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Original Article

Study to Establish the Clinical Correlation between Chemical Constituents of Gallstones and Serum Biochemical Parameters

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Abstract

Introduction: Gallstones are solid crystalline precipitates in the biliary tract usually formed in the gall bladder. Gallstones may occur as one large stone or hundreds of tiny stones almost in any combination. Cholesterol and calcium bilirubinate are the two main substances involved in gallstone formation.

Materials & Methods: This study is a prospective cohort study which included 100 consecutive patients over a period of 18-month (October 2013 to August 2015) with imaging studies suggestive of cholelithiasis. A detailed history was recorded from the patients as per the prescribed pro forma and through clinical examination was performed. The recorded data included demographics and details such as onset, duration, location, and progression of abdominal pain, associated symptoms of patient and relevant clinical findings.

Results: In this study it was found that 76% had cholesterol stones, 19% had mixed stones and 5% had pigment stones which suggests that majority of the patients had cholesterol stones. Gallstones represent a major problem in many countries.

Conclusion: Incidence of gallstones is most often correlated with dietary factors and gallstones were found to be more common in patients having a mixed diet as compared to strict vegetarians which might be correlated to increased cholesterol content of the food. A significant correlation was also found between serum bilirubin levels and gallstone formation

Key words: Gallstone, Cholesterol, Bilirubin

INTRODUCTION

The gallbladder is a pear-shaped digestive storage organ that is situated under the liver on the upper right side of the human abdomen. Its job is to store and slowly release bile into the digestive system for the digestion of fats. Bile is excreted from liver and gallbladder in the stomach for digestion. After digestion, if the gallbladder is not emptied out completely, the bile that remains in the gallbladder can become too concentrated with cholesterol and gallstones are created. Gallstones are one of the most common problems associated with the gallbladder, affecting millions of people throughout the world.[1]

The gallstones are solid crystalline precipitates in the biliary tract usually formed in the gallbladder. Gallstones may occur as one large stone or hundreds of tiny stones almost in any combination. Cholesterol and calcium bilirubinate are the two main substances involved in gallstone formation.[2] Gallstones derived from bile consist of mixture of cholesterol, bilirubin with, or without calcium. Based on their chemical composition, gallstones found in the gallbladder are classified as cholesterol, pigmented, or mixed stones. Gallstones can be mostly white, yellow,

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brown, black, and green colored. Approximately 80% of the gallstones are cholesterol gallstones, which chiefly consist of cholesterol plus bile salts.[3]

Cholesterol stones are usually yellow but are sometimes white in color. These are divided into two subtypes as pure (90-100% cholesterol) or mixed (50-90% cholesterol). Cholesterol gallstones develop when bile contains too much cholesterol or not enough bile salts. Pure stones often are solitary, whitish, and larger than 2.5 cm in diameter. Mixed stones usually are smaller, multiple in number, and occur in various shapes and colors. The remaining 20% are usually referred to as pigment gallstone, which mainly consists of bilirubin (the pigment) and calcium salts such as calcium carbonate. These occur in two subtypes brown and black. Brownstones are usually made up of calcium bilirubinate. Black stones typically form in the gallbladder result when excess bilirubin enters the bile and polymerizes into calcium bilirubinate. Bilirubin stones are formed from cholesterol and bilirubin, which are mainly seen in people who have hereditary blood disorders such as thalassemia, sickle cell anemia, biliary tract infections, and cirrhosis.[4] Composite (mixed) stones also occur in the gallbladder, i.e., those consisting of a mixture of cholesterol, bilirubin, and calcium. Diets high in cholesterol and fat, increase the chance of developing stones. Gallstones have a high prevalence among elderly adults.

Aims and Objectives

Aim

To establish the clinical correlation between chemical constituents of gallstone and serum biochemical parameters.

Primary objective

To analyze all gallstones removed after cholecystectomy to detect the presence of cholesterol, pigment, calcium, and phosphate and their relative quantitative and qualitative analysis.

Secondary objective

- To estimate serum cholesterol and serum iron in the patients
- Attempt to establish a correlation with gallstones and decrease serum iron levels
- Attempt to correlate cholesterol gallstones formation with dietary factors, and diabetes mellitus
- To correlate increase serum bilirubin levels with gallstones.

