

# A Study of Chemical Composition of Gall Stones in a Tertiary Care Hospital

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## ABSTRACT

### BACKGROUND

Gallstones are a major health problem & have been recognized since antiquity. Gallstones are generally classified into pure cholesterol, pigment and mixed type. Knowing the chemical composition of gallstones is essential for determining etiopathogenesis of gallstone disease. Fourier Transform Infrared Spectroscopy (FTIR) method is the most widely used technique in chemical analysis of gall stones. Reports on chemical analysis of gallstones are available from different endemic regions of India. However, no reports are available about the composition and etiopathogenesis of gallstones in Himachal Pradesh.

### METHODS

Gallstones from 400 patients of cholelithiasis were collected after cholecystectomy at Indira Gandhi Medical College Hospital (I.G.M.C.), Shimla, between June 2016 to June 2018, and were subjected to chemical analysis by FTIR method. The data was further correlated with regard to age, gender, socio-economic status, various life style factors like diet, obesity, physical activity, with stone number and colour.

### RESULTS

Chemical analysis of gall stones revealed that pure cholesterol, mixed and pigment variety were 94 %, 2 % and 4 % respectively. Majority of the patients were below the age of 50 years (66 %). Male to female ratio was 2.7:1. Both sexes had predominantly pure cholesterol stones. Most of the patients belonged to the lower middle class (56.5 %) & all socio-economic classes showed predominantly pure cholesterol stones. Similarly, there was no predilection of any type of stone to a particular physical work category. 53.5 % patients were non-vegetarians and 46.5 % were vegetarians. There was no correlation between diet and BMI with type of stones. In our study, 66 % of patients had multiple stones. Multiple stones were seen in all varieties of stones. Pure cholesterol stones showed a variety of colours contrary to traditional classification.

### CONCLUSIONS

In Himalayan population, the predominant type of gall stones were pure cholesterol stones. Mixed stones and pigment containing stones were found in much smaller frequencies. This result is in contrast to that of rest of the Indian subcontinent from where larger percentage of pigment and mixed stones has been reported. And pure cholesterol stones showed maximum variation in colours contrary to our expectation.

### KEY WORDS

Gall Stone Disease (GSD), Fourier Transform Infrared (FTIR) Spectroscopy, Chemical Composition, Gall Stones

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**BACKGROUND**

Gallstones are still a major health problem. Gallstones even have been found in Egyptian mummies dating back to 1000 BC.<sup>1</sup> Langenbuch performed the first successful cholecystectomy, setting the path for therapeutic intervention. Gallstone is more prevalent in developed nations.<sup>2</sup> In India, this disease is seven times more common in the North than in South India. Gallstone composition has not attracted adequate attention in current population-based studies leaving uncertainty regarding the confirmation of this assumption. The impact of lifestyle and ethnicity is underscored by the profoundly different gallstone compositions in studies from sub-Saharan Africa,<sup>3</sup> and China.<sup>4</sup>

Gall stones are generally classified into three major types: pure cholesterol, pigment and mixed gall stones. Pigment gall stones are subdivided into laminated brown stones and amorphous black stones and are more common in Asia and India. In Western countries, about 80 % of gallstones are cholesterol stones, about 15% to 20 % are black pigment stones,<sup>5</sup> & brown pigment stones form a small percentage.

In Northern India, pigment and mixed stones are prominent as compared to cholesterol stones & vegetarian/non-vegetarian diet did not influence the chemical composition. While, South India reported 47 % mixed stones, 41.1 % brown pigment stones and 11.7 % black pigment stones & no cholesterol stones.<sup>5</sup>

Studies from Saudi Arabia reported low incidence of cholesterol stones.<sup>6</sup> While study from China,<sup>7</sup> reported 36.8 % cholesterol stones with variety of colours, 26.9 % pigment stones & 36.3 % mixed stones & BMI of patients with cholesterol stones was higher.

