Original Article

Comprehensive Analysis of Gallstone Composition and Association with Risk Factors in Bangladesh: Insights into the burden and outlook for prevention and management.

Farhad-Ul-Hassan ASM[1] , Hossain ABMB[2] , Akther SMQ[3], Ahmed[4] , Islam MJ[5], Suma SA[6], Khan MS[7]

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ABSTRACT:
Gallstone is as ancient as human civilization. It has been found in the gallbladders of Egyptian mummies, dating back to 1000 BC. Gallstone is one of the diseases prevalent in developed nations but it is less prevalent in the developing populations that still consume traditional diets. Gallstones are formed in the gallbladder, from the constituents of bile cholesterol and calcium bilirubinate are the main chemical compounds present in gallstones and their precipitation in bile is induced by multiple aetiological factors. The prevalence and the chemical composition of gallstones vary from population to population. This indicates the involvement of multiple risk factors in the pathogenesis of gallstones. Therefore investigation of the chemical composition of gallstones is primarily important to recognize the correlation with the risk factors for gallstones disease in a community.

Key Words:
Gallstone,Chemical composition

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1. Junior Consultant, Department of Surgery, Shaheed Suhrawardy Medical College Hospital
2. Principal and Professor Of Surgery, Green Life Medical College Hospital
3. Professor and Head, Department of Surgery, Shaheed Suhrawardy Medical College Hospital
4. Associate Professor and Head, Department of Hepatobiliary Surgery, Shaheed Suhrawardy Medical College Hospital
5. Junior Consultant, Department of Surgery, Shaheed Suhrawardy Medical College Hospital
6. Lecturer, Department of Anatomy, Shaheed Suhrawardy Medical College
7. Resident, Department of hepatobiliary and pancreatic Surgery, Shaheed Suhrawardy Medical College Hospital, Dhaka.

Correspondence: Dr. A.S.M Farhad-Ul-Hassan (Author), Email:dr.farhadul.hassan @gmail.com, Mobile: +01716505270, Junior Consultant, Shaheed Suhrawardy Medical College Hospital, Sher-e-Bangla Nagar, Dhaka-1207

Introduction:

Gallstone disease is one of the most prevalent gastrointestinal diseases with a substantial burden to health care systems that is supposed to increase in ageing populations at risk. Aetiology, pathogenesis and correlations of risk factors of gallstones still not well defined, so exact chemical composition is very important to identify the aetiopathogensis and relations with risk factors of gallstone disease[1]. Cholelithiasis or gallbladder stones are one of the major surgical problems in many hospital admission world wide and need surgical interventions. This problem is probably related to obesity, cardiovascular disorders (CVD), metabolic syndrome, and dietary habits, especially excessive consumption of faty food, which is known to contain large amounts of cholesterol [2]. Obese individuals with a BMI-30 kg/m2 have 95% cholesterol-dominant gallstones and are at a high-risk for cholesterol stones. A quantitative chemical analysis of total cholesterol, total bilirubin, fatty acids, triglycerides, phospholipids, bile acids, soluble proteins, calcium, sodium potassium, magnesium, copper, oxalate and chlorides as minor components were found in all types of gall stone [3].Studies on gallstone composition carried out in different parts of the world indicate a close link with

