Original Research Article

Study of gall stone composition inNorth Karnataka

Kalghatgi S[1], K. Aravind[2], Mukund Kulkarni[3], Vipin T[4], Sanganal B[5]

[1]Kalghatgi S, Assistant Professor, Department of Surgical Gastroenterology, Karnataka Institute of Medical Sciences, Hubli, Karnataka, [2]K. Aravind, Associate Professor, [3]Mukund Kulkarni, [4]Vipin T, [5]Sanganal B, All authors are affiliated with Department of Surgical Gastroenterology, Karnataka Institute of Medical Sciences, Hubli, Karnataka, India.

Address for Correspondence: K Aravind, Email: dr.suhasrk@gmail.com

Abstract

Introduction:Gall stone disease is common problem in Indian subcontinent. While majority of gallstone disease are cholesterol in north India (similar to western world) they are predominantly pigment or mixed type in south India. There are recent reports of changing trends in composition of gallstones with a shift towards cholesterol gall stones especially in south Asian countries, and this has been attributed to changes in life style and dietary factors. **Material and Methods**: Descriptiveanalyticstudywere conducted on 39 gallstones from KIMS, HUBLI hospital were analysed by semiquantitative titrimetric and colourimetric methods over a period of 18 months from dec 2014 to 2016. The proportion of different types of gallstones was described using 95% confidence interval based on exact method. **Results**: The biliary calculi collected from 39 gallstone patients were divided into 3 groups based on their colour: cholesterol calculi, mixed calculi and pigment calculi. Out of the 39 stones collected , 15 were mixed calculi, 14 were pigment calculi and 10 were cholesterol calculi indicating the incidence of gallstones in the studied population from KIMS, Hubli as follows : Mixed calculi (38%) > pigment calculi (36%)> cholesterol calculi (26%). The incidence of gallstone was higher in age group 41-50 yrs followed by 51- 60 yrs and 31-40 yrs in which females were higher than males. **Conclusion:** A quantitative chemical analysis of total cholesterol, total bilirubin, fatty acids, triglycerides, phospholipids, bile acids, soluble proteins, sodium potassium, magnesium, copper, oxalate and chlorides of biliary calculi (10 cholesterol, 15 mixed and 14 pigment) retrieved from surgical operation of 39 patients from KIMS, Hubli hospital was carried out. Although total cholesterol was a major component of cholesterol, mixed and pigment gall stone in KIMS, Hubli, the content of most of the other lipids, cations and anions was different in different gall stones indicating their different mechanism of formation.

Keywords: Gall stone, Biliary calculi, Cholesterol, Calcium carbonate, Chloride.

Introduction

Gallstone is one of the diseases prevalent in developed nations, but it is less prevalent in the developing populations that still consume traditional diets [1]. Gallstone chemical analysis gives important evidences for the origin, aetiology, and the metabolic basis of its formation, and helps in the identification of risk factors that predispose certain individuals to the calculi formation. Many studies to identify risk factors for biliary lithiasis in the West have focused on hyper saturation of cholesterol in bile in the nucleation process, a critical step in the genesis of bile stones [2,3]. The high concentration of cholesterol in gallstones has been the basis for the widespread use of bile acids, a nonsurgical treatment for the dissolution of gall bladder stones. These stones account for as much as 80% of Western stones[3].

Manuscript Received: 4th October 2017
Reviewed: 14th October 2017
Author Corrected: 20th October 2017
Accepted for Publication: 25th October 2017

Unfortunately, gall bladder stone composition is heterogeneous, and differs within and without populations around the world [4-9].

Gallstone disease is increasing in India. In the west, the majority of stones can be dissolved with bile salts, since the major component is cholesterol. This medical therapy is expensive and not readily accessible to poor populations. It was therefore necessary to analyze the chemical composition of biliary stones in a group of patients, so as to make the case for introducing bile salt therapy.

Material and Material

Patients admittedwith a diagnosis of gall stones in the department of general surgery Karnataka Institute of Medical Sciences, Hubli over a period of 18 months from December 2014 to June 2016 will includes in study.

Study Design- Descriptive analytic design study was conducted using questionnaire to collect required data.

Inclusion criteria- patients with definitive diagnosis of gallstone based on ultra sonogram of abdomen

Exclusion criteria- Patients less than 15 yearsold, overt hemolysis, co morbidities like pancreatitis and those who are not willing for participation in the study.