Fourier Transform Infra-Red (FTIR) spectroscopy has been established as a means of gallstones analysis by Japanese and US investigators<sup>8</sup> and mainly used in the large-scale analysis of GS samples.<sup>9</sup>

There is no data available on chemical composition of gallstones from Himachal Pradesh & our aim was to determine the chemical composition of gall stones and to correlate the chemical composition data with age, gender, diet, physical activity, socio-economic status, stone colour and stone number in this unstudied population having different geographic, environmental and dietary habits.

**METHODS**

This was a prospective observational study of gallstones from 400 patients of cholelithiasis which were collected in a single surgical unit of I.G.M.C. Shimla, after cholecystectomy, between June 2016 to June 2018. These were subjected to chemical analysis by Fourier transform infrared spectroscopy (FTIR) method. The data was correlated in relation with age, gender, socio-economic status, diet, obesity, physical activity and with number and colour of stones. After cholecystectomy, stones were removed after sectioning the gall bladder, washed with sterile water and were examined for shape, size and colour. A single gallstone (heaviest one in case of multiple stones) from each patient was subjected to chemical analysis. More than 95 % relative amount of cholesterol was taken as

minimum percentage for classifying a stone as pure cholesterol stone. Modified B.G. Prasad scale for socio-economic status,<sup>10</sup> revised WHO range for Asia- Pacific region for Body Mass Index & Worker's Compensation Board of Nova Scotia levels (2014) of physical activity were used.<sup>11</sup>

**Inclusion Criteria**

All patients undergoing cholecystectomy in single surgical unit in I.G. Medical College, Shimla.

**Exclusion Criteria**

Patients with: acute cholecystitis, choledocholithiasis, haemolytic anaemias, pregnancy, and liver cirrhosis.

**Statistical Analysis**

Statistical correlations between patient age, gender, food habits, physical activity, stone number, stone color and chemical composition of gallstones were performed, using SPSS software version 8.

**RESULTS**

In this study, the observed chemical composition was as per table 1. Age ranged between 10 years to 84 years. Maximum patients were in the age group of 41 - 50 years (26 %) with mean age of 44.07 years and 66.5 % patients were below 50 years. Mean age for pure cholesterol stones was 44.22 ± 14.11 years, for mixed stones was 23.25 ± 2.06 years and for pigment stones was 50.88 ± 24.48 years (Fig. 1). Female patients were 73 %, while 27 % were male. Among females, 95.9 % had pure cholesterol stones, 2.7 % had mixed stones and 1.4 % had pigment stones. Among males, 88.9 % had pure cholesterol stones, none had mixed stones and 11.1 % had pigment stones.

In socio-economic status, 56.5 % patients belonged to lower middle class, middle class constituted 18 % of patients, upper middle class constituted 18.5 % of patients, 3 % of patients and upper class constituted 4 % of patients. In all socio-economic classes, cholesterol stones were prominent.

Most patients (67.5 %) with gall bladder stones indulged in mild daily physical activity, 31.5 % patients were involved in moderate daily activity and 1 % indulged in severe/heavy physical activity work. In all categories of physical activity, cholesterol stones were prominent. Regarding diet, 53.5 % patients were non-vegetarian and 46.5 % were vegetarians. Among patients with pure cholesterol stones, 53.7 % were non- vegetarians and 46.3 % were vegetarians. Among patients with mixed stones, 50 % were non vegetarian and 50 % were vegetarians. Among patients with pigment stones, 50 % were non vegetarian and 50 % were vegetarians (Fig. 2). In BMI study, 40 % patients belonged to pre-obese/obese category, 41.5 % had normal BMI and 8.5 % were underweight BMI (Fig. 3).

