Quantitative Analysis of Chemical Composition of Gallstones in North Indian Population (Rohilkhand Region, Uttar Pradesh)

Dr. Biswajit Das*, Dr.Ayaz Khurram Malik, Dr. Amineur Rehman***,**
Mr. Sumeru Samanta, Dr Marya Ahsan******

*Associate Professor, **Assistant Professor, Department of Biochemistry, ***Ex. Professor, Department of Sugery, **** Assistant Professor, Department of Pharmacology, Rohilkhand Medical College, Bareilly- 243006.

Abstracts: **Background And Objectives:** In India, it has been recognised that gallstones and gallbladder cancer are common in the Gangetic belt comprising of Uttar Pradesh, Bihar, West Bengal, and Assam. States in South India do not have a high incidence of these diseases. Aim: The present study was done to describe an extensive quantitative chemical analysis of gallstones and to find the association of gallstones with age distribution and also to compare this finding with previous workers. In this study we analysis the cholesterol, triglycerides, phospholipids, bilirubin, bile acids, calcium, phosphorus, magnesium,and oxalates in 3 different types of gallstones. Methods: Total 43 gallstones were collected from surgical operation and quantative analysis of these gallstones was done in the department of Biochemistry. The stones were classified into cholesterol (CS), pigment (PS), and mixed stones (MS). Total cholesterol was estimated by CHOD-PAP, total bilirubin by Diazo method, triglycerides by GPO-PAP method, oxalate by the method described by Satyapal and Pundir based on colorimetric enzymatic method (21), calcium by O-Cresolphthalein-Complexone method, Phospholipid and inorganic phosphate were determined according to Fiske and Subba Rao. Magnesium was measured by Calmagite method. **Results:** In our study, the incidence of gallstones was highest in age group of 37-46 years and Male : Female ratio was 1: 3.8. Out of 43 gallstones, 16 were pigment stones, 15 were mixed stones and 12 were cholesterol stones. Total cholesterol was a major component of all gall stones and triglycerides, total bilirubin, phospholipids, bile acids, calcium, magnesium, inorganic phosphate and oxalate were found in all types of gallstones. The cholesterol stones had higher content of total cholesterol, phospholipids, inorganic phosphate as compared to mixed and pigment stones. The mixed stones had higher content of triglycerides than to cholesterol and pigment stones. The pigment stones were richer in total bilirubin, bile acids, calcium, oxalate, and magnesium compared to cholesterol and mixed stones**. Interpretation And Conclusion :** Pigment stone was the most common type of gallstones in our studies and common age group for gallstone development was 37-46 years. The content of the total cholesterol and other metabolites in different gall stones indicating their different mechanism of formation. High cholesterol and triglyceride content in CS and MS suggests that dyslipidemic changes contribute to etiology. [Das B NJIRM 2014; 5(4) :4-12]

Key Words: cholesterol stones, pigment stones, mixed stones, oxalate, bilirubin

Author for correspondence: Dr. Biswajit Das, Associate Professor, Department of. Biochemistry, Rohilkhand Medical College and Hospital, Bareilly 243006; M: 9411922086 Email: biswajitdas1971@gmail.com

Introduction: Gallstone is a crystalline concretion Formed within the gall-bladder by accretion of bile component. Presence of stones in the gallbladder is referred to as cholelithiasis. Migration of gall stones into the ducts of the biliary tract, the condition is referred to as choledocholithiasis. Choledocholithiasis is frequently associated with obstruction of the biliary tree, leading to acute ascending cholangitis. Gallstones within the ampulla of Vater can obstruct the exocrine system of the pancreas, resulting in pancreatitis [1]. The Gall bladder is a slate blue, pyrifom sac sunken in fossa in the right hepatic lobe s inferior surface. It is 7-8 cm long, 3 cm broad at the widest and 30-50 ml in capacity [2]. Its job is to store and slowly release bile into the digestive system for the digestion of fats. When we eat fat, both the liver and the gall bladder excrete bile to the duodenum for digestion[3].

Gallstones may occur as one large stone or hundreds of tiny stones almost in any combination. The two main substances involved in gallstone formation are cholesterol and calcium bilirubinate[4]. Gallstones found in the gall bladder are classified as cholesterol, pigmented or mixed stones based on their chemical composition.

