

## RESEARCH ARTICLE

### QUALITATIVE AND QUANTITATIVE ANALYSIS OF BILIARY CALCULI IN A TERTIARY CARE HOSPITAL

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**ABSTRACT: Background** :- Gallstone disease remains a serious health concern for human beings, affecting millions of people throughout the world. **Material and method**:- The cross sectional study was conducted in department of Biochemistry and Surgery in Guru Gobind Singh Medical College and Hospital, Faridkot. 100 patients of cholelithiasis were enrolled for study. Physical and chemical analysis of stones was done. **Result**:- Out of 100 gallstone patients, 36 were male and 64 were females. The biliary calculi divided into three categories, these are cholesterol stone(33), pigment stone (25)and mixed stones(42). The qualitative and quantitative analysis of biliary calculi showing the maximum amount of cholesterol was present in cholesterol stones( $452.24 \pm 37.02\text{mg/gm}$ ). The maximum amount of bilirubin was present in pigment stones ( $4.26 \pm 0.42\text{mg/gm}$ ).The maximum amount of calcium was present in pigment stones( $19.28 \pm 2.10\text{mg/gm}$ ). Mixed type of stone is more common than the cholesterol and pigmented stones. **Conclusion**:- From the present study, we concluded that the mean age of the patients with gall stones was  $48.19 \pm 10.72$  years with a male-female ratio of 1:1.7. Mixed type of stone is more common than the cholesterol and pigmented stones. The categorization of gall stones based on chemical composition is required for understanding path physiology of gall stones. These manual methods are easy and cheap to do quantitative analysis of gall stones.

**KEY WORDS**:- gall stones, cholelithiasis

### INTRODUCTION:

The gall bladder is a pear shaped digestive storage organ that is situated under the liver on the upper right side of the human abdomen<sup>[1]</sup>. The main purpose of the gallbladder is to store bile, also called gall, needed for the digestion of fats in food<sup>[2]</sup>. Gall stones are the most common biliary pathology. The estimated prevalence of gallstone disease in India has been reported as 2% to 29%.

In India, this disease is seven times more common In the North than in South India<sup>[3-5]</sup>. Gallstone disease remains a serious health concern for human beings, affecting millions of people throughout the world. The term cholelithiasis may refer to the presence of stones in the gallbladder or to the diseases caused by gallstones<sup>[6]</sup>. Cholesterol gallstones develop when bile contains too much

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cholesterol and not enough bile salts<sup>[7]</sup>. Mixed/pigment stones typically arise secondary to infection of the biliary tract which results in the release of  $\beta$ -glucuronidase (by injured hepatocytes and bacteria) which hydrolyzes bilirubin glucuronides and increases the amount of unconjugated bilirubin in bile. Between 4% and 20% of stones are mixed.<sup>[26]</sup> Pigment stone happen due to increase of unconjugated bilirubin in bile<sup>[8]</sup>.

Gallstones can vary in size and shape from as small as a grain of sand to as large as a golf ball.<sup>[9]</sup> The gallbladder may contain a single large stone or many smaller ones. The composition of gallstones is affected by age, diet and ethnicity.<sup>[10]</sup>

The present study describes an qualitative and quantitative analysis of gallstones which includes cholesterol, bilirubin, calcium and phosphorus.

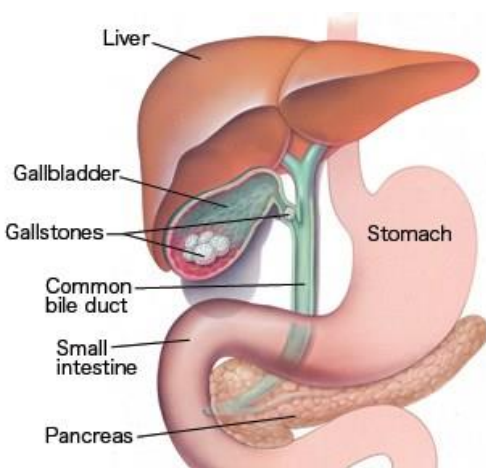


Figure no 1. Gall Bladder with stones.