Type of Stone	Frequency	Percent
Cholesterol > 95 %	376	94.0
Bile Salts 75 %, Cholesterol 25 %	8	2.0
Cholesterol 30 % Bile Salts 50 %, Biliverdin 20 %	16	4.0
<b>Total</b>	<b>400</b>	<b>100.0</b>

*Table 1. Number of Gall Bladder Stones Based on Chemical Analysis*

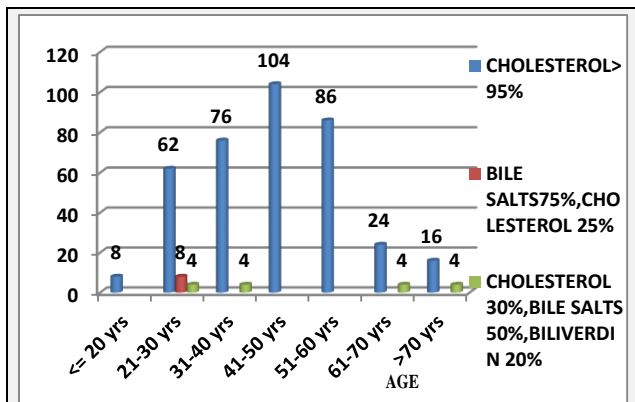


Figure 1. Age Wise Distribution of Different Types of Gall Bladder Stones

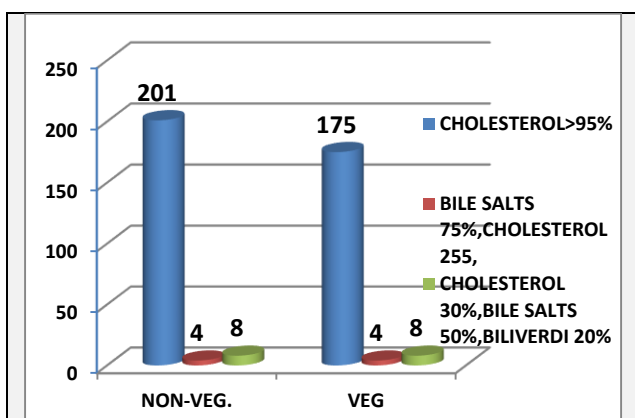


Figure 2. Distribution of Different Types of Gall Bladder Stones as per Diet of Patients

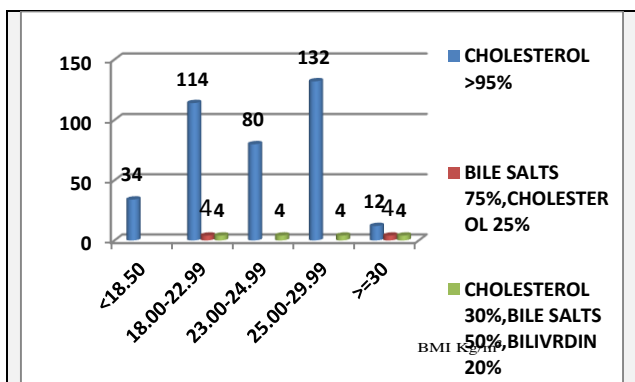


Figure 3. Distribution of Different Types of Gall Bladder Stones as per BMI

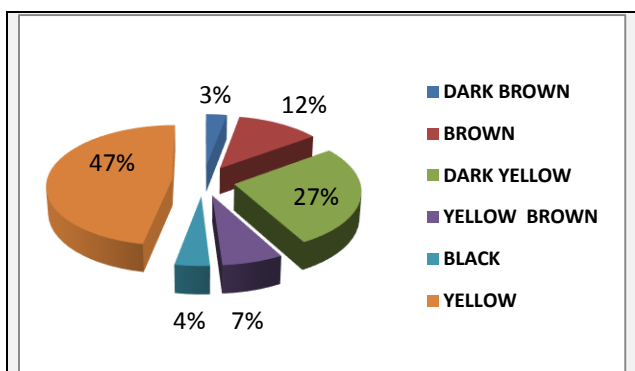


Figure 4. Percentage Distribution of Gall Bladder Stones as per Colour

Multiple stones were found in 66 % of patients & 34 % of patients had single stone. Among pure cholesterol stones, 64.9 % were multiple and 35.1 % were single & 100 % mixed stones were multiple. Among pigment stones, 75 % were multiple and 25 % were single. A variety of stone colours were observed. Pure cholesterol stones showed maximum variety of colour with yellow (50 %), dark yellow (29.3 %), yellow-brown (7.4 %), brown (12.2 %) and dark brown (1.1 %) stones. None of the pure cholesterol stones was black in colour (Fig. 4). Whereas all mixed stones were dark brown, and all pigment stones were black in colour.