Cholesterol Stones: Cholesterol stones have been noted in Egyptian mummies indicating that the

disease have existed for more than 35 centuries [5]. Cholesterol stones chiefly consist of cholesterol plus bile salts. They are usually green, but are sometimes white or yellow in color. Approximately 80% of the gallstones are cholesterol gallstones. These are divided into two sub type as pure (90-100% cholesterol) or mixed (50-90% cholesterol). Cholesterol gallstones develop when bile contains too much cholesterol and not enough bile salts. Pure stones often are solitary, whitish, and larger than 2.5 cm in diameter [3].

Pigment Stones: Pigmented gallstone chiefly consists of bilirubin (the pigment) and calcium salts such as calcium carbonate. These occur in two sub types: brown and black. Brown stones are usually made up of calcium bilirubinate. Black stones typically form in the gall bladder result when excess bilirubin enters the bile and polymerizes into calcium bilirubinate. Pigment stones tend to develop in people who have cirrhosis, biliary tract infections and hereditary blood disorders such as sickle cell anemia [3,6].

Mixed Stones: Mixed gallstones typically contain 20–80% cholesterol (or 30–70%, according to the Japanese- classification system). Other common constituents are calcium carbonate, palmitate Phosphate, bilirubin and other bile pigments. Because of their calcium content, they are often radiographically visible. Mixed and pigment stones are common in northern India [7].

Gallstone disease is one of the most common and costly of all digestive diseases. Removal of the gall bladder by surgical method is the only solution available to the gallstone disease today [8]. A Fat,Fertile, Flatulent, Female of 50 years is classic sufferer of Gall Bladder stones [9]. Epidemiological studies show that gallstones formation is a complex multifactorial disease. The prevalence varies with age, sex, and ethnic group [10]. 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 (stone belt) than in South India. Dietary differences in the two regions are suspected to be responsible for the difference in the prevalence rate. The average age of these patients in India, is a decade younger than those in the West.[11-13].

Chemical analysis of gallstone gives important evidences for the aetiology, origin and the metabolic basis of its formation, and helps in the identification of risk factors that predispose certain individuals to the stone formation. Very little reports on gallstone disease are available from Rohilkhand region of Uttar Pradesh and the disease prevalence at the country level is increasing, but no thorough study has been conducted. The present study was done to describe an extensive quantitative chemical analysis of gallstones removed from patients receiving treatment in Rohilkhand Medical College and Hospital, Bareilly, Uttar Pradesh, India and to find the association of gallstones with age distribution and also to compare this finding with previous workers. In this study we analysis the cholesterol, triglycerides, phospholipids, bilirubin, bile acids, calcium, phosphorus, magnesium, and oxalates in 3 different types of gallstones.

Material And Methods: Quantitative chemical analyses of 43 gallstones were conducted in Rohilkhand Medical College and Hospital (RMCH), Bareilly at Biochemistry Department. These stones were removed surgically from June 2012 to May 2014. All the procedures reported here in the study have followed the guidelines approved by the locally appointed ethical committee. In all, 43 gallstones were collected from 34 females and 09 male.

The patient's information regarding, name, age, sex, chief complaints, present, past and family history of cholelithiasis, and also number of gallstones were obtained from hospitals' records. The physical parameters of the gallstones, such as colour, number, shape, texture, and cross section, were noted. The gallstones were classified into three groups on the basis of their morphology (Table 1). Out of 43 gallstones, cholesterol stones (CS), pigment stones (PS), and mixed stones (MS) were collected as 12 (28%), 16 (37.2%) and 15 (34.8%). The stones were powdered using a mortar and dissolved in different solvents depending on the type of chemical constituent to be analyzed. To determine total cholesterol and

total bilirubin, 30 mg stone powder was dissolved in 3 ml chloroform in a test tube. The tube was kept in boiling water bath for 2 min. Aliquots from these samples were used for determination of total cholesterol and total bilirubin. To determine calcium, oxalate, inorganic phosphate, magnesium and triglycerides 30 mg of the powdered stone was dissolved in 3 ml of HCl in a graduated 10-ml tube and the volume was made up to 10 ml with distilled water. The tubes were kept in a boiling water bath for one hour. To analyze phospholipids, 20 mg of powdered stone was dissolved in 15 ml of a 2:1 mixture of chloroform and methanol containing 1 N HCl. To measure bile acids, the stones were dissolved in chloroform-methanol (2:1) mixture. The solutions were preserved at 4 C until they were used.