dietary habits and ethnicity [4] .Other studies have shown that dietary intake of total calories in the form of carbohydrates and fats were associated with high triglyceride levels in gallstone patients. Gallstone formation is relatively increased with consumption of dietary fats rich in saturated fatty acids[5].Gallstones might induce biliary inflammation and cholecystectomy is typically followed by chronic calculus cholecystitis. This study describes an extensive quantitative analysis of gallstones, including calcium, magnesium, phosphate, uric acid and oxalate and their association to risk factors of gallstone formation. Increased patho-physiological interest in the formation of gallstones made the knowledge of their exact chemical composition very important for all types of gallstones. Analysis of chemical composition of gallstones can provide a significant reference to the treatment and prevention[6] .With regard to chemical composition the majority of human gallstones are cholesterol and pigment stones. Cholesterol gallstones consist of more than 70% cholesterol and also a small amount of pigment residues and trace elements. Pigments gallstones consist of more than 75% of pigments and also a small amount trace elements and mixed stones consist of cholesterol and pigments. Other substances found in gallstones include calcium salts of phosphate, mucin, glycoprotein, phospholipids and some metals in all types of gallstone. It has been reported that some elements play a significant role in the formation of gallstones. It was observed that 14 minor or trace elements namely Sulfur, Chloride, Potassium, Vanadium, Chromium, Manganese, Iron, Nickel, Copper, Zinc, Bromide and Lead were present in the gallstones collected from Indian stone formers. In the Japanese classification system, these two main types are further subdivided based on the cross sectional morphology [7] . In this classification, cholesterol stones are sub grouped as pure, mixed and combination stones. Pigment stones are further classified as black and calcium bilirubinate (brown) stones [8] .

The prevalence and the chemical composition of gallstones vary from population to population. This indicates the involvement of multiple aetiological and risk factors in the pathogenesis of gallstones [9] .Therefore investigation of the chemical composition of gallstones is primarily important to recognize the aetiological factors for gallstones disease in a community. Gallstones disease has not been adequately investigated in Bangladeshi population despite its high prevalence according to the hospital records. Apart from the identification of bacterial colonies in bile, patients with gallstones disease [10] .Studies to identify the mechanism of formation of gallstones have not yet been carried out. Moreover, a high prevalence (10-12%) of gallstones disease and its complications (eg; GB carcinoma) are reported from India, the closest neighbor of Bangladesh [11] .Based on these facts, an extensive investigations on gallstone disease among Bangladeshies can be considered imperative. Further, identification of chemical composition of gallstones among Bangladeshies would be an important gateway to explore the aetiopathogenesis and risk factors of gallstones disease.

Fourier Transform Infrared Spectroscopy (FTIR) is the most widely used technique in the analysis of chemical composition of gallstone, as it is accurate, less time consuming and cost effective [12]. Thus it is the main technique used in the large scale analysis of gallstone samples [13] .Moreover the chemical analysis of gallstone by X—ray Powder Diffraction (XRD) and colorimetric assays are also available. XRD is used to describe the crystalline composition of gallstone [14].

The objectives of this study were to describe the chemical composition of gallstone of patients with gallstone disease, undergone cholecystectomy as well as the evaluate the relationship with socio-demographic and clinicopathological factors to understand the correlations between chemical composition of gallstone and risk factors of gallstone formation. The present study also aimed to find out the frequency of different type of gallstones in Bangladeshi population to identify patients and management of gallstone disease for future.

MATERIALS AND METHOD

This cross sectional analytical study was conducted in department of Surgery in SSMCH, Dhaka, during the period from November 20016 to October 2017. The study was performed among the patients who were admitted with diagnosis of Chronic Calculus Cholecystitis having various clinical presentation in the Department of Surgery, Sir Salimullah Medical College and Mitford Hospital, Dhaka and subsequently who were undergone cholecystectomy. Then stones were sent to the laboratory for assessment of the chemical compositions of the stones and bile for culture and sensitivity test.

RESULTS

Table I: Age distribution of the patients (n=100).

Table-I shows the age distribution of the study patients, age range between 20-70 years. Mean± SD was 43.14±11.38 years and median 45.0 years. 56% patients were in between 31-50 years,19% patients were in upto 30 years and 25% patients age above 50 years.

Age Group	Frequency (n)	Percentage (%)
Up to 30	19	19.0
31-50	56	56.0
>50	25	25.0
Total	100	100.0

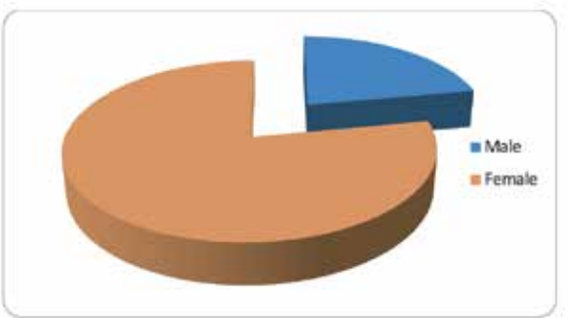

Figure-1: Sex distribution of the study populations.

