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Chemical analysis of gallstones and quantitative analysis of its biochemical components viz total cholesterol, total bilirubin, calcium and inorganic phosphate

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Abstract
To understand gallstone disease, the knowledge of it's chemical composition becomes very important. In our study, we have done the quantitative chemical analysis of gallstones collected from 40 patients and have classified them according to their biochemical composition. In our result we have found that pigment gallstones (n=16) were the most frequent type gallstones encountered followed by mixed gallstones (n=13) and cholesterol gallstones (n=11). Cholesterol was found to be a major component irrespective of the type of gallstones. The cholesterol stones had a higher amount of total cholesterol and inorganic phosphate compared to pigment and mixed gallstones. The pigment gallstones had higher amount of total bilirubin and calcium when compared with cholesterol gallstones and mixed gallstones. The mixed gallstones had higher amount of total bilirubin and calcium when compared with cholesterol gallstones.

Keywords: *gallstones, chemical analysis, classification of gallstones, total cholesterol, total bilirubin, calcium,*

Introduction

Gallstone disease is the most common disorder affecting the biliary system. Gallstones are formed by the super saturation of cholesterol in bile in the presence of other enucleating factors and calcium salts of bilirubin. In understanding gallstone disease or cholelithiasis, the knowledge of the chemical composition of the gallstones becomes of paramount importance. Studies on chemical composition of gallstones has been carried all over the world and in various parts of India[1][2], but such a study has not yet been carried in the north-eastern region of India. In our present study we have determined the chemical composition of cholesterol, bilirubin, calcium and inorganic phosphate in the gallstones and have classified the gallstones biochemically according to the Japanese classification of gallstones[3][4], which classifies gallstones as cholesterol gallstones, mixed gallstones or pigment gallstones according to their cholesterol content.

Table.1 Japanese classification of gallstones:

Cholesterol gallstone: • Pure cholesterol stone (cholesterol content ≥70%) • Mixed stone (30%≤ cholesterol content≤70%)
Pigment gallstone (cholesterol content ≤ 30%) It can be either black or brown in colour.

* The cholesterol content is based on the total dry weight of the respective stones

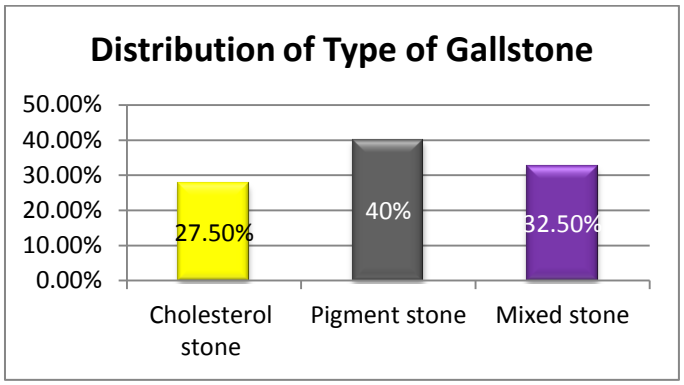
Materials and Methods

From 40 patients, gallstones were collected after cholecystectomy performed either by laparoscopically or by the open route in Assam medical college and hospital, Dibrugarh, Assam. All the gallstones were identically processed. The gallstones were washed with distilled water and air dried. The gallstones were pulverized in a mortar and pestle and dessicated to a constant weight. The mass of each stone as well as it's powder was measured using an analytical scale. To determine total cholesterol and total bilirubin 30 mg of the dried gallstone powder was taken in a test tube and dissolved in 3 ml of acidified methanol-chloroform mixture (1:1 v/v) containing 1% of 1N HCL[5]. The tube was kept in boiling water bath for two minutes. The stone solution thus obtained was used for determination of total cholesterol and total bilirubin. To determine calcium and inorganic phosphate, 30 mg of the gallstone powder was dissolved in 3 ml 1N HCL in a graduated 10 ml tube and its final volume was made upto 10 ml with distilled water. The tube was kept in boiling water bath for 1 hour[1]. The stone solution thus obtained was used to determine calcium and inorganic phosphate. All the tests were done by using enzymatic colorimetric methods, as described. The gallstone powder which was insoluble, was dried, weighed and designated as residue which was no further analyzed. Compositions were expressed as milligrams per gram of dry initial weight.

After each use, the mortar and pestel was carefully washed clean with 1N HCL and distilled water, and completely dessicated before the next use.