Total cholesterol was estimated by CHOD-PAP, end point (manufactured by: Transasia biomedical LTD) [14]. Total bilirubin by Diazo method, end point[15], triglycerides by GPO-trinder method [16], oxalate by the method described by Satyapal and Pundir based on colorimetric enzymatic method[17], calcium by O-Cresolphthalein-Complexone method [18], bile acids were estimated by colorimetric method of Carey [19] . Phospholipid and inorganic phosphate were determined according to Fiske and Subba Rao[20]. Magnesium was measured spectrophotometrically at 530 nm. (Wavelength range: 500-550 nm) using Calmagite method[21] .
Statistical analysis of the data was carried out by ANOVA Test.

Result: Table -1and Figure 1 & 2 represents the physical features of some studied stones, such as size, shape, surface and color. The size of cholesterol stone was in the range 0.2-2.6 cm and 0.5- 3.5 cm with an average of 1.3 cm and 2.1 cm, while for pigment stone it was 0.1-1.85 cm and 0.2-2.0 cm with an average of 0.71 cm and 0.82 cm and for mixed stone it was in the range of 0.1-2.3 cm and 0.1-2.8 cm with an average of 0.80 cm and 1.00 cm. The shapes of the collected gallstones were irregular (26) and round (17). Smooth and rough surface gallstones were found in 28 and 15 out of 43 gallstones. The colors of the studied gallstones were whitish yellow, black, brown, greenish brown and green.

The age of the patients ranged 17-66 years. Out of 43 gallstones of 43 patients, 34 (79 %) stones belonged to females while 09 (21 %) stones belonged to male; male to female ratio was 1: 3.8. The incidence of gallstones was highest in age group of 37-46 years (Table 1).

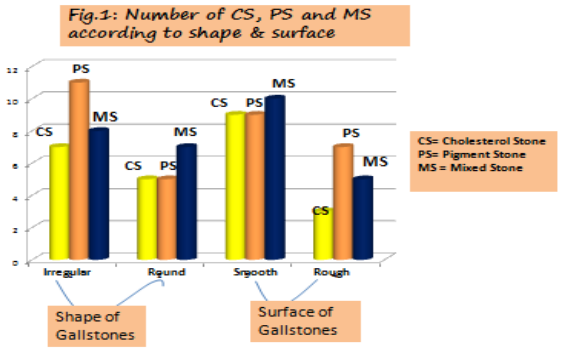
Table 1: (A), (B). Physical Properties And Incidence Of Various Types Of Gallstones According To Age

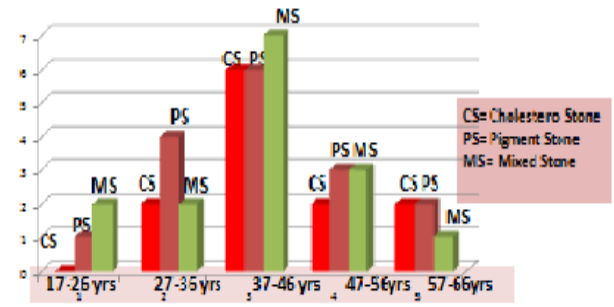
(A)

	Cholesterol stones	Pigment stones	Mixed stones	Total
Shape	Irregular-07 Round-05	Irregular-11 Round-05	Irregular-08 Round-07	Irregular-26 Round-17
Surface	Rough-03 Smooth-09	Rough-07 Smooth-09	Rough-05 Smooth-10	Rough-15 Smooth-28
Size (cm, mean)	1.3 X 2.1	0.71 X 0.82	0.80 X 1.00	
Colour	Yellow-07 Whitish-05	Black-07 Brown-09	Green-11 Greenish brown-04	

(B)

Sex	CS	PS	MS	M: F
Female	10	12	12	
Male	02	04	03	1: 3.8
Age group (Years)				Total
17-26	00	01	02	03
27-36	02	04	02	08
37-46	06	06	07	19
47-56	02	03	03	08
57-66	02	01	02	05