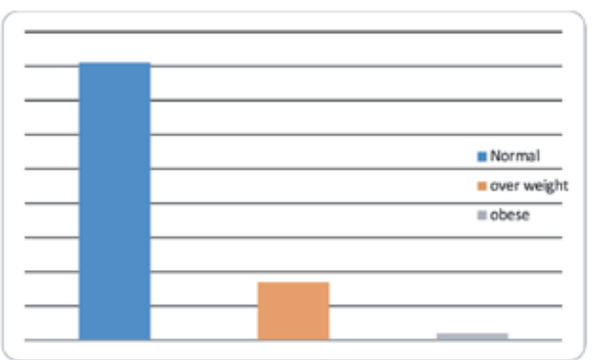

Figure- 2: Distribution of the patients by BMI (n=100).

Figure shows (n= 81) 81% patients were in BMI(18.5 – 25),within normal range, (n=17) 17% patients were overweight, BMI (25.1- 30),only 2% obese.

Table II: Distribution of the patients by dietary habit (n=100).

Dietary Habit	Frequency	Percent (%)
Normal	74	74.0
Fatty Food	26	26.0
Total	100	100.0

Table shows out of 100 patients fatty food intake only 26% patients and rest of all 74% on normal Bangladeshi diet.

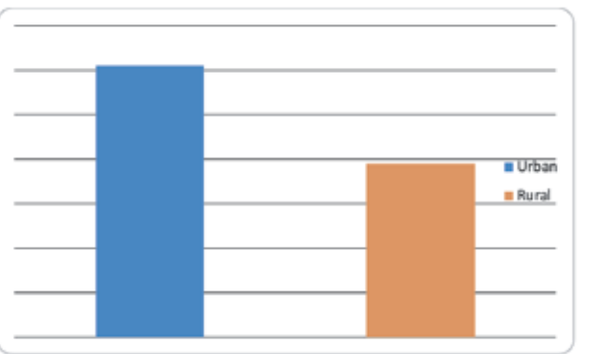

Figure – 3: Distribution of gallstone patients based on area of residence.

Table shows- distribution of patients based on area of residence 61% of patients reside in urban area and the remaining patients from rural area.

Table-III: Occupational status of gallstone disease patients.

Occupation	Frequency	Percent (%)
Housewife	65	65.0
Farmer	11	11.0
Service	11	11.0
Businessman	5	5.0
Others	8	8.0
Total	100	100.0

Table shows occupational status of gallstone disease patient majority (65%) housewives. service holders were 11%, farmers were 11% and businessman were 5%.

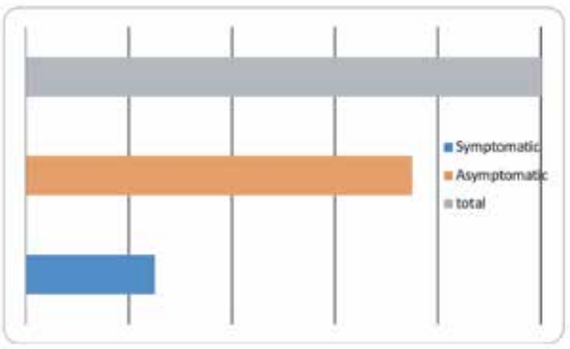

Figure 4: Presenting complaints of gallstone diseases.

Figure shows, some patients presented with symptoms such as abdominal pain, vomiting but majority of patients (75 out of 100) were found asymptomatic .

Table-IV: Status of serum lipid profile in Gallstone disease patients.

Lipid Profile	Frequency(n)	Percentage (%)
Normal	70	70.0
Dyslipidaemic	30	30.0
Total	100	100.0

Table shows-majority of patients (70%) had desirable serum lipid profile with in normal level. 30 out of 100 patients were found to have lipid profile above the desirable level.

Table V: Distribution of the patients by number of gallstone present in gall bladder (n=100).