## MATERIAL AND METHOD:

The study was conducted in Dept of Biochemistry in collaboration with Dept of Surgery in GGS Medical College Faridkot. Ethical clearance was taken from institutional ethical committee. Proper informed consent was taken from all the participants. Gallstones from 100 patients of cholelithiasis were collected after cholecystectomy. In all, 100 gallstones were

collected from 36 males and 64 females. The various physical parameters of stones such as number, shape, size, margin and consistency were noted.

The stones were powdered in a pestle and mortar and dissolved in different solvent according to chemical constituents to be analyzed.

To determine **cholesterol and bilirubin**, 30 mg stone powder were dissolved in 3 ml chloroform in a test tube. The tube was kept in boiling water bath for 2 minutes.

To determine **calcium and phosphorus** the stones were powdered in a pestle and mortar. 30 mg stone powder was dissolved in 3 ml 1 N HCl in a 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 hour.

The contents were then analyzed quantitatively and qualitatively.

The Cholesterol analyzed by CHOD/PAP method,<sup>[11,12,13]</sup> bilirubin by Malloy and Evelyn method,<sup>[14]</sup> calcium by o-cresolphthalein complexon method<sup>[15]</sup> and phosphorus by Gomorri's method<sup>[16]</sup> were estimated.

Statistical Analysis was done by IBM® SPSS® V.20.00 software. Independent Mann Whitney test was applied to do calculations

## RESULTS AND OBSERVATIONS:

The biliary calculi collected from 100 gallstone patients were divided into three categories. Depending on content of stones these were cholesterol stone, pigment stone and mixed stones. Out of 100 stones, 42 were mixed calculi, 33 were cholesterol calculi and 25 were pigment calculi. Out of 100 gallstone patients, 36 were male and 64 were females thus the male to female ratio was 1:1.7. Out of the total number of stones

collected the incidence of gallstone was higher in age group 51-60 yrs (Table 1).

**Table 1: Showing Age and sex Distribution Of Patients**

AGE (YEARS)	MALE	FEMALE	TOTAL	PERCENTAGE
21-30	1	3	4	4.0
31-40	9	18	27	27.0
41-50	8	18	26	26.0
51-60	14	18	32	32.0
61-70	4	7	11	11.0
Total	36	64	100	100.0

Physical examination of stones showed that the multiple number of stones were present in mixed calculi followed by pigment calculi and then cholesterol calculi. Pigment calculi showed highest weight than cholesterol and mixed calculi. The collected gallstone specimens were of variable sizes. The size of calculi was large in cholesterol followed by mixed calculi and pigment stones. The colour of cholesterol calculi was yellow to yellowish green, colour of pigment calculi was brown and black and colour of mixed calculi was whitish grey. Shape of stone varied from round, pyramid to irregular.

#### Determination of Metabolites :

The cholesterol, bilirubin, calcium and phosphorus were found in all 100 gallstones. The qualitative analysis of biliary calculi showing the maximum amount of cholesterol was present in cholesterol stones (++++), it is comparatively lesser in mixed stones (++) and very low in pigment stones (+). The maximum amount of bilirubin was present in pigment stones (++) followed by cholesterol stones (++) and minimum amount in mixed stones (+). The maximum amount of calcium was present in mixed stones (++++) followed by pigment stones (++) and cholesterol stones (+)

respectively. The maximum amount of phosphorus was present in cholesterol stones (++++), it is comparatively lesser in mixed stones (++) and very low in pigment stones (+) (Table 2).

**Table 2. Qualitative analysis.**

No of patients	cholesterol	Bilirubin	Calcium	Phosphorus
33	++++	++	+	+++
25	+	+++	+++	+
42	++	+	++++	++

The mean value and standard deviation of cholesterol contents found in the 3 types of stones showed slight variation having its highest values in cholesterol stones ( $452.24 \pm 37.02$ ) followed by mixed stones ( $347.20 \pm 53.47$ ) and pigment stones ( $310.45 \pm 65.21$ ) respectively. The bilirubin content was significantly high in pigment stone ( $4.26 \pm .42$ ) as compared to cholesterol ( $3.62 \pm 6.57$ ) and mixed stones ( $1.55 \pm .34$ ) respectively. The calcium content was high in pigment stone ( $19.28 \pm 2.10$ ) followed by mixed stone ( $18.87 \pm 2.28$ ) and least in cholesterol stone ( $9.79 \pm 1.23$ ) respectively. The phosphorus content was highest in cholesterol stone ( $13.94 \pm 3.75$ ) as compared to mixed ( $9.28 \pm 1.85$ ) and pigment stone ( $7.98 \pm .69$ ) respectively (Table 3).