MATERIALS AND METHODS

This study was conducted by enrolling the patients admitted to the ward of K. J. Somaiya Hospital and Research Centre, Mumbai, with imaging studies suggestive of cholelithiasis.

Methodology

This study is a prospective cohort study which included 100 consecutive patients over a period of 18-month (October 2013 to August 2015) with imaging studies suggestive of cholelithiasis. A detailed history was recorded from the patients as per the prescribed pro forma and through clinical examination was performed. The recorded data included demographics and details such as onset, duration, location, and progression of abdominal pain, associated symptoms of patient and relevant clinical findings. Basic investigations included complete hemogram, liver function test, lipid profile, serum iron, serum iron binding capacity, serum creatinine, random blood sugar levels, urine routine microscopy, and electrocardiogram. Imaging studies included plain abdominal radiographs and ultrasound studies.

Patients were subjected to cholecystectomy after complete workup and anesthetic fitness. Gallbladder was sent for histopathological examination, and physico-chemical analysis of gallstones was done. The gallstone surgically removed was crushed with mortar and pestle and then analyzed for cholesterol, calcium, phosphate, and bilirubin (pigment).

Statistical Analysis

This was performed as follows.

Data entry

All patients' data were entered using Microsoft Excel as per the study pro forma which is mentioned under a separate heading below. This included all details of all the blood investigations and stone analysis.

Data tabulation

The data were tabulated, charted, and graphical representation were done using bar diagrams.

Data analysis

Data were analyzed using SPSS (Statistical Package in Social Sciences) version 16 to present frequency and percentage distribution. The following tests were used, and results interpreted in the overall context of the aim of the study.

Analysis of variance (ANOVA)

One-way ANOVA is the parametric test of significance used to compare mean of quantitative data across three or more groups if the data is following normal distribution. It calculates the F-statistics and derives *P* value. The significance of association of test variable (quantitative) with grouping variable (qualitative) can be commented using ANOVA.

Chi-square test

A non-parametric test of significance used for qualitative data having frequency distribution. The Chi-square value is calculated and using the standard table value at calculated

degree of freedom, *P* value is derived. The data should be mutually exclusive and the percentage of cells with expected count <5 should not be more than 20%.

Fischer's exact test

A non-parametric test of significance used for qualitative data having frequency distribution and applied only to, 2 × 2 contingency table when the percentage of cells with expected count <5 is more than 20%.

Inclusion Criterion

- Pain in right upper quadrant of abdomen
- Basic investigations within normal limits
- Imaging studies revealing cholelithiasis.

Exclusion Criterion

- Patient's undergone cholecystectomy
- Pregnant women
- Patients with coagulation defects
- Patients with critical illness or sepsis
- Medically unfit for surgery
- Patients not subjected to imaging studies.

RESULTS

Gallstones

The most common type of gallstone in our study was found to be cholesterol stone followed by mixed stone and pigment stone (Table 1).

Table 1: Frequency of gallstones

Gallstone	Number of patient (%)
Cholesterol stone	76 (76)
Mixed stone	19 (19)
Pigment stone	5 (5)
Total	100 (100)

Table 2: Age-wise distribution of gallstones

Age groups (years)	Number of patients (%)
10-20	4 (4)
21-30	14 (14)
31-40	31 (31)
41-50	24 (24)
51-60	16 (16)
61-70	10 (10)
71-80	1 (1)
Total	100 (100)

Table 3: Sex distribution of gallstone patients

Sex	Number of patients (%)
Male	15 (15)
Female	85 (85)
Total	100 (100)

Age Distribution

Patient with the lowest age was of 13 years and oldest was of 72 years.

Mean age of presentation in years was 42-98 ± 13.186 years.

The peak incidence of gallstone formation in this study was seen in 31-40 years age group (Table 2).

Sex Distribution

The incidence of pain in abdomen in males was 15% and in females 85% (Table 3).

Sex and Gallstone

In our study of the 15 (15%) males, 10 (66.7%) patients had cholesterol stones and 5 (33.3%) patients mixed stones.