Number of Stone	Frequency(n)	Percentage(%)
Single	25	25.0
Multiple	75	75.0
Total	100	100.0

Table-shows the distribution of number of stone, there were single stone in 25 patients and 75 patients were multiple. Percentage of single and multiple stone was 25 and 75 respectively.

Table-VI: Distribution of the patients by colour of stone.

Color of Stone	Frequency(n)	Percentage (%)
Yellow	23	23.0
Dark Yellow	55	55.0
Black	22	22.0
Total	100	100.0

Table-shows the distribution by colour of gallstones, 55% patients had dark yellow/ brown, 23% were Yellow and 22% were black stones.

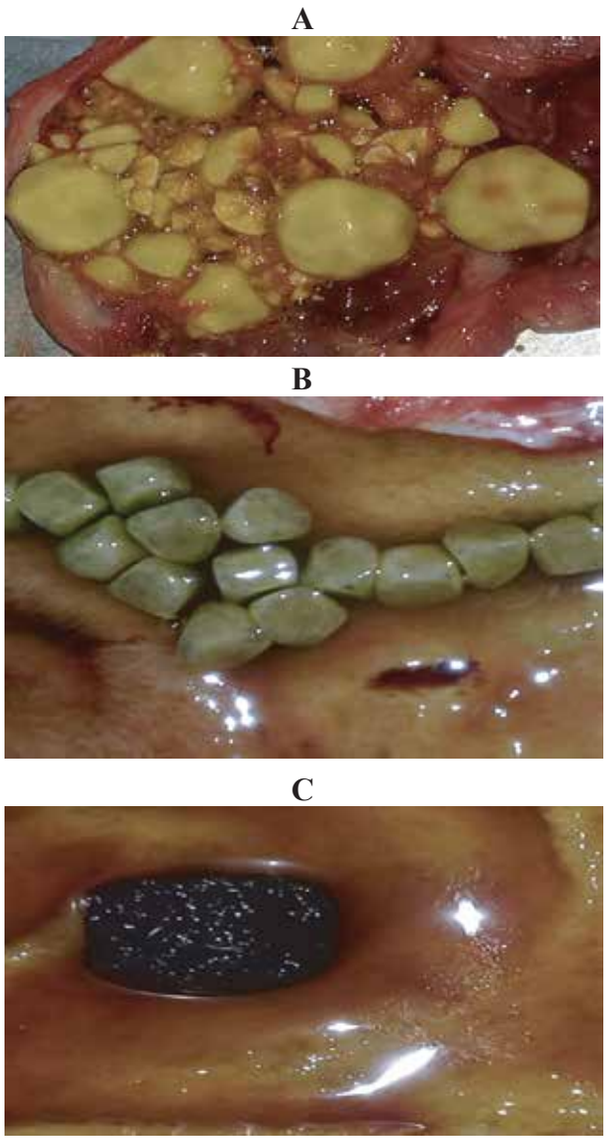

Figure 5: A. yellow stone B. dark yellow/ brown stone C. black stone

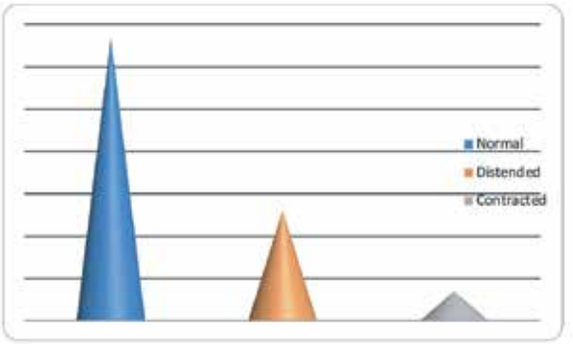

Figure 6: Distribution of the patients by condition of gall bladder.

Figure shows the distribution by condition of gall bladder, normal size 67%, distended 26% and 7% were contracted.