The quantitative analysis of 43 gallstones revealed that cholesterol was higher in cholesterol stones than in the other two type of stones but difference was significant compared to pigment stones (p= 0.007) and in mixed stones as compared to pigment stones (p= 0.022) [Table 2 & 3; figure 3(a)]. Total bilirubin content was significantly higher in pigment stones compared to cholesterol stones (p= 0.008). Triglycerides content was lowest in pigment stones but it was significantly different as compared cholesterol stones and mixed stones. The phospholipids content was highest in cholesterol stones and there was a significant difference between phospholipid content of cholesterol stones and mixed stones (p= 0.009) and pigment stones (p= 0.001). Insignificant difference was observed between pigment stones and mixed stones (p= 0.73). Bile acids were significantly higher in pigment stones as compared to cholesterol stones (0.039). Bile acids contents in cholesterol stones also significantly different in comparisons to mixed stones (0.026) [Table 2 & 3; Figure 3(c)].

The mean calcium content was highest in pigment stones (9.7 mg/gm) and lowest in cholesterol stones (5.4 mg/gm). It was significantly higher in pigment stones as compared to cholesterol stones (p=0.001) and mixed stones (p= 0.009) [table 2 & 3; figure 3b]. Calcium content in mixed stones were also statistically significant in comparison to cholesterol stones (p=0.009). The magnesium content was highest in pigment stones. It was significantly higher than cholesterol stones (p= 0.016).The difference of magnesium content of cholesterol stones and mixed stones (p=0.605) and between pigment stones and cholesterol stones (p=0.074) was however insignificant. The oxalate content was significantly higher in pigment stone as compared to mixed stones (p=0.003) and in cholesterol stones compared to mixed stones (p= 0.023). Insignificant difference was found between cholesterol stones and pigment stones (p= 0.094).The inorganic phosphorus content was highest in cholesterol stones (11.1 mg/gm). There was a significant difference between cholesterol stones and pigment stones (p=0.001) and cholesterol stones and mixed stones (p=0.026) but no significant difference between mixed stones and pigment stones (p= 0.17).

Table 2: Contents of Metabolites In Different Types Of Gallstones (Mg/ Gm)

Metabolites	Cholesterol stones	Pigment stones	Mixed stones
Cholesterol	545.19 ± 61.8	421.36 ± ± 79.4	498.0 ± 64.04
Bilirubin	1.10 ± 0.28	1.63 ± 0.50	1.48 ± 0.38
Triglycerides	36.1 ± 5.6	28.0 ± 5.7	40.8 ± 7.1
Phospholipids	6.40 ± 0.73	5.04 ± 1.05	4.9 ± 0.78
Bile acids	11.05 ± 2.3	15.6 ± 3.2	14.2 ± 1.9
Calcium	5.4 ± 0.81	9.7 ± 3.1	8.00 ± 2.8
Magnesium	5.9 ± 1.09	7.0 ± 0.94	6.05 ± 3.0
Oxalate	6.8 ± 1.2	7.3 ± 0.93	6.3 ± 1.2
Inorganicphosphate	11.19 ± 2.3	8.03 ± 1.2	9.02 ± 1.06

Fig. 3 (a) Mean value of Cholesterol in CS, PS and MS

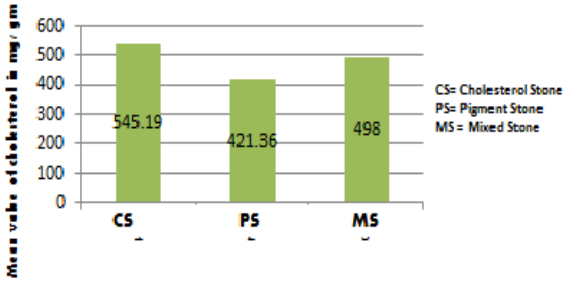


Fig. 3 (b) Mean value of metabolites (Bilirubin, Calcium, Magnesium, Oxalate and Phospholipids) in CS, PS and MS

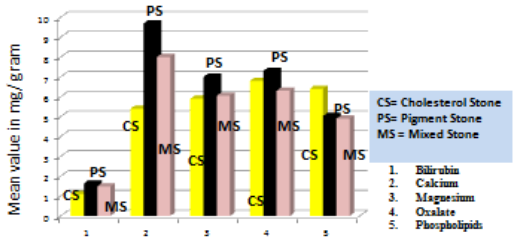


Fig. 3 (c) Mean value of metabolites (Triglyceride, Bile acids and Inorganic Phosphate) in CS, PS and MS