### DISCUSSION

Reports on gallstone disease are mentioned as early as 2000 BC, when the Babylonians first described the bile duct system. The first chemical analysis of the composition of gallstones was made in 1789 by Fourcroy. Surgical treatment of gall stones was established in 1867 by John Bobbs.<sup>12</sup>

Gallstones are common in the Western world, with prevalence rate of 10 - 15 % for men and about 20 % for women. While prevalence of gallstone disease in India has been reported as 2 % to 29 % & average age of Indian patients is a decade younger than those in the West.<sup>13</sup> It is a disorder with increasing life expectancy and changes in life style in "westernised" societies, particularly an increase of cholesterol gallstones. This disease is seven times more common in the North than in South India. Dietary differences in the two regions are suspected to be responsible for this difference.<sup>12</sup>

Gallstones are divided into three major types: cholesterol, black pigment and brown pigment stones. Black pigment stones are common among patients with haemolytic diseases and liver cirrhosis.<sup>14,15</sup> Brown stones are often caused by stasis and infection in the biliary system. In the Western world, the major constituent of gallstones is cholesterol, which comprises 50 - 98 % of the dried substance of the stone. Other constituents may include fatty acids, triglycerides, proteins, polysaccharides, calcium bilirubinate, calcium carbonate and calcium bicarbonate. Gallbladder stones vary in size from less than a millimetre up to few centimetres in diameter. Thus, application of novel preventive or therapeutic approaches will likely be limited to certain classes of gallstones as defined by their composition and aetiology.<sup>16,17</sup>

We used FTIR, in order to define the target populations for future therapeutic and preventive therapies and to investigate the gallstones composition in Himachal Pradesh. As there have been no studies to evaluate the chemical composition of gall stones from the Himalayan region of South East Asia and one has to rely on and extrapolate data related to epidemiology and chemical composition of gall stones which is generalised to Northern plains of India.

In our study, 94 % of the patients had pure cholesterol stones, 2 % of patients had mixed stones and 4 % of patients had pigment stones. Findings of our study are also in consonance with other studies in the Western world which all showed that about 80 % - 90 % of gallstones are cholesterol stones while about 10 % to 20 % are black pigment stones and mixed stones in 0 % - 5 % patients.<sup>18,19</sup> Brown pigment stones accounts for a small percentage. Both types of pigment stones are more common in Asia as well as in India.<sup>19,20</sup>

However, findings of our study are in contrast to studies done in the Indian subcontinent<sup>21</sup> which reported pigment stones in 36 %-40 % patients, mixed stones in 35 % -58 % patients and cholesterol stones in only 25 % - 42 % patients. While studies from South India,<sup>22</sup> reported 47 % mixed stones, 41.1 % brown pigment stones and 11.7 % black pigment stones. There were no cholesterol stones. Our findings were also not in consonance with studies from the Middle East, Africa and China<sup>4</sup> which reported 49.3 % -17 % cholesterol stones, 31.3 % - 36.3 % mixed stones and 17.3 %-51.07 % pigment stones.

Age and sex distribution of gall stones was found similar to other Indian and International studies<sup>23</sup>, with maximum occurrence in the age group of 41 - 50 years and with a female predominance. This might be due to the basic hormonal differences between males and females. Both sexes showed a predominance of cholesterol stones with 100 % of mixed stones occurring in females. And 75 % of pigment stones occurring in males, this finding is similar to finding of other study,<sup>23</sup> that there is a correlation between stone types and gender; male patients predominantly tend to develop mixed stones or pigment stones. However, our findings are concurrent with study by Schafmayer et al,<sup>13</sup> which reported that cholesterol stones were predominant in both sexes, while 58 % of pigment stones were seen in males.