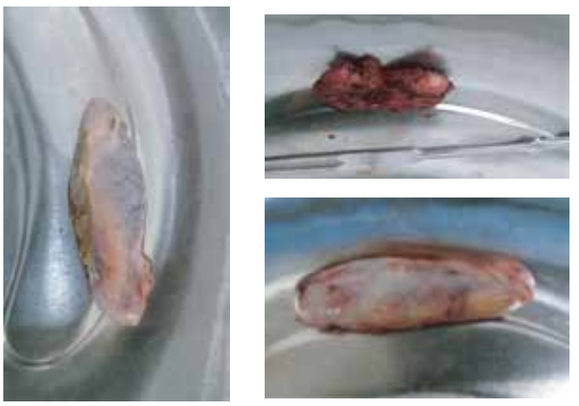

Figure 7: a. normal gall bladder b. distened gall bladder. c . contracted gall bladder.

Table VII: Distribution of the patients by condition of gallbladder wall thickness.

Wall thikness of GB	Frequency	Percent (%)
Normal	37	37.0
Thickened	63	63.0
Total	100	100.0

Table shows the distribution of patients by wall thickness of gall bladder (n=63) 63% patients having thickened gall bladder wall rest of the gall bladder were normal.

A

B

Figure 8: a. normal walled GB b. thicke walled GB

Table VIII: Distribution of the patients by bile infection (n=100).

Infection of Bile	Frequency(n)	Percentage (%)
Yes	18	18.0
No	82	82.0
Total	100	100.0

Table shows the distribution of patients by bile infection (n=18) 18% patients were positive for bile culture and sensitivity test.

Table IX: Distribution of the patients by types of gall stone (n=100).

Type of Stone	Frequency(n)	Percentage (%)
Cholesterol	7	7.0
Pigmented	18	18.0
Mixed	75	75.0
Total	100	100.0

Table- shows the distribution by types of gallstones, cholesterol stone 07, pigmented stone 18 and mixed stone 75 patients. So percentage of stone types cholesterol, pigmented and mixed stones 07%, 18% and 75% respectively.

Table XII: The Relationship of chemical compositions of gallstone and different factors.

Factors		chemical compositions of gallstone				P- value
		Cholesterol (07) No (%)	Pigmented (18) No (%)	Mixed (75) No (%)	Total (100) No(100.0%)	
Age group	Upto 30	1 (5.3%)	0 (.0%)	18 (94.7%)	19 (100.0%)	
	31-50	3 (5.4%)	12 (21.4%)	41 (73.2%)	56 (100.0%)	.142ns
	>50	3 (12.0%)	6 (24.0%)	16 (64.0%)	25 (100.0%)	
Sex	Male	2 (9.1%)	11 (50%)	9 (40.9%)	22 (100.0%)	
	female	5 (6.4%)	7 (8.9%)	66 (84.6%)	78 (100.0%)	.127ns
BMI	Obese	0 (0%)	0 (0%)	2 (100.0%)	2 (100.0%)	
	Overweight	2 (11.8%)	2 (11.8%)	13 (76.5%)	17 (100.0%)	.769ns
	Normal	5 (6.2%)	16 (19.8%)	60 (74.1%)	81 (100.0%)	
Dietary Habit	Normal	5 (6.8%)	10 (13.5%)	59 (79.7%)	74 (100.0%)	
	Fatty Food	2 (7.7%)	8 (30.8%)	16 (61.5%)	26 (100.0%)	.132ns

s= significant ,ns= not significant, p value reached from chi square test

Analysis of chemical composition of gallstone and its relation with demographic factors, shown in Table XII. There was no significant association with composition of gallstone and patients age, sex, dietary habit and body mass index (p >0.05).

Table XIII: Relationship between chemical composition of gallstone and other clinico-pathological factors.