Of the 85 (85%) females, 66 (77.6%) had cholesterol stones, 14 (16.5%) had mixed stones, and 5 (5.9%) had pigment stones (Table 4).

Food Habits

Out of the 100 gallstone patients, 35 were pure vegetarians and 65 had a mixed diet, i.e., vegetarian and non-vegetarian (Table 5).

Food Habits and Stone

Among 35 vegetarians, 30 had cholesterol stones and 5 had mixed stones.

Among 65 mixed diet patients, 46 had cholesterol stones, 14 had mixed stones and 5 had pigment stones.

Table 4: Sex and gallstone

Sex	Gallstone			Total (%)
	Cholesterol stone (%)	Mixed stone (%)	Pigment stone (%)	
Male	10 (66.7)	5 (33.3)	0	15 (100)
Female	66 (77.6)	14 (16.5)	5 (5.9)	85 (100)
	76 (76)	19 (19)	5 (5)	100 (100)

Table 5: Food habits of gallstone patients

Food habits	Number of patients (%)
Veg	35 (35)
Veg+non-veg	65 (65)
	100 (100)

Table 6: Food habits and stone

Food habits	Gallstone			Total (%)
	Cholesterol stone (%)	Mixed stone (%)	Pigment stone (%)	
Veg	30 (85.7)	5 (14.3)	0 (0)	35 (100)
Veg+non-veg	46 (70.8)	14 (21.5)	5 (7.7)	65 (100)
	76 (76)	19 (19)	5 (5)	100 (100)

These findings were nearly significant ($P = 0.052$) (Table 6).

Cholesterol Status

About 86 patients had increased cholesterol level, whereas 14 patients had a normal level. None of our patients had decreased cholesterol (Table 7).

Cholesterol Status and Gallstones

The mean cholesterol level was highest among mixed gallstone patients followed by cholesterol gallstone then pigment gallstone patients. This difference was found to be significant ($P = 0.04$) (Table 8).

Serum Iron Status

About 93 patients had decreased serum iron, whereas only 7 had normal serum iron. None of our patients had increased serum iron levels (Table 9).

Serum Iron Status and Gallstone

The mean serum iron was found to be highest in mixed gallstones followed by cholesterol stones followed by pigment stone. However, this difference was found to be insignificant ($P = 0.96$) (Table 10).

It was seen that patients having increased serum cholesterol had decreased serum iron though this finding was not statistically significant, the mean cholesterol level among different gallstone patients was statistically significant ($P = 0.04$) (Table 11).

Cholesterol Status and Diabetes Mellitus

Among the 14 patients who had normal cholesterol, only 1 patient was diabetic, whereas 13 were non-diabetic.

Among the 86 patients who had increased cholesterol, 8 patients were diabetic, whereas 78 were non-diabetic. However, this association was found to be insignificant ($P = 0.632$) (Table 12).

Serum Bilirubin and Gallstones

The mean serum bilirubin was found to highest in pigment gallstone patients followed by mixed gallstone patients followed by cholesterol gallstone patients. The

Table 7: Cholesterol status

Cholesterol status	Number of patients (%)
Increased	86 (86)
Normal	14 (14)
	100 (100)

Table 8: Cholesterol status and gallstones

Gallstone	Number of patients	Serum cholesterol (mean±SD)
Mixed stone	15	255.11±43.721
Cholesterol stone	76	254.99±53.879
Pigment stone	5	192.00±73.736

SD: Standard deviation

Table 9: Serum iron status

Serum iron	Number of patients (%)
Decreased	93 (93)
Normal	7 (7)

Table 10: Serum iron status and gallstone

Gallstone	Number of patients	Serum iron (mean±SD)
Mixed stone	19	54.42±18.15
Cholesterol stone	76	53.42±16.56
Pigment stone	5	52.40±12.56