Collection of data- Controls are age and sex matched asymptomatic subjects with a normal gall bladder from same population coming to KIMS Hubli for regular health check up or admitted in other department, taking ultra sonogram for purposes not related with gall stone. Cases (n = 39) from all age groups and both sexes with sonographically proven gallstones were recruited over a duration of 18 months from the surgical wards of a KIMS, HUBLI Hospital from December 2014 to 2016.

The stones were divided into 3 groups depending upon their colour: pale yellow and whitish stones as cholesterol calculi, black and blackish brown as pigment calculi and brownish yellow or greenish with laminated features as mixed calculi. The other relevant information about the patients such as age, sex and number of calculi were obtained from hospital records. The various physical parameters of stones such as number, shape, size, texture and cross-section were noted. The stones were powdered in a pestle and mortar and dissolved in different solvents depending upon the type of chemical constituent to be analyzed. To determine total cholesterol and total bilirubin, 30mg stone powder was dissolved in 3 ml chloroform in a test tube. The tube was kept in boiling water bath for 2 min. The stone solution thus obtained was used for determination of total cholesterol and total bilirubin. To determine calcium, oxalate, inorganic phosphate, magnesium, chloride, soluble protein, triglycerides, iron, copper, sodium and potassium, 30 mg stone powder was dissolved in 3 ml IN HCl in 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 hr. To analyze phospholipids, stone powder (20 mg) was dissolved in 15 ml CHCl3+CH3OH in 2:1 ratio, containing 1N HCl. To measure bile acids and fatty acids, the stones were dissolved in chloroform-methanol (2:1) mixture and ethyl alcohol-solvent ether in (3:1 mixture) respectively. Total cholesterol by enzymic colorimetric method of Bayer Diagnostic India Ltd [10], total bilirubin by colorimetric method method of Accurex Biomedical Pvt. Ltd [11], triglycerides by enzymatic colorimetric method of Bayer Diagnostics India Ltd [11], soluble protein by colorimetric method of Lowry et al [12].

Statistical analysis- Statistical analyses were performed with Graph Pad software (Graph Pad Software Inc., USA). The unpaired t-test was used for comparison of group means. A p-value of <0.05 was considered significant. Descriptive analysis was done using, mean and standard deviation for quantitative variables, frequency and percentages for categorical variables. The comparison between explanatory and outcome variables was assessed by comparing the means for quantitative variables and proportions for categorical variables. Independent sample t-test and chi square test were used appropriately to assess the statistical significance. Appropriate graphs like, bar diagram and pie diagram were used to visually represent the data. IBM SPSS version 21 was used for statistical analysis

Results

The biliary calculi collected from 39 gallstone patients were divided into 3 groups based on their colour: cholesterol calculi, mixed calculi and pigment calculi. Out of the 39 stones collected, 15 were mixed calculi, 14 were pigment calculi and 10 were cholesterol calculi indicating the incidence of gallstones in the studied population from KIMS, Hubli as follows : Mixed calculi (38%) > pigment calculi (36%)> cholesterol calculi (26%).

Table No.-1: Incidence of different types of gallstones in relation to age.

Age group (yrs)	Cholesterol	Mixed	Pigment	Total gallstones
1-10	-	-	-	-
11-20	1	-	1	2
21-30	1	3	1	5
31-40	2	2	4	8
41-50	4	4	3	11
51-60	2	2	4	8
>60	1	1	2	4
Total	**10**	**15**	**14**	**39**

Out of the total number of stones collected, the incidence of gallstone was higher in age group 41-50 yrs followed by 51- 60 yrs and 31-40 yrs.

Table-2: Descriptive analysis of cases & controls in study group (N=79)

Cases & Controls	Frequency	Percent
Cases	39	49.4
Controls	40	50.6

Out of the 79 subjects, 39 were cases and remaining 40 were controls.

Table-3: Comparison of Age and gender parameters across the two study groups (N=79)

Parameter	Cases(N=39)	Controls(N=40)	P-value
Age	45.56±14.65	40.20±15.79	0.12
Female: Male	1:0.25	1:0.29	0.83

Above table shows mean age was 45.56 ±14.65 for gall stones and females were higher than males.

Table-4: Comparison of bilirubin parameters across the two study groups (N=79).