Most of the patients belonged to lower middle class (56.5 %). Patients belonging to all social classes had predominantly pure cholesterol stones. Our findings were not in consonance with other study that gallstone disease was more frequently seen in high socioeconomic class. Most of the patients (67.5 %) were involved in mild daily physical activity while 31.5 % patients were involved in moderate daily activity. Patients involved in severe/heavy work were only 1 %. All three categories had predominantly pure cholesterol stones. This finding of a negative relation between physical activity and occurrence of gall stones, confirms the findings of previous studies.<sup>23</sup> There was no correlation between type of diet and gallstones composition. These findings are concurrent with other studies, that vegetarian/non-vegetarian diet did not influence the prevalence and chemical composition of gallstones.

In BMI study, 40 % patients belonged to pre-obese/obese category with BMI > = 25 Kg / m<sup>2</sup>, 51.5 % had BMI in normal range and 8.5 % had below normal BMI. Pure cholesterol stones were predominant (> 90%) in all categories of BMI. Our study showed no correlation between BMI and gallstones. There was also no specific predilection of any type of gallstone to a particular BMI range. These findings are in consonance with study by Singh A et al<sup>24</sup> who reported that body mass index did not influence the prevalence of gallstone disease. Our findings are not in consonance with findings of Schafmayer et al<sup>13</sup> who reported that BMI > 25 Kg / m<sup>2</sup>, was significantly associated with gallstones predominantly of cholesterol.

In our study, majority (66 %) of patients had multiple stones and 34 % of patients had single stones. Multiple and single stones were seen in all three type of gall stones. These findings are in consonance with findings by Bansal et al<sup>24</sup> who reported multiple stones in 75% and single stones in 25 % patients. Pure cholesterol stones showed maximum variation in colour. None of the pure cholesterol stones was black in colour. All mixed stones (100 %) were dark brown and all pigment stones (100 %) were black in colour. These findings are in

consonance with other studies,<sup>25</sup> which reported various colours of cholesterol stones as; brownish yellow, amber, grey, celadon or black while pigment stones were amorphous, brittle, granules having black, charcoal grey or greyish brown colour. Other studies,<sup>20</sup> have classified the stones prior to analysis of chemical composition, as either cholesterol, mixed or pigment, based on colour and later performed the analysis.

Based on our findings, we are of the opinion that, classifications based solely on colour may be misleading giving rise to misreporting of actual occurrence of different types of stones. Classifications must be based on the more accurate and reliable chemical composition reports rather than visual inspection of colour of the stones.

This is possibly the first study to evaluate the chemical composition of gall stones from the Himalayan region of India in South East Asia and compare the same with various epidemiological and life style factors. Studies from Northern India have by and large focussed on chemical composition of gall stones of patients from the plains of North India.

This study showed a predominance of cholesterol stones, which is in consonance with studies done in the Western World showing percentage occurrence of cholesterol stones above 90 %, more so in European countries with cold climate like Germany and Sweden,<sup>25</sup> where climatic conditions are similar to Himachal Pradesh.

However, our findings were in contrast to other Indian studies from both North India and South India,<sup>15,16</sup> which have described a higher occurrence of pigment and mixed stones as compared to cholesterol stones. Our findings were also not in consonance with findings from Middle East, China and Africa,<sup>6,7</sup> all of which showed a much lower prevalence of cholesterol stones. Higher occurrence of cholesterol stones in patients from Himachal Pradesh could be related to similar environmental and dietary factors as in Western populations.

## CONCLUSIONS

All patients showed a predominance of cholesterol stones with 100 % of mixed stones occurring in females and 75 % of pigment stones occurring in males. Most of the patients with gall stones belonged to the lower middle socio-economic strata & maximum number of patients were in mild physical activity group. There was no specific co - relation to diet or BMI with the occurrence of gall stones or with their chemical composition. Most patients had multiple stones and stones showed varying colours with pure cholesterol stones showing maximum variation in colour.

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