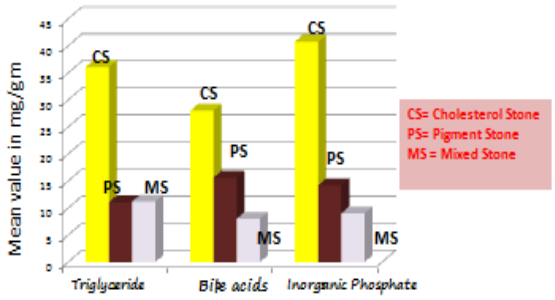


Table 3: P-Values for Differences In Chemical Composition Of Gall Stones

Metabolites	Cholesterol stones vs Pigment stones	Cholesterol stones vs Mixed stones	Pigment stones vs Mixed stones
Cholesterol	0.007	0.076	0.022
Bilirubin	0.008	0.59	0.225
Triglycerides	0.026	0.160	0.001
Phospholipids	0.009	0.001	0.73
Bile acids	0.039	0.026	0.192
Calcium	0.001	0.009	0.009
Magnesium	0.016	0.605	0.074
Oxalate	0.094	0.023	0.003
Inorganic phosphate	0.001	0.026	0.173

Discussion: An interesting finding in this study is that out of the 43 collected stones , 16 were pigment stones, 15 were mixed stones and 12 were cholesterol stones indicating, the incidence of gallstones in the studied population follows : pigment stones (37.2 %) > Mixed stones (34.8 %)>cholesterol stones (28.0 %). Similar finding observed of some authors from Haryana [22] , Kanpur[23] , Aligarh regions[24]. But different in some regions like Japan [25] and Assam[26] .This may be due to different dietary conditions and habitats and different socio-economic status of the people in these areas[24] .

On analyzing the results of our study (Table-1; Figure 1), we observed that the size of cholesterol stones were bigger as compared to mixed stones and pigment stones. This is in agreement with studies conducted by Raha P.K. et al [27]. and Pundir C. S, et al [28]. The shape and surface of the collected gallstones specimens were of variable, out of 43 gallstones irregular and round shape gallstones were observed 26 and 17, smooth and rough surface gallstones were collected 28 and 15.

From table 1; Figure 2 we have been observed that gallstone disease was predominantly seen in females (79.0%) as compared to males (21.0%), in a ratio of female: male was (3.8:1). This is in agreement with studies conducted by other authors [22, 29]. The exact cause of increase tendency to formation of gallstones in female was not identified. Some authors believes that under the influence of female sex hormones, the muscle may relax which causes to dilate biliary passage and duodenal content of pancreatic secretion regurgitates into gallbladder and promote conditions which favour the formation of gallstones [30]. Another study suggested that the female are at higher risk of cholelithiasis development than male because of high percentage of female with high risk factors such as multiparty, use of oral contraceptives and obesity [31]. It has also been observed that the commonest age group for the development of cholelithiasis in our study was 37-46 years.

Chemical Analysis of Gallstones: The cholesterol, bilirubin, triglycerides, phospholipids, bile acids, calcium, magnesium, oxalate and inorganic phosphate present in all the 3 type of gallstones were analyzed. The results of quantitative analysis of the constituents of 43 gallstones are expressed as mg/gm dry wt. of stone powder (Table- 2 & 3; figure: 3 a,b,c).

Cholesterol stones (CS): Cholesterol content was found to be highest in CS. This is because the cholesterol saturation index is more than one between cholesterol and bile salts [32] . In our study, the total cholesterol was significantly higher in cholesterol stones, compared to pigment stones (p= 0.007). However, there was an insignificant difference between total cholesterol content of cholesterol stones and mixed stones (p= 0.076) [Table 1 & 2; figure: 3(a)]. Similar results observed by P.Chandran et al.[22], Pundir CS [28]. Several studies suggests that formation of cholesterol stones have been related to high carbohydrate diet, which lead to cholesterol supersaturation in the bile[33,34]. Atherosclerosis, hyperlipidaemia, and hyperinsulinism[35] and also gallbladder hypomotility and mucus hyper secretion have been encountered as risk factors for cholesterol gallstone disease[36]. From table-1 it has also been observed that cholesterol containing stones were more frequent in female than in male. This is in line reports from other countries, such as Saudi Arabia [34] and Korea [37].