SD: Standard deviation

Tables 11: Correlation

Correlation of	Pearson coefficient (*R*)	R^2	Significance (*P* value)
Serum iron with cholesterol	−0.001	1.876	0.989
Serum iron and cholesterol with cholesterol stones	−0.122	0.015	0.295
Serum iron and cholesterol with mixed stones	0.355	0.126	0.136
Serum iron and cholesterol with pigment stones	0.697	0.485	0.191
Hemoglobin with cholesterol	0.148	0.022	0.142
Hemoglobin and cholesterol with cholesterol stones	0.165	0.027	0.155
Hemoglobin and cholesterol with mixed stones	0.072	0.005	0.770
Hemoglobin and cholesterol with cholesterol stones	0.622	0.387	0.263

Table 12: Cholesterol status and diabetes mellitus

Cholesterol status	Diabetes mellitus	
	Present	Absent
Normal	1	13
Increased	8	78

Table 13: Serum bilirubin and gallstones

Gallstone	Number of patients	Serum bilirubin (mean±SD)
Pigment stone	5	1.84±0.114
Mixed stone	19	1.39±0.66
Cholesterol stone	76	0.81±0.95

SD: Standard deviation

mean bilirubin level was found to be significant ($P < 0.05$) (Table 13).

DISCUSSION

Gallstones represent a major problem in many countries. The incidence of gallstones is most often correlated with socio-economic conditions and dietary factors.

The treatment of gallstone diseases as suggested by Shi *et al.*,[5] Ertan[6] and many others run the gamut from bile salts dissolution to fragmentation with laser, pulverization with extracorporeal shock wave lithotripsy, endoscopic extraction, and classical surgery, whereas non-invasive medical therapy is appealing, bile acid therapy is only effective in some cholesterol gallstones.

Our study included a total of 100 gallstone patients all of which underwent cholecystectomy (laparoscopic or open). The gallstones thus obtained were subjected to chemical analysis for the presence of cholesterol, bilirubin, calcium, and phosphate. Conventionally, the stones were classified into 3 categories, namely, cholesterol stones, mixed stones, and pigment stones.

It was found out that of the 100 patients we studied, 76% had cholesterol stones, 19% had mixed stones, and 5% had pigment stones, which suggests majority of our patients had cholesterol stones which are in accordance with the studies carried out by Taher[7] (cholesterol stones 49%, mixed stones 25%, and pigment stones 18%), Tassaduqe *et al.*[8] and Shareef *et al.*[9] (cholesterol stones 54%, mixed stones 40%, and pigment stones 6%), Jarrar and Al-Rowaili.[10] (cholesterol stones 54%, mixed stones 43%, and pigment stones 3%), and Channa *et al.*[11] (cholesterol stones 68%).

The youngest patient in our study was 13 years and the eldest was 72 years. Furthermore, studies reported by Channa *et al.*,[11] Saqib *et al.*,[12] and Ahmed *et al.*,[13] the peak age of occurrences were 45-59 years for males and 30-44 years for females, 30-50 years and 41-50 years, respectively. Our study also revealed that gallstones are uncommon in infants and children which was in accordance with Shaffer.[14]

Our study concluded that gallstones are more prevalent in females as compared to males with ratio of 5.6:1. This finding is consistent with the results reported by Central Department of Statistics in Saudi Arabia.

While Chen *et al.*[15] concluded that age and fatty liver in both sexes were found to be risk factors for gallstone disease in the study population. Serum cholesterol was found to be increased in 86 patients. In our study, the mean serum cholesterol was found to be highest in patients with mixed gallstones followed by cholesterol gallstones followed by pigment gallstone patients which was found to significant ($P = 0.04$).

CONCLUSIONS

- The most common type of gallstone was found to be cholesterol type of gallstone followed by mixed and pigment gallstones
- Serum cholesterol levels were found to be raised in the majority of the patients which might be a predisposing factor for cardiovascular diseases, etc. Similarly, serum iron was found to be low in majority of the patients indicating iron deficiency as a cause of gallstone formation
- Gallstones were more common in patients having mixed diet as compared to strict vegetarians which might be correlated to increased cholesterol content of the food. As diabetic patients have increased cholesterol, they were found to be more prone for gallstone formation
- A significant correlation was found to be between serum bilirubin levels and gallstones formation indicating that increased bilirubin may predispose to gallstone formation.

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