Parameter	Cases(N=39)	Controls(N=40)	P-value
Total protein	8.25±8.54	7.88±8.50	0.85
Albumin	3.75±0.64	3.48±0.733	0.08
Total Bilirubin	1.42±2.17	0.68±0.33	0.03
Direct Bilirubin	0.67±1.03	0.31±0.22	0.03
AST	45.40±36.73	34.57±17.51	0.09
ALT	40.23±49.34	24.63±17.69	0.06
ALP	131.20±120.07	77.37±33.65	0.008

The mean total protein at cases was 8.25±8.54 and 7.88±8.50 was at controls which was statistically not significant. The mean albumin was 3.75±0.64 at cases and 3.48 at controls with a p value 0.08 which was not significant.

Table-5: Quantitative analysis of metabolites in different types of biliary calculi expressed as mg/gm dry stone powder.

Parameter	Mean±STD	Median	Max	Min	95% C.I.for EXP(B)	
					Lower	Upper
Weight in Gms	5.58±13.82	2.00	59.0	1.0	-1.51	12.69
Cholesterol	13.45 ±30.45	1.46	110.00	0.12	-2.20	29.10
TG mg/dl	7.04±18.23	1.90	76.90	0.11	-2.33	16.41
Bilirubin	0.81 ± 0.75	1.00	2.10	0.01	0.42	1.20
Protein	2.01 ±1.40	2.10	4.8	0.2	1.29	2.74
Chloride	98.84 ±73.17	63.00	196.0	0.72	61.22	136.46
Magnesium	2.66 ± 1.84	2.15	6.08	0.82	1.72	3.61
Phosphorous mg/dl	31.93±61.56	2.90	200.18	0.00	0.28	63.58
Calcium	16.20 ±8.28	20.05	29.00	0.04	11.95	20.46
Copper	444.50±721.11	179.90	2535	14.40	73.73	815.26
Iron	530.83 ±414.57	732.50	998.6	6.4	317.67	743.99

The mean weight of the gall stone was 5.58 ±13.01 in study population. The mean cholesterol was 13.45±30.45 and 7.04±18.23 was triglycerides in study population. The mean bilirubin was 0.81±0.75 and the mean protein was 2.01± 1.40 respectively. The mean Chloride, magnesium and phosphorouswere 98.84 ± 73.17, 2.66±1.84 and 31.93±61.56 respectively in study population.

Discussion

The biliary calculi collected from 39 gallstone patients were divided into 3 groups based on their colour: cholesterol calculi, mixed calculi and pigment calculi. Out of the 39 stones collected, 15 were mixed calculi, 14 were pigment calculi and 10 were cholesterol calculi indicating the incidence of gallstones in the studied population from KIMS, Hubli as follows : Mixed calculi (38%) > pigment calculi (36%)> cholesterol calculi (26%), which is similar to study done by chandran et al [13] where they have reported quantitative chemical analysis of total cholesterol bilirubin, calcium, iron and inorganic phosphate in 120 gallstones from haryana. To extend this chemical analysis of gall stones by studying more cases and by analyzing more chemical constituents.

A quantitative chemical analysis of total cholesterol, total bilirubin, fatty acids, triglycerides, phospholipids, bile acids, soluble proteins, sodium potassium, magnesium, copper, oxalate and chlorides of biliary calculi (52 cholesterol, 76 mixed and 72 pigment) retrieved from surgical operation of 200 patients from Haryana state was carried out.

Total cholesterol as the major component and total bilirubin, phospholipids, triglycerides, bile acids, fatty acids (esterified), soluble protein, calcium, magnesium, iron, copper, sodium, potassium, inorganic phosphate, oxalate and chloride as minor components were found in all types of calculi.

Jaraari AM et al [14] study was conducted to determine the composition of gallstones and their possible etiology in a Libyan population. The chemical composition of gallstones from 41 patients (six males and 35 females) was analyzed. The stones were classified into cholesterol, pigment, and mixed stones (MS). Cholesterol stones (CS) showed a significantly higher cholesterol content than pigment stones (PS) (p=0.0085) though not significantly higher than MS, which is similar to our study.

In our study, Out of the total number of stones collected, the incidence of gallstone was higher in age group 41-50 yrs followed by 51- 60 yrs and 31-40 yrs. mean age was 45.56 ±14.65 for gall stones and females were higher than males. similarly Hussain SM et al[15] found that the highest incidence of gallstones in the age group 40-49 was 13 cases followed by 11, 8 and 4 cases for age groups 30-39, 50-59, 20-29 and 60 and above, respectively. The chemical analysis showed the majority of gallstones were mixed, 38 containing calcium followed by 37 cases with uric acid, 28 with magnesium, and 25 and 22 stones with oxalate and phosphate, respectively.