Phospholipid content was also highest in CS. There were significantly more phospholipids in CS than in MS (p=0.001) and PS (p=0.009).The difference was insignificant between pigment stones and mixed stones (p>0.05). This might be due to accumulation of phospholipids along with cholesterol during CS formation. The accumulation of phospholipids could either be due to their enhanced biosynthesis or decreased utilization. These results are comparable to reports from Rohtak [22].

The inorganic phosphate content was highest in cholesterol stones and lowest in pigment stone. However, there was a significant difference between cholesterol stone and mixed stone (p = 0.02) and cholesterol stone and pigment stone (p=0.001).It is likely that phosphorus may play a more important role than calcium in CS formation by forming a salt with calcium, which might be responsible for the hardness of the CS [38].

Bile acids, calcium and oxalate content in cholesterol stone was significantly different than mixed stone (p = 0.026, p=0.009, p=0.02). But there was no significant difference (p>0.05) between magnesium content of Cholesterol stones vs Mixed stones (p= 0.6). The other component of cholesterol stones likes bilirubin; bile acids, calcium, magnesium and oxalate contain less amount than pigment stones. The triglyceride content was also lowest in cholesterol stones in comparison to mixed stones [Table 2 & 3; figure 3(b) & 3 (c)].

Pigment stones: The bilirubin content was highest in pigment stone as compared to cholesterol stone and mixed stones. Other authors from Haryana [28] Delhi [39], and Kanpur [40] also reported similar observation. Decreased secretion of biliary acids, increased secretion of unconjugated bilirubin into the bile, and infection of the biliary tract are the most important causative factors [41]. In addition, beta glucuronidase of bacterial origin of conjugated bilirubin, forming free bilirubin in the form of calcium bilirubinate salt [42].

The bile acid content in pigment stones was significantly higher than that in cholesterol stone (p= 0.039). But the difference was insignificant between mixed stone versus pigment stone (p=0.192).

The calcium content in various gallstones was in the following order. Pigment stone > Mixed stone > Cholesterol stone. The mean calcium content was significantly higher in pigment stone as compared to cholesterol stone (p = 0.001) and mixed stone (p = 0.009). This is in conformity with observations made by others [40, 43]. It is known that bilirubin combines with calcium to form a precipitate of calcium bilirubinate. Since PS has excess bilirubin, calcium forms calcium bilirubinate [44] .

As like calcium, the magnesium content in various gallstones in the following order; Pigment stone > Mixed stone > Cholesterol stone. But there was no

significant difference (p>0.05) between magnesium content of pigment stones vs Mixed stones (p= 0.07). However, the difference was significant between mixed stone versus pigment stone (p= 0.01). This is in conformity with observations made by Chandran et al [22].

The oxalate content was highest in pigment stone and lowest in mixed stone. There was no significant difference of oxalate content between cholesterol stone and pigment stone (p = 0.09) However, there was a significant difference between pigment stone and mixed stone (p = 0.003). Since the higher oxalate content in PS is likely to be associated with higher magnesium content, the formation of magnesium oxalate may be responsible for the hardness of PS. The triglyceride content in pigment stones was also significantly different as compared to cholesterol stones (p= 0.026).

Cholesterol, triglyceride, phospholipids, inorganic phosphate content in pigment stones were lesser than cholesterol stones and mixed stones.
Mixed stones

The triglyceride content was highest in mixed stones as compared to cholesterol stones and pigment stones. The content of triglyceride in mixed stones was highly significant (p=0.001) in comparison to pigment stones. However, the difference was insignificant between cholesterol stones and mixed stones (p>0.05). These observations are in agreement with Abdalla M. Jaraari et al[45]. The triglycerides get accumulated along with cholesterol salts to form gallstones. In mixed stone or cholesterol stone compared to pigment stone the higher content of triglyceride might be due to more deposition of calcium salts of cholesterol and esters of fatty acids in mixed and cholesterol stone as compared to pigment stone, where calcium bilirubinate is the major salt [45]. Cholesterol, calcium and oxalate content in mixed stone were significantly different in comparison to pigment stones. There was no significant difference of bilirubin, bile acids, phospholipids, magnesium and inorganic phosphorous were observed between mixed stone and pigment stone (p>0.05) (Table 2 & 3; figure 3 a,b,c).

