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Biochemical Analysis of Gallstone in Patients Operated at BPKIHS.

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ABSTRACT

Gallstone (GS) is a crystalline concentration formed within the gallbladder by accretion of bile components. Cholesterol stones formed in gallbladder, while pigment stones mostly formed in the bile canaliculi of liver and mixed stones in entire biliary tract and ultimately grow and settle down in gallbladder. This study aimed to analyze the biochemical contents of gallstones and to differentiate the composition of different types of gallstones qualitatively. Thirty gall stone samples collected from the operation theatre after cholecystectomy by purposive sampling technique. Qualitative analysis done for Cholesterol, Oxalate, Calcium and Inorganic Phosphate but quantitative analysis done only for total Cholesterol. Data expressed on frequency, percentage, mean±SD and median (IQR). Parametric and non-parametric tests applied depending upon the nature of data considering $p \leq 0.05$ as statistically significant. Gall stones were identified by color; black (n=8), brown (n=3), green (n=5), white (n=4) and yellow (n=10), texture; rough surface (n=15) and smooth surface (n=15) and shape; bean (n=2) crystal (n=6), irregular (n=7), oval (n=5) and round (n=10. GS were classified as cholesterol stone (n=14), mixed stone (n=5) and pigment stone (n=11). The total cholesterol concentration in GS was 572.18±226.73 mg/dL and median (IQR) of weight of stone and Cholesterol content in stone were 0.59 (0.28, 1.30) mg and 0.35 (0.12, 0.74) gm respectively. Cholesterol stone is the most commonly occurring stones in the studied population consistent with other reports.

Keywords: Gall stone, Cholesterol stone, Gall Stone Analysis, Mixed Stone, Pigment Stone

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INTRODUCTION

Gallstone (GS) is a crystalline concentration formed within the gallbladder by accretion of bile components. Presence of stones in the gallbladder is called cholelithiasis.[1] If GS migrate into the ducts of biliary tract; the condition is referred to as choledocholithiasis. GS within the ampulla of vater can obstruct the exocrine system of pancreas, which in turn can result in pancreatitis.[2]

GS are divided into the three different types cholesterol, pigment and mixed stone.[3] Cholesterol stones are light yellow to dark green or brown in color and oval in shape and2-3 cm long in length. It contains 80% cholesterol containing more than 50% of crystalline cholesterol monohydrate. Pigment stones are small in size, dark in color and comprise bilirubin and calcium salts found in bile. They contain less than 20% of cholesterol. Mixed stones typically contain 20-80% cholesterol and other common constituents are calcium carbonate, palmitate phosphate, bilirubin and other bile pigments. Because of their calcium content, they are often radio-graphically visible.

Cholesterol stones are formed in gallbladder, while pigment stones are mostly formed in the bile canaliculi of liver and mixed stones in entire biliary tract and ultimately grow and settle down in gallbladder.[4] Qualitative analysis will be done for cholesterol, calcium, oxalate, and phosphate in GS collected from patients operated at BPKIHS Dharan.[5] Chemical analysis of these GS has revealed that cholesterol stones contain cholesterol as a chief constituents, while pigment stones have calcium carbonate as principle constituents and mixed stones consist of salts of cholesterol and bilirubin as major constituents. The other substances found in GS are calcium salts of phosphates, carbonate, fatty acids, phospholipids and some trace elements like Sodium, Potassium, Copper, Magnesium, Manganese and Iron.[4] This study aimed to analyze the biochemical composition of gall stone

MATERIALS AND METHODS

A total of 30 gallstones from the patients were collected from surgery ward/operation theatre at BPKIHS, Dharan (Nepal), in an analytical study covering a three- month period in 2014. Out Of the 30 patients, 86.67% (n=26) were female and 13.33% (n=4) were male, with a mean age of 43.17±15.28 years ranges from 21 to 76 years.

Gallstones were collected in airtight plastic bag and stored them in dark and well ventilated place maintaining the ambient temperature. Gallstones were crushed into powder with mortar and piston. A pinch of powder was heated with small portions of ether in a test tube by inserting the tube in warm water. After heating, the mixture was filtered, evaporated off the ether.

Cholesterol was detected by Salkowski's reaction. In the detection of inorganic phosphate, calcium and oxalate in gallstone, the residue remaining after ether extraction was treated with 25% Hydrochloric acid to dissolve any inorganic salts and filtered and this filtrate was divided into three parts. In the three aliquots presence of inorganic, presence of calcium and presence of oxalate were detected. Quantitative estimation of total cholesterol in gallstone was done by using cholesterol oxidase peroxidase method using commercial test kit.

