cholesterol concentration is not always a good indicator of biliary cholesterol content and cholesterol supersaturation of bile is a key element of cholesterol gallstone formation. Several factors contribute to the formation of gallstones such as the composition of lipids in the bile, gallbladder motility, nucleation factors, genetic susceptibility and local biliary environment.[12]

The present study has shown that serum bilirubin was the only biochemical parameter, among the ones evaluated, related in a significant way to the type of gallstone. Results drive the bilirubin metabolism concept for pigment stone pathogenesis and stress the multifaceted pathophysiology of gallstones.[13]

Limitations: The study was done in a single tertiary care centre on a fairly small population. There is no control group to assess the causal associations between biochemicals and gallstone disease. Also, detailed chemical analysis was not conducted, and classification of gallstones was according to their gross morphology.

Conclusion

In the current study, the key demographic findings for gallstone disease were that it predominantly affected middle-aged people and there was a strong female predominance. Pigment stones were the most common type of gallstones followed by mixed and cholesterol stones.

Of these biochemical parameters, serum bilirubin was found to be statistically significant and it was the highest in pigment stones. Serum calcium, phosphorus and cholesterol did not significantly vary by type of gallstone. In addition, no significant relationships were found between the various

biochemical parameters, there were no notable relationships between the gallstone type and age, sex, or number of stones.

The results indicate that there is a possibility that bilirubin metabolism has a significant role in the pathogenesis of pigment gallstones. Serum biochemical parameters, however, do not fully explain gallstone composition, reflecting the multifactorial nature of gallstone formation. Further, large scale study with detailed biochemical analysis of gallstones and biliary constituents is needed to gain a better understanding of the association between systemic biochemical factors and the composition of the gallstones.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Lammert F, Gurusamy K, Ko CW, Miquel JF, Méndez-Sánchez N, Portincasa P, et al. Gallstones. Nat Rev Dis Primers. 2016;2:16024.
2. Wang DQH, Afdhal NH. Gallstone disease. In: Feldman M, Friedman LS, Brandt LJ, editors. Sleisenger and Fordtran's Gastrointestinal and Liver Disease. 11th ed. Philadelphia: Elsevier; 2021. p. 1104–1128.
3. Portincasa P, Di Ciaula A, Wang DQH. An update on the pathogenesis and treatment of cholesterol gallstones. Nat Rev GastroenterolHepatol. 2018;15(8):503-521.
4. Tazuma S. Gallstone disease: epidemiology, pathogenesis, and classification of biliary stones (common bile duct and intrahepatic). Best Pract Res ClinGastroenterol. 2006;20(6):1075-1083.
5. Singh D, Gupta V, Shukla VK. Quantitative analysis of gallstones and serum biochemical parameters in patients with cholelithiasis. Indian J Surg. 2015;77(Suppl 2):376-380.
6. Lammert F, Acalovschi M, Ercolani G, van Erpecum KJ, Gurusamy K, van Laarhoven CJ, et al. EASL Clinical Practice Guidelines on the prevention, diagnosis and treatment of gallstones. J Hepatol. 2016;65(1):146-181.
7. Ramaswami K, Praveenraj P, Senthilnathan P, Parthasarathi R, Rajapandian S, Palanivelu C. Clinical profile and pattern of gallstone disease in South India. Indian J Gastroenterol. 2013;32(6):381-385.
8. Cirillo DJ, Wallace RB, Rodabough RJ, Greenland P, LaCroix AZ, Limacher MC, et al. Effect of estrogen therapy on gallbladder disease. JAMA. 2005;293(3):330-339.
9. Nath G, Gulati AK, Shukla VK. Role of bacteria in gallstone pathogenesis. Indian J Surg. 2010;72(4):281-285.
10. Tazuma S. Gallstone disease: epidemiology, pathogenesis, and classification of biliary stones. Best Pract Res ClinGastroenterol. 2006;20(6):1075-1083.
11. Singh D, Gupta V, Shukla VK. Quantitative analysis of gallstones and serum biochemical parameters in patients with cholelithiasis. Indian J Surg. 2015;77(Suppl 2):376-380.
12. Portincasa P, Di Ciaula A, Wang DQH. An update on the pathogenesis and treatment of cholesterol gallstones. Nat Rev GastroenterolHepatol. 2018;15(8):503-521.
13. Wang DQH, Cohen DE, Carey MC. Biliary lipids and cholesterol gallstone disease. J Lipid Res. 2009;50(Suppl):S406-S411.