Conclusion: In our study gall stones were in following order; PS > MS > CS and more common age group for development of gall stones was 37-46 years and female was more predominant than male. Total cholesterol was a major component of cholesterol, mixed and pigment gall stones in Rohilkhand region of Uttar Pradesh. The content of the other metabolites in different gall stones indicating their different mechanism of formation. High cholesterol and triglyceride content in CS and MS suggests that dyslipidemic changes contribute to etiology.
Our studies suggest that dyslipidemia consequent to high intake of fats by Rohilkhand region population may be responsible for gallstones, and dietary modification might reduce the incidence of gallstones.

References:

1. Everhart JE, Khare M, Hill M, Maurer KR. Prevalence and ethnic differences in gallbladder disease in the United States. Gastroenterology. 1999; 117 (3): 632. 2.
2. Russel RCG , Williams NS, Blustrade GJK – Bailey stones. Short Practice of surgery , 23 Edition Arnold Publishers , 2009,74 – 973.
3. S.Sikkandar1, S. Jayakumar, S. Gunasekaran, T.S .Renugadevi and B.Alwar. Study on the Analysis of Human Gallstones using Fourier Transform Infrared Spectroscopic Technique. International Journal of ChemTech Research. Jan-Mar 2011Vol.3, No.1, pp 149-154.
4. Gall bladder Problems" St. Lukes Episcopal Hospital. March 1998. Regi.3.
5. Berci G. Historical overview of surgical treatment of biliary stone disease. In: MacFadyen BV, Arregui M, Eubanks S, Olsen DO, Peters JH, Soper NJ, et al., editors. Laparoscopic surgery of the abdomen. New York (NY): Springer; 2004. pp. 139–142.
6. Ahmed, M.D.Ramsey.C .Cheung M.D. and Emmet B. Keeffe,M.D. Management of Gallstones & their Complication Stanford University school of Medicine, California Published in American Family Physician Clinical Journal, March,2000; 15.
7. GokulaKrishnan S, Murugesan R, Mathew S,Prasanthi R,Ashok AC, Ramesh H et al. Predicting the composition of gall stones by