Clinicopathological factors		Types of gall stone				P-value
		Cholesterol (07) No(%)	Pigmented (18) No(%)	Mixed (75) No(%)	Total (100)No(100.0%)	
Symptoms	present	0 (.0%)	10 (40.0%)	15 (60.0%)	25 (100.0%)	
	absent	7 (9.3%)	8 (10.7%)	60 (80.0%)	75 (100.0%)	.002s
Lipidaemic status	normal	5 (7.1%)	3 (4.3%)	62 (88.6%)	70 (100.0%)	
	dyslipidaemia	2 (6.7%)	15 (50.0%)	13 (43.3%)	30 (100.0%)	.001s
Condition of gall bladder	normal	3 (4.5%)	11 (16.4%)	53 (79.1%)	67 (100.0%)	
	distended	4 15.4%	3 11.5%	19 73.1%	26 (100.0%)	.022s
	contracted	0 (.0%)	4 (57.1%)	3 (42.9%)	7 (100.0%)	
Wall thickness of GB	normal	6 (16.2%)	1 (2.7%)	30 (81.1%)	37 (100.0%)	
	thickned	1 (1.6%)	17 (27.0%)	45 (71.4%)	63 (100.0%)	.001s
Bile culture	positive	0 (.0%)	7 (38.9%)	11 (61.1%)	18 (100.0%)	
	negative	7 (8.5%)	11 (13.4%)	64 (78.0%)	82 (100.0%)	.024s

s= significant ,ns= not significant, p value reached from chi square test

Analysis of chemical composition of gallstone and its relation with clinico-pathological factors, shown in Table XIII. There was a significant association with composition of gallstone and patients presenting symptoms, Lipidaemic status , conditions of gall bladder, wall thickness of gall bladder and bile infection (p <0.05).

DISCUSSIONS

Gallstones represent a major problem in many countries, including Bangladesh. Reports from different parts of the world indicate that the incidence of gallstones is correlated with socio-economic conditions and dietary factors; other risk factors are marked obesity, family history of gallstone disease, high energy intake, aging, multiple parity, cholecystitis, and sedentary lifestyle. Gallstones are more frequent among patients with certain conditions, such as Crohn's disease and liver cirrhosis.

Pathogenesis of gallstone is multifactorial and detection of chemical composition of gallstone is primarily important to identify their mechanism of formation. To the best of our knowledge this is the first attempt made to identify the aetio-pathogenesis of gallstone among Bangladeshi people. Of the three types of gallstone, mixed cholesterol stones were the commonest types identified in this study.

In this study, FTIR was used as the basic tool in the analysis of chemical composition of gallstone as it is an analytical technique with a higher accuracy. One of the major advantages of FTIR in gallstone analysis is the ability to use only a small amount of sample for testing . The identification of chemical compounds was done by matching wave numbers obtained for the samples with the standards. The best possible matching was ensured to minimize the errors in the interpretation. Moreover the obtained wave numbers were cross checked with the existing literature data to improve the accuracy. Absorption peaks at 1465, 1381 and 1056cm-1 were taken as finger print wave numbers for cholesterol while, absorption peaks 1658, 1627, 1566 and 1249 cm-1 were used to detect calcium bilirubinate . Presence of two strong broad peaks at 1080 and 1033cm-1, and two small peaks at 609 cm-1 and 547 cm-1 were used to identify calcium phosphate . Absorption peaks at 1462/1450, 871 and 698/694 cm-1 were used to detect calcium carbonate and 2916, 2846, 1543, 1103 and 756 cm-1 were taken as the characteristic wave numbers for calcium palmitate.

XRD was also used to identify the chemical composition of gallstone as it is an accurate method to identify the crystalline compounds. XRD results confirmed the presence of all the other chemical compounds identified by FTIR other than calcium bilirubinate. Calcium bilirubinate was not detected by XRD and was considered as an amorphous material. This fact was compatible with the findings of the current study. Failure to detect

calcium bilirubinate can be recognized as the main reason for infrequent use of XRD to characterize the chemical composition of gallstone. Being the very first attempt to analyze the composition of gallstone in Bangladesh, XRD findings are supportive evidence for FTIR analysis in the this study.

The basic parameters of the patients like age, gender, BMI, food habit, clinical presentations, Lipid profile were taken from patient data sheets. Gall bladder stones were taken, morphology, number of stone, macroscopic features of gall bladder and its wall thickness were observed and recorded by naked eye observation. Identified the chemical compositions of the stones, bile culture and sensitivity test was done and recorded. All data were collected and correlated.