Total cholesterol was done using the CHOD/PAP principle[8][9]. Calcium was done using the principle of OCPC method[10][11]. Inorganic phosphate was done using the principle of Molybdalate U.V method[12]. Total bilirubin was estimated using the Mod. Jendrasik & Grof's method[13]. Triton X was required to prevent the precipitation present. All the kits were provided by Coral Clinical Systems by Tulip Diagnostics.

Results



In our study we observed the highest number patients n= 16 (40 %) were suffering from pigment gallstones. 13 (32.5 %) of the patients were suffering from mixed gallstones, and 11 (27.5 %) patients had cholesterol gallstones.

Table 2: Table Comparing Total Cholesterol, Total Bilirubin, Calcium, Inorganic Phosphate between Stones

Type of stone	Total cholesterol	Total bilirubin	calcium	Inorganic phosphate
Cholesterol gallstone	784. 36 ± 30.85	1.38 ± 0.22	5.37 ± 1.0	12.41 ± 0.87
Pigment gallstone	264 ±38.27	5.81 ± 0.79	9.14 ±1.24	4.04 ± 0.54
Mixed gallstone	580.54 ± 43.31	3.7 ± 0.38	8.22 ±1.88	9.34 ±1.08

The total cholesterol level was highest in cholesterol gallstones (784.36±30.85), followed by mixed gallstones (580.54 ± 43.31), and least in pigment gallstones (264±38.27).

The total bilirubin level was highest in pigment gallstones (5.81±0.79), followed by mixed gallstones (3.7±0.38); while in cholesterol gallstones (1.38±0.22) it was lowest.

The total calcium level was highest in pigment gallstones (9.14±1.24), followed by mixed gallstones (8.22±1.88); while the least amount was observed in cholesterol gallstones (5.37±1.0).

The amount of inorganic phosphate was highest in cholesterol gallstones (12.41±0.87), followed by mixed gallstones (9.34±1.08); while the lowest amount was found in pigment gallstones (4.04±0.54).

Photo 2: Pure Cholesterol Stone with Mulberry Appearance with Cut Surface. Very Little Pigmentation in the Centre Seen

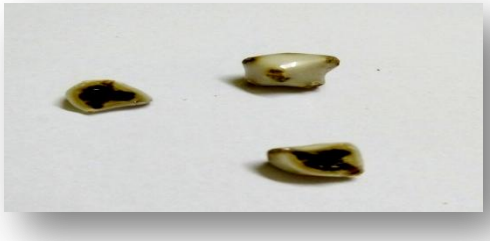


Photo 4: Mixed Stone, Multifaceted Smooth Appearance. Prominent Pigmentation Seen In Cut Surface

Photo 9: Pigment Stones. Multifaceted, Irregular Appearance. Cut Surface Shows Heavy Pigmentation

Table 3: Table Showing The Significance Of The Parameters Analysed Inbetween Stones.

groups	Total cholesterol	Total bilirubin	calcium	Inorganic phosphate
Pigment stones vs. cholesterol stones	< 0.05	< 0.05	< 0.05	< 0.05
Pigment stones vs. mixed stones	<0.05	< 0.05	> 0.05	< 0.05
Cholesterol stones vs. mixed stones	< 0.05	< 0.05	< 0.05	< 0.05

The total cholesterol level was significantly higher in cholesterol gallstones when compared with both pigment gallstones and mixed gallstones, and in mixed gallstones when compared with pigment gallstones.

The total bilirubin level was significantly higher in pigment gallstones when compared with both cholesterol gallstones and mixed gallstones, it was also observed to be significantly higher in mixed gallstones when compared with cholesterol gallstones.

The inorganic phosphate level was significantly higher in cholesterol gallstones when compared with both pigment gallstones and mixed gallstones, it was also observed to be significantly higher in mixed gallstones when compared with pigment gallstones.

The calcium level was significantly higher in pigment gallstones, and mixed gallstones when compared with cholesterol gallstones, and insignificantly higher in pigment gallstones when compared with mixed gallstones.

Table 4: Table Showing the Results of Anova, of the Parameters Between Stones

	Total cholesterol	Total bilirubin	Calcium	Inorganic phosphate
Cholesterol stones / pigment stones / mixed stones	p-value <0.05	p-value <0.05	p-value <0.05	p-value <0.05

On performing the ANOVA, it was observed that there was a significant difference in the levels of total cholesterol, total bilirubin, calcium and inorganic phosphate between the different stone types.

All paragraphs must be justified, i.e. both left-justified and right-justified.

Discussion

The total cholesterol content was highest in cholesterol gallstones, followed by mixed gallstones and pigment gallstones.