Atamanalp SS et al [16] found that High serum cholesterol and LDL levels were associated with high cholesterol stone rates (86.7% vs. 40.0%, $P < 0.001$; 75.0% vs. 48.9%, $P < 0.05$, respectively). Similarly, high serum cholesterol and LDL levels were correlated with high gallbladder stone cholesterol concentrations (63.6% vs. 44.4%, $P < 0.001$; 62.3% vs. 46.0%, $P < 0.001$, respectively).

In contrast, low serum HDL levels do not seem to affect the occurrence of gallbladder cholesterol stones (60.0% vs. 58.3%, respectively, $P > 0.05$) or gallbladder stone cholesterol concentrations (50.8% vs. 52.4%, respectively, $P > 0.05$). Cholesterol content was found to be highest in CS. This is because the cholesterol saturation index is more than 1 between cholesterol and bile salts [17].

The finding that the highest cholesterol content was in CS reiterates that these type of stones are formed primarily because of supersaturation of cholesterol in the bile, which precipitates as a stone.

The co-existence of nucleating factors, gallbladder hypomotility [18], and mucus hyper secretion also contribute to cholesterol precipitation leading to the development of gallstones [18]. Triglyceride content was higher in MS than in the other two types of stones, but the difference was significant only compared to PS (p=0.0004). Triglycerides accumulate along with cholesterol salts to form gallstones.

The higher content of triglycerides in MS or CS compared to PS might be due to a higher deposition of calcium salts of cholesterol and esters of fatty acids in MS and CS when compared to PS in which calcium bilirubinate is the major salt [19].

The incidence of gallstones increases with an increase in age, with females more likely to form gallstones than males. Age 50 – 65 approximately 20% of women and 5% of men have gallstones. Overall, 75% of gallstones are composed of cholesterol, and the other 25% are pigmented. Despite the composition of gallstones the clinical signs and symptoms are the same[20].

Ramana Ramya J et al [21] foundRepresentative gallstones from north and southern parts of India were analyzed by a combination of physicochemical methods: X-ray diffraction (XRD), infrared spectroscopy (IR), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), CHNS analysis, thermal analysis and Nuclear Magnetic Resonance (NMR)

spectroscopy (^{1}H and ^{13}C). The stones from north Indian were predominantly consisting of cholesterol monohydrate and anhydrous cholesterol which was confirmed by XRD analysis. FTIR spectroscopy confirmed the presence of cholesterol and calcium bilirubinate in the south Indian gallstones.

Jayanthi V et al [22] Gallstones (GS) in south India (SI) are predominantly pure pigment or mixed, while in North India (NI), these are either pure cholesterol or mixed. While cholesterol rich gallbladder (GB) bile predicts cholesterol GS, constituent of bile in primary pigment GS is not known.

Gallbladder bile in controls and patients with GS from north India had significantly high cholesterol concentration. In south India, patients with mixed GS had cholesterol rich bile while pigment GS had higher concentrations of bile salts.

Conclusion

Total cholesterol as the major component and total bilirubin, phospholipids, triglycerides, bile acids, fatty acids (esterified), soluble protein, calcium, magnesium, iron, copper, sodium, potassium, inorganic phosphate, oxalate and chloride as minor components were found in all types of calculi.

The cholesterol stones had higher content of total cholesterol; phospholipids, fatty acids (esterified), inorganic phosphate and copper compared to mixed and pigment stones.

Further, considering that cholesterol levels in the gallstones mirrors the serum cholesterol levels, health issues associated with increased cholesterol levels, such as cardiovascular diseases, might be associated. However, larger randomized studies are required to study this association and to confirm these observations.

Conflict of interest: None declared.
Funding: Nil, **Permission from IRB**: Yes

References

1. Alsaif MA. Variations in dietary intake between newly diagnosed gallstone patients and controls. Pakistan J Nut. 2005; 4(1):1–7.