Our study showed, age of the patients range between 20-70 years, Mean± SD was 43.14±11.38 years and median 45.0 years. 56% patients were in between 31-50 years and 25% patients age above 50 years and sex of the study subjects, 78 were female and 22 were male out of 100 patients. So 78% were female and 24% male, male, female ratio was 1 : 3.5. Most of the patients were married (93%), only 7% patient was unmarried, (n= 81) 81% patients were in BMI (18.5 – 25), within normal range, (n=17) 17% patients were overweight, BMI (25.1- 30 kg/m2), only 2% were obese (BMI>30 kg/m2), fatty food intake only 26% patients and rest of all (74%) on normal Bangladeshi diet. Distribution of patients based on area of residence 61% of patients resided in urban area and the remaining patients from rural area. Occupational status of gallstone disease patients, majority (65%) housewives. service holders was 11%, farmers were 11% and businessman was 5%. Patients presented with symptoms (such as abdominal pain, vomiting) only 25%, majority of patients (75%) were found asymptomatic. Forty five percent of patients had come from low income, patients had come from medium income family were 43%, only 12 patients had high economic background, 70% patients had desirable serum lipid profile level, 30% patients were found to have lipid profile above the desirable level. The distribution of number of stone, there were single stone in 25% patients and 75% patients were multiple, the distribution by colour of gallstones, 55% patients had dark yellow/ brown, 23% were Yellow and 22% were black stones, the distribution by condition of gall bladder, normal size 67%, distended 26% and 07% were contracted and maximum gall bladder (n=67), 67% were thick walled rest of the gall bladder were normal. Distribution of patients by bile infection (n=18) 18% patients were positive for bile culture and sensitivity test. The types of gallstones, cholesterol stone 07, pigments stone 18 and mixed stone 75 patients. So percentage of stone type's cholesterol, pigments and mixed stones were 08%, 18% and 73% respectively. Distended gall bladder was more in mixed type of stone and contracted gall bladder was more in pigmented and mixed stones. Bile infection was more in mixed type of stones and also present in pigmented stones. Symptoms of disease was present in the patients having mixed and pigmented stones. Prevalence of dyslipidaemia was observed more in patients having mixed and pigmented stone.

Evidence from the current study indicates that the risk factors and pathogenesis of gallstone in our population is likely to be different from that of the Western population, where cholesterol gallstones were the most predominant. However, common risk factors and etiology for the development of gallstone can be anticipated for South Asian region, considering the data from other South Asian countries like India and Srilanka, where majority of gallstone were mixed or pigment gallstone.

Our results showed that females were more affected with cholelithiasis than males, with a female to male ratio of 3.5:1. According to the Statistics, in Bangladesh, female to male ratio of the total population was 100:104 in 2015. Reports from other countries, such as India, Srilanka and, other countries of the world also indicate that females are more affected by the disease than males. This might be due to the basic hormonal differences between males and females, together with the differences that might exist due to co-expression of sex hormone receptors in the gallbladder of both sexes. The medical records of the patients of the present study showed that most of the female patients had parity. This is also in agreement with other studies, which concluded that fertility and multiple pregnancies appeared to be a risk factor for gallstones among the women. Increased levels of sex hormones as a result of pregnancy may increase cholesterol in the bile and decrease gallbladder movement, resulting in cholelithiasis.

In our study, in Bangladeshi population there was no significant association with composition of gallstone and

sociodemographic factors like patients age, sex, dietary habit and body mass index etc ($p > 0.05$). But there was a significant association with clinicopathological factors like patients presenting symptoms, lipid profile, conditions of gall bladder, wall thickness of gall bladder and bile infection ($p < 0.05$).

CONCLUSIONS

It is apparent from the findings of the present investigation that the most common gallstone in Bangladesh was mixed stone, and that the age group at risk of having gallstones was the third to fifth decade of life, with a higher risk for females than males. There was a significant association with chemical composition of gallstone and patients presenting symptoms, dyslipidaemia, conditions of gall bladder, wall thickness of gall bladder and bile infection ($p < 0.05$). However, patients age, sex, dietary habit, body mass index and others demographic factors were not associated with the types of gall stones.