infrared spectroscopy. Trop Gastroentero 2001; 22: 87-89.
8. Kratzer W, Mason RA, and Kachela V. Prevalence of gallstones in Sonographic Surveys worldwide. J. Clin. Ultrasound 1999,27,1-7.
9. Anamika Gaharwar. Factors Favouring Cholelithiasis in North Indian Population. IOSR Journal Of Pharmacy. Volume 3, Issue 5 (June 2013), Pp 01-03.
10. Johnson DE, Kaplan MM. Pathogenesis and treatment of gallstones. New Engl J Med 1993;328:412-21.
11. Jayanthi V, Palanivelu C, Prasanthi R, Methew S, Srinivasan V. Composition of gallstones in Coimbatore district of Tamil Nadu State. Ind J Gastroenterol 1998;17:134-35.
12. Malhotra SL. Epidemiological study of cholelithiasis among railroad workers in India with special reference to causation. Gut 1968;9:290-95.
13. Tandon RK. Pathogenesis of gallstones in India. Trop Gastroenterol 1988; 9:83-91
14. Allain C.C., Poon L.S., Chan C.S.G., Richmond W. and FuP., Clin. Chem., 1974. 20 (470).
15. Pearlman, P.C. & Lee, R.T. Clin.Chem. 1974, 20:447.
16. Fossati P. Ann Clin Biochem 1969; 6; 24-7.
17. Pundir CS. Purification and properties of an oxalate oxidase from leaves of grain sorghum hybrid. Biochim Biophys Acta.1993; 1161: 1-5.
18. Gindler EM & King J.D. Am J. Clin Pathol. 1972; 58: 376.
19. Carey JB. The serum trihydroxy-dihydroxy bile acid ratio in liver and biliary tract disease. J Clin Invest 1958; 17:1494-1502.
20. Fiske CH, Subba Row Y. The colorimetric determination of phosphorous. J Biol Chem. 1925; 66: 375- 400.
21. Gindler EM, Heth DA. Colorimetric determination with bound calmagite of magnesium in human blood serum. Clin Chem. 1971; 17: 662.
22. P.Chandran, N. K. Kuchhal, P. Garg* and C.S. Pundir. AN EXTENDED CHEMICAL ANALYSIS OF GALLSTONE.Indian Journal of Clinical Biochemistry, 2007 / 22 (2) 145-150
23. Tyagi SP, Tyagi N, Maheshwari V, Ashraf SM, SahooP.Morphological changes in diseased gall bladder: A study of 415 cholecystectomies at Aligarh. J Ind Med Assoc 1992; 90: 178-81
24. Bansal SK, Gupta SK, Bansal A, Rajput VS, Joshi LD. Chemical composition of Biliary Calculi from Kanpur region.Ind J Clin Biochem1992; 7: 27-9
25. Nakayama F. Quantitative microanalysis of gallstones. J Lab Clin Med 1968; 72: 602-11
26. Goswami M. An analysis of 160 cholecystectomies at Guwahati.Ind J Surg 1999; 61: 252-5
27. Raha PK, Sengupta KP, Aikat BK. X-Ray Diffraction analysisof gallstones. Ind J Med Res1966; 54: 729-34
28. Pundir CS, Chaudhary R, Rani K, Chandran P, Kumari M,Garg P. Chemical analysis of biliary calculi in Haryana. Ind JSurg 2001; 63: 370-73.
29. Saadeldin Ahmed Idris, Kamal Elzaki Elsiddig, Mohamed Mahmoud Hafiz, Aamir Abdullahi Hamza, Mohammed H. F. Shalayel. Minerals' composition of different types of gallstones in Sudanese population. Open Science Journal of Analytical Chemistry.Vol. 1, No. 1, 2014, pp. 1-5.
30. Horn G. Observations on the aetiology of cholelithiasis. Bri Med J 1965; 2: 732.
31. Taher MA. Descriptive study of cholelithiasis with chemicalconstituents' analysis of gallstones from patients living in Baghdad, Iraq. International Journal of Medicine and Medical Sciences 2013; 5(1): 19-23.
32. Smith JL, Nathanson LK, Riottot M. Effect of statins on biliary lipids and cholesterol gallstones. J Fu¨ r Kardiologie. 2002; 9: 295-8.
33. Singh A, Bagga SPS, Jindal VP, Singh K, Rao SS. Gall bladder disease: An analytic report of 250 cases. J Ind Med Assoc. 1989;87: 253–656.
34. Bashir M Jarrar and Meshref A Al-Rowaili. Chemical Composition of Gallstones from Al-Jouf Province of Saudi Arabia. Malays J Med Sci. 2011 Apr-Jun; 18(2): 47–52.
35. Amigo L, Zanlungo S, Mendoza H, Miquel JF, Nervi F. Risk factors and pathogenesis of cholesterol gallstones: State of the Art. Eur Rev Med Pharmacol Sci. 1999;3(6):241–246.
36. Apstein MD, Carey MC. Pathogenesis of cholesterol gallstones: a parsimonious hypothesis. Eur J Clin Invest. 1996; 26: 343-52.
37. Kim E, Lee Y. A descriptive study of gall stone patient's dietary habits and nutritional status. Korean J Comm Nutr. 2007; 12(6): 826–877.
38. Udupa KN, Chansouria JPN, Gode JD, Gupta S. Studies on etiology of gallstone. Indian J Surg. 1968; 68: 120-8.

39. Kumar D, Garg PK, Tandon RK. Clinical and biochemical comparative study of different types of common bile duct stones. Ind J Gastroenterol 2001; 20: 187-90.
40. Bansal SK, Gupta SK, Bansal A, Rajput VS, Joshi LD.Chemical composition of Biliary Calculi from Kanpur region. Ind J Clin Biochem1992; 7: 27-9.
41. Lee SP, Lamont JT, Carey MC. Role of gallbladder mucus hypersecretion in the evolution of cholesterol gallstones studies in the Prairie dog. J Clin Invest. 1981; 67: 1712_23.
42. Lamont. Mucin glycoprotein content of human pigment stone. Hepatology 1983; 3: 372-82.
43. Verma GR, Pandey AK, Bose SM, Prasad R. Study of serum calcium and trace elements in chronic cholelithiasis. ANZ J Surg 2002; 72: 596-9.
44. Nakama T, Furusawa T, Itoh H, Hisadome T. Correlation of cholesterol and bilirubin solubilization in bile salt solution. Gastroenterol Jpn. 1979; 14: 565-72.
45. Abdalla M. Jaraari et al. Quantitative analysis of gallstones inLibyan patientsLibyan J Med 2010, 5: 4627.

Conflict of interest: None
Funding: None