2. Bartoli E, Capron JP. Epidemiology and natural history of cholelithiasis.Rev Prat. 2000 Dec 1;50(19):2112-6.

3. Kalloo AN, Kantsevoy SV. Gallstones and biliary disease. Prim Care. 2001 Sep;28(3):591-606, vii.

4. Kratzer W, Mason RA, Kächele V. Prevalence of gallstones in sonographic surveys worldwide. J Clin Ultrasound. 1999 Jan; 27(1):1-7.

5. Walker TM, Hambleton IR, Serjeant GR. Gallstones in sickle cell disease: observations from The Jamaican Cohort study. J Pediatr. 2000 Jan;136(1):80-5.

6. Akute OO, Marinho AO, Kalejaiye AO, Sogo K. Prevalence of gall stones in a group of antenatal women in Ibadan, Nigeria. Afr J Med Med Sci. 1999; 28(3-4): 159–161.

7. Kalloo AN, Kantsevoy SV. Gallstones and biliary disease. Prim Care. 2001 Sep;28(3):591-606, vii.

8. Stringer MD, Taylor DR, Soloway RD. Gallstone composition: are children different? J Pediatr. 2003 Apr; 142 (4):435-40.

9. Kim MH, Lim BC, Myung SJ, Lee SK, Ohrr HC, Kim YT, Roe IH, Kim JH, Chung JB, Kim CD, Shim CS, Yun YB, Min YI, Yang US, Kang JK. Epidemiological study on Korean gallstone disease: a nationwide cooperative study. Dig Dis Sci. 1999 Aug; 44(8):1674-83.

10. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol.Clin Chem. 1974 Apr;20(4):470-5.

11. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem. 1973 May;19(5):476-82.

12. Lowry OH, Rosebrough NI, Farr Al, Randall RJ. Protein measurement with the Folin phenol reagent.J Biol Chem. 1951 Nov;193(1):265-75.

13. Chandran P, Kuchhal NK, Garg P, Pundir CS. An extended chemical analysis of gallstone.Indian J Clin Biochem. 2007Sep;22(2):145-50.doi:10.1007/BF02913334

14. Jaraari AM, Jagannadharao P, Patil TN Quantitative analysis of gallstones in Libyan patients. Libyan J Med. 2010 Jan 7;5. doi: 10.4176/091020.

15. Hussain SM, Al-Jashamy KA. Determination of chemical composition of gallbladder stones and their association with induction of cholangiocarcinoma. Asian Pac J Cancer Prev. 2013;14(11):6257-60.

16. Atamanalp SS, Keles MS The effects of serum cholesterol, LDL, and HDL levels on gallstone cholesterol concentration. Pak J Med Sci. 2013 Jan;29(1):187-90.

17. Smith JL, Nathanson LK, Riottot M. Effect of statins on biliary lipids and cholesterol gallstones. J Für Kardiologie. 2002;9:295–8.

18. Portincasa P, Di Ciaula A, Vendemiale G, Palmieri V, Moschetta A, Vanberge-Henegouwen GP, Palasciano G. Gallbladder motility and cholesterol crystallization in bile from patients with pigment and cholesterol gallstones. Eur J Clin Invest. 2000 Apr; 30(4):317-24.

19. AulakhR, Mohan H, Attri AK, Kaur J, Punia RP. A comparative study of serum lipid profile and gallstone disease. Indian J Pathol Microbiol.2007Apr;50(2):308-12.

20. Hassler KR, Jones MW. Gallbladder, Cholecystectomy, Laparoscopic. Stat Pearls [Internet]. Treasure Island (FL): Stat Pearls Publishing; 2017-. 2017 Oct 16.

21. Ramana Ramya J[1], Thanigai Arul K Chemical and structural analysis of gallstones from the Indian subcontinent. Mater Sci Eng C Mater Biol Appl. 2017 Sep 1;78:878-885.

22. Jayanthi V, Sarika S, Varghese J, Vaithiswaran V, Sharma M, Reddy MS, Srinivasan V, Reddy GM, Rela M, Kalkura S. Composition of gallbladder bile in healthy individuals and patients with gallstone disease from north and South India. Indian J Gastroenterol. 2016 Sep;35(5): 347-353. Epub 2016 Sep 16.

.....

How to cite this article?

Kalghatgi S, K. Aravind, Mukund Kulkarni, Vipin T, Sanganal B. Study of gall stone composition inNorth Karnataka. Surgical Update: *Int J surg Orthopedics.* 2017;3(4):93-98.doi:10.17511/ijoso.2017.i04.01.

.....