RESULTS

Gallstones were identified by color, shape, texture as their physical characteristics and sex of the patients. Biochemical analysis was done to find out the composition stones. In this study, stones were analyzed for presence or absence of cholesterol, calcium, inorganic phosphate and oxalate.

The color of stones were recorded as black (n=8), brown (n=3), green (n=5), white (n=4) and yellow (n=10). Texture of stones were found through rough surface (n=15) and smooth surface (n=15). Shape of the gall stones were identified as bean (n=2), crystal (n=6), irregular (n=7), oval (n=5) and round (n=10) shaped. Cholesterol was detected in all stone samples but oxalate was not detected in all samples. Calcium was detected in 16 stone samples and inorganic phosphate was detected in 23 stone samples. Gallstones were classified based on color and cholesterol content. Gallstone collected in this study were cholesterol stone (n=14), Mixed stone (n=5) and Pigment stone (n=11) (Table 1).

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Table 1: General Characteristics of the stone

Group	Subgroups	Number of Stone
Sex	Male	4
	Female	26
Color of Stone	Black	8
	Brown	3
	Green	5
	White	4
	Yellow	10
Texture	Rough Surface	15
	Smooth Surface	15
Shape	Bean	2
	Crystal	6
	Irregular	7
	Oval	5
	Round	10
Calcium	Present	16
	Absent	14
Inorganic Phosphate	Present	23
	Absent	7
Types of Stone	CS	14
	MS	5
	PS	11

Cholesterol stones were white and yellow, Mixed stones were green and Pigment stones were black and brown in color which was statistically significant (P<0.001). Calcium was detected in only few (n=2) cholesterol stones and not detected in majority (n=12) cholesterol stones but detected in all pigment stones (n=11) which was statistically significant (P<0.001). Inorganic phosphate was detected in all cholesterol stone (n=14) but detected in only five pigment stones which was statistically significant (P=0.006) (Table 2).

Table 1: Comparison of different parameters with types of stone.

Parameters		Types of Stone			P value*
		CS	MS	PS	
Sex	Male	0	1	3	0.123
	Female	14	4	8	
Color of stone	Black	0	0	8	<0.001
	Brown	0	0	3	
	Green	0	5	0	
	White	4	0	0	
	Yellow	10	0	0	
Texture of stone	Rough Surface	5	3	7	0.339
	Smooth Surface	9	2	4	
Calcium	Present	2	3	11	<0.001
	Absent	12	2	0	
Inorganic Phosphate	Present)	14	4	5	0.006
	Absent	0	1	6	
Shape of Stone	Bean	2	0	0	0.159
	Crystal	5	0	1	
	Irregular	4	1	2	
	Oval	0	2	3	
	Round	3	2	5	

*Chi- square test used with considering p<0.05 is statistical significant.

The total cholesterol was significantly higher in cholesterol stone, 774.01 mg/gm as compared to pigment (318.81 mg/gm) and mixed stone (564.44 mg/gm) (P<0.001). Median (IQR) in cholesterol stone

0.561(0.289-0.877) gram was significantly higher as compared to pigment 0.375(0.311-0.658) gm and mixed stones 0.114(0.770-0.541) (p=0.032) (Table 3).

Table 3: Comparison of different parameters in three types of stone.

Different parameters	Types of stone			P value
	CS	MS	PS	
Age	38±16	47±13	48±15	0.434[a]
Total Cholesterol (mg/dl)	774.0164±102.69	564.44±42.60	318.811±83.47	<0.001[a]
Weight of stone (mg)	0.685 (0.365-1.190)	0.650 (0.519-1.210)	0.390 (0.260-1.740)	0.429 [b]
Cholesterol in stone(gm)	0.561 (0.289-0.877)	0.375 (0.311-0.658)	0.114 (0.770-0.541)	0.32 [b]

[a] ANOVA, data expressed as mean± SD; p < 0.005 was considered as statistically significant.

[b] Kruskal Wallis test, data expressed as median (interquartile range Q_1-Q_3); p< 0.005 was considered as statistically significant.