**Table 3. Mean value of different chemical composition in biliary calculi.**

Group	Type of stone	Cholesterol (mg/gm)	Bilirubin (mg/gm)	Calcium (mg/gm)	Phosphorus (mg/gm)
1	Cholesterol	$452.24 \pm 37.02$	$3.62 \pm 6.57$	$9.79 \pm 1.23$	$13.94 \pm 3.75$
2	Pigment	$310.45 \pm 65.21$	$4.26 \pm .42$	$19.28 \pm 2.10$	$7.98 \pm .69$
3	Mixed	$347.20 \pm 53.47$	$1.55 \pm .34$	$18.87 \pm 2.28$	$9.28 \pm 1.85$

Out of 100 stones the 42% had mixed stones, 33% had cholesterol stone and 25% had pigment stones.

in males 14 stones (38.8%) were cholesterol stone, 16 stones (16.6%) were mixed stones and 6 stones (44.4%) were pigment stones present. And in females 19 stones (29.6%) were pigment stones, 26 stones (40.6%) were mixed stones and 19 stones (29.6%) were cholesterol stone present (Table 4).

**Table 4. Stone distribution according to sex.**

Gender	Types Of Stones			Total
	Cholesterol	Pigment	Mixed	
Male	14	6	16	36
Female	19	19	26	64
Total	33	25	42	100

## **DISCUSSION:**

Gallstones are one of the most common problem associated with the gall bladder and can become dangerous if left untreated. Gallstone disease remains a serious health concern for human beings, affecting millions of people throughout the world. The mean age of study group (as shown in table 1) showed the maximum number (32%) of patients were from the age group of 51-60 years. Mean age of patients was  $48.19 \pm 10.72$  years. Out of 100 patients included in the present study, there were 36 males and 64 females giving a male to female ratio of 1:1.7. Females have more chances susceptible to gall stone due to estrogen level. With presence of estrogen the biliary cholesterol secretion are increase which cause cholesterol super saturation of bile. The cholesterol calculi were mostly in yellow in colour. The cholesterol stones also found in yellowish green, yellowish brown, green and blackish yellow in colour. The pigment stones were mostly in brown and black in colour, some stones were dark brown, creamish brown and light brown. And the mixed stones were multiple in colour. These were white, grey, white grey, creamish brown, light grey, blackish

white, greenish, whitish brown, greenish brown, yellowish brown and dark brown in colour.

The cholesterol in cholesterol stones was ( $452.24 \pm 37.02$ ) followed by mixed stones ( $347.20 \pm 53.47$ ) and pigment stones ( $310.45 \pm 65.21$ ) respectively. The bilirubin content was significantly high in pigment stone ( $4.26 \pm .42$ ) as compared to cholesterol ( $3.62 \pm 6.57$ ) and mixed stones ( $1.55 \pm .34$ ) respectively. The calcium content was high in pigment stone ( $19.28 \pm 2.10$ ) followed by mixed stone ( $18.87 \pm 2.28$ ) and least in cholesterol stone ( $9.79 \pm 1.23$ ) respectively. The phosphorus content was highest in cholesterol stone ( $13.94 \pm 3.75$ ) as compared to mixed ( $9.28 \pm 1.85$ ) and pigment stone ( $7.98 \pm .69$ ) respectively, this is in agreement with P Chandran et al<sup>[17]</sup> and B Das et al.<sup>[18]</sup>

Bilirubin stones are usually rich in cholesterol and bilirubin. These are majorly seen in patients of cirrhosis, biliary tract infections. Diet also plays an important role in formation of gall stones. Quantitative analysis of these stone can help in better management of gall stone patients.

## **CONCLUSION:**

From the present study, we conclude that the mean age of the patients with gall stones was  $48.19 \pm 10.72$  years with a male-female ratio of 1:1.7. Mixed type of stone is more common than the cholesterol and pigmented stones.

The categorization of gall stones based on chemical composition is required for understanding pathophysiology of gall stones. These manual methods are easy and cheap to do quantitative analysis of gall stones.

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