The total cholesterol level was significantly higher in cholesterol gallstones when compared with both mixed gallstones and pigment gallstones. It was also observed that the total cholesterol content was also significantly higher in mixed gallstones when compared with pigment gallstones. This is in accordance with various other studies that have concluded that cholesterol is a major component of gallstones, irrespective of the type of gallstones[14]. C.S. Pundir etal[1] observed in their study that the total cholesterol level was highest in cholesterol gallstones, followed by mixed gallstones and pigment gallstones. They observed that the total cholesterol level was significantly higher in cholesterol gallstones when compared with pigment gallstones, but observed an insignificant difference when compared with mixed gallstones.

A multitude of factors has been postulated in the formation of gallstones, and amongst them the concept of cholesterol supersaturation in the formation of cholesterol gallstone is the most widely accepted one. The cause of cholesterol supersaturation is usually due to the hypersecretion of cholesterol into the bile by liver. This may be due to increased de-novo synthesis of cholesterol by the liver; this hypothesis is given ground by some clinical trails in which it was observed that use of statin group of drugs decreases the incidence of gallstone disease[15]. Supersaturation of cholesterol can also be due to the defect in bile acid synthesis or metabolism. Cholesterol being a non-polar molecule is transported in clusters of bile acids called micceles. Increased cholesterol to bile acid ratio may also lead to the bile being supersaturated with cholesterol, thus predisposing to the formation of gallstones.

The total bilirubin content was highest in pigment gallstones, followed by mixed gallstones. The total bilirubin content was observed to be the lowest in cholesterol gallstones. Das Biswajit et al[2] and Saddeldin A. Idris etal[16] observed similar findings, with total bilirubin content to be highest in pigment gallstones, followed by mixed gallstones and cholesterol gallstones. In contrast C.S. Pundir et al[1] observed that the total bilirubin content to be highest in pigment gallstones, followed by cholesterol gallstones, while mixed gallstones was observed to have the lowest content of total bilirubin. The easily recognizable colour of the pigment stones has been attributed to the colour of bilirubin.

The calcium content was observed to be highest in pigment gallstones, followed by mixed gallstones. The calcium content was observed to be lowest in the cholesterol gallstones. The calcium content was significantly higher in pigment gallstones when compared with cholesterol gallstones, but there was an insignificant difference in the calcium content between pigment gallstones and mixed gallstones, while the calcium content in mixed gallstone was significantly higher when compared with cholesterol gallstones. Similar findings were also observed by C.S.Pundir et al[1], Das Biswajit et al[2] and S.A.Idris et al[16]. The inorganic phosphate content was highest in cholesterol gallstones, followed by mixed gallstones, and the lowest content of inorganic phosphate was observed in pigment gallstones. Similar findings were also observed by C.S. Pundir et al[17], Das Biswajit et al[2] and K.M. Shareef et al[18]. We also observed that the inorganic phosphate content was significantly higher in cholesterol gallstones when compared with both pigment gallstones and mixed gallstones, and was significantly higher in mixed gallstones when compared with pigment gallstones.

Both calcium and phosphate are very tightly regulated in our body. Calcium as well as phosphate plays a critical role in the pathogenesis of gallstones, but the accurate study of calcium content in the gallbladder is very difficult, because calcium's entry as well as regulation in bile occurs by passive mechanism and as such any artificial maneouveor may either lead to increase or decrease of it's level. It has been observed that the concentration of calcium increases significantly in early gallstone formation contributing to the increased saturation of each calcium salt in bile[19]. Calcium salt saturation is dependent on the concentration of calcium sensitive anions in bile, including bilirubinate, carbonate and phosphate.

Conclusion

Pigment gallstones were the most frequent type of gallstones encountered, and cholesterol was found to be a major component irrespective of the type of the gallstone. Cholesterol gallstones, which is the most frequent type of gallstone afflicting the western countries, apparently attracts the attention of scientific commumity, at large. Similar in-depth research on pigment gallstones, more prevelant in the developing countries, is scarcely seen. Detailed research with careful morpho-constitutional examination of pigment gallstones, involving layer by layer morphological examination of the calculi and analysis of the complete chemical composition using modern and more accurate methods such as FTIR (Fourier Transform Infrared Spectroscopy) is needed. A multi-centric trial involving geneticists, surgeons, nutritionists, and social scientists could draw out concrete evidence on the etiopathogensis and progression of lith formation. It could also go a long way in illuminating unforeseen territories of research on prevention and treatment of pigment gallstones, specifically and cholelithiasis in general.

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