DISCUSSION

Gallstone (GS) is a crystalline concentration formed within the gallbladder by accretion of bile components. Presence of stones in the gallbladder is called cholelithiasis.[1] If GS migrate into the ducts of biliary tract; the condition is referred to as choledocholithiasis. GS within the ampulla of vater can obstruct the exocrine system of pancreas, which in turn can result in pancreatitis.[2]

GS are divided into the three different types; cholesterol, pigment and mixed stone.[3] Cholesterol stones are light yellow in color, pigment stones are black or black-brown and mixed stones are greenish, red or brownish yellow in color. Pigment stones are mostly oval/round shaped having rough surface, Mixed stones have smooth surface mostly with varying shape and cholesterol stones have both types of texture either rough and smooth surface and are mostly crystal shaped. Chandran P et. al. (2007) had found that PS had rough surface, CS had smooth surface, while MS had both smooth and rough surface.[20]Cholesterol stone contains 54 to 94.4% cholesterol, mixed stone contains 30 to 70% cholesterol and pigment stone contains 20 to 57.78 % cholesterol. They are often radio-graphically visible, because of their calcium content.

Cholesterol stones are formed in gallbladder, while pigment stones are mostly formed in the bile canaliculi of liver and mixed stones in entire biliary tract and ultimately grow and settle down in gallbladder.[4]In this study qualitative analysis had been done for cholesterol, calcium, oxalate, and inorganic phosphate in GS collected from patients operated at BPKIHS Dharan. Calcium is detected in all PS compared to CS, detected in only few (n=2) which is statistically significant (p<0.001) .Inorganic phosphate is detected in all CS compared to PS, detected only in five stone which is statistically significant (p<0.001). The other substances found in GS are calcium salts of phosphates, carbonate, fatty acids, phospholipids and some trace elements like Sodium, Potassium, Copper, Magnesium, Manganese and Iron.[4]ShalayelFaris et.al (2013) had observed that the CS had highest composition of cholesterol, while MS had a high content of triglycerides and PS were composed mostly of bilirubin.[21]

The present study shows that, the most common types of stone collected are cholesterol stone. Out of 30 gallstones collected, 14 were cholesterol stones, 11 were pigment stones and 5 were mixed stones. The difference may be attributed to different dietary conditions and habitats and different socio-economic status of the people in these areas. Out of total stones collected, 26 were from female and four were from male. Rains (1964) advocated that concentration of bile salts in bile is reduced by estrogen and thereby making it lithogenic.[22]

Horn (1965)postulated that under the influence of female sex hormone, the muscle may relax, biliary passage dilates and duodenal content of pancreatic secretion regurgitate into gallbladder and promote conditions which favor the formation of GS.[23] Females had a greater risk of GS disease, especially if they had used oral contraception and/or had four or more children.[24]

In our study we revealed that total cholesterol was significantly higher in CS (774.01 mg/gm) as compared to pigment (318.81mg/gm) and mixed (564.44mg/gm) stones which is similar to a study done by Cuevas A et.al who stated that the total cholesterol was significantly higher in cholesterol calculi, compared to PS ($p<0.05$) and MS ($p<0.01$).[25] GS are believed to form, when the concentration of cholesterol exceeded in mixed micelle solution with bile acids and phospholipids. Super-saturation is the basis for gall stone formation composed of cholesterol. The high level of cholesterol in cholesterol stone has been related to high carbohydrate diet.[25]

Pradhan SB et.al (2009) had observed that MS was the commonest stone followed by CS and PS comprising 78.5%, 12.5% and 7.5% respectively.[26]In this study CS was the most common stone followed by PS and MS comprising 46.67%, 36.67% and 16.67% respectively which is comparable with study done by Kamiya T et. al. (1992) in Bolivia, in which the incidence of CS was 56.67%.[6] Studies done in Singapore and San Francisco, the incidence of PS comprised 12% and 30.5% respectively.[27]

The most common age group for cholelithiasis found to be 31-40 years (30%) with female predominance (M:F=1:6.5), similar findings were found by Pradhan SB.et.al. (2009).[26]Shrestha HG et.al (1991) found the higher incidence of cholelithiasis among younger age group of 20-30 years with male to female ratio 1:4.[28] and Maskey CP et.al (1990) in a retrospective study also found that the most commonest age group for cholelithiasis was below 30 years comprising 37.5%.[29]

CONCLUSION

Cholesterol stone found to be the commonest stone and there is a relation between stone types and gender: male patients predominantly tend to develop MS or PS while, female tend to develop all types of stones especially.

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