LIMITATIONS OF THE STUDY

This is a small hospital based study, so findings cannot be applied to the whole population. The most accurate correlations of chemical composition of gallstone and other demographic and clinicopathologic risk factors of gallstone diseases can only be estimated in large population or community based cohort study.

The study in the Sir Salimullah Medical College and Mitford Hospital, Dhaka is the tertiary level government hospital of Bangladesh & situated in capital city of Bangladesh. So people of remote, rural area of Bangladesh may not get access to Salimullah Medical College and Mitford Hospital. So this result may not reflect actual situation in the community of Bangladesh. So large population based study should done to find out the correlations of chemical composition of gallstone and other demographic and clinic-pathologic risk factors of gallstone diseases in the country.

RECOMENDATIONS

Further studies with larger number of cases are necessary to allow multivariate analyses and to confirm these findings to establish more precisely the relationships between chemical composition of gall bladder stone and other demographic and clinicopathologic parameters of gallstone diseases.

REFERENCES

1. Fitzgerald JEF, Fitzgerald LA, Maxwell-Armstrong CA, Brooks AJ. Recurrent gallstone ileus: time to change our surgery?. J Dig Dis. 2009;10(2):149-51.
2. Kaechele V, Wabitsch M, Thiere D, Kessler AL, Haenle MM, Mayer H, et al. Prevalence of gallbladder stone disease in obese children and adolescents: Influence of the degree of obesity sex and pubertal development. J Pediatr Gstroenterol Nutr. 2006;42(1):66-77.
3. Wesdrop I, Bosman D, de Graaff A, Aronson D, vanderBlif FM, Taminiau J. Clinical presentations and predisposing factors of cholelithiasis and sludge in children. J Pediatr Gastroenterol Nutr. 2000;31(4):411-7.
4. Rome Group for the Epidemiology and Prevention of Cholelithiasis (GREPCO). The Epidemiology of gallstone disease in Rome, Italy. Prevalence data in men. Hepatology. 1988;8(4):904-6.
5. Singh V, Trikha B, Nain CK, Singh K, Bose SM. Epidemiology of gallstone disease in Chandigarh: A community-based study. J Gastroenterol Hepatol. 2001;16(5):560-3.
6. Van Erpecum KJ. Biliary lipids, water and cholesterol gallstones. Biol Cell. 2005;97(11):815-22.
7. Venneman NG, van Erpecum KJ. Pathogenesis of gallstones Gastroenterol Clin North Am. 2010;39(2):171-83.
8. Friedman GD, Raviola CA, Fireman B. Prognosis of gallstones with mild or no symptoms: 25 years of follow-up in a health maintenance organization. J Clin Epidemiol. 1989;42(2):127-36.
9. Russo MW, Wei JT, Thiny MT, Gangarosa LM, Brown A, Ringel Y, et al. Digestive and liver diseases statistics. Gastroenterology. 2004;126(5):1448-53.
10. Dowling RH. Review: pathogenesis of gallstones. Alimentary Pharmacol Therapeut. 2000;14(Suppl 2):39-47.
11. Van Erpecum KJ, van Berge Henegouwen GP, Stoelwinder B, Stolk MF, Eggink WF, Govaert WH. Cholestrol and pigment gallstone disease: comparison of the reliability of three bile tests for differenciation between two stone types. Scand J Gastroenterol. 1988;23(8):948-54.
12. Vitek L, Carey MC. Enterohepatic cycling of bilirubin as a cause of black pigment gallstones in adult life. Eur J Clin Invest. 2003;33(9):799-810.
13. Ontiveros AG, Hinojosa JC, Extremera BG, Moral JMD. Differences in gallstone structure in primary common bile duct lithiasis and gallbladder lithiasis. Klin Wochenschr. 1990;68(10):496-502.
14. Mukaihara S. Chemical analysis of gallstones: classification and composition of human gallstones. Arch Jpn Chir. 1981;50(3):476-500.