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Original Research Article

Etiology and chemical composition of gall stone disease: a prospective observational study from the developing world

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ABSTRACT

Background: Aim of the study was to assess the etiology and determine the chemical composition of gallstones in our population.
Methods: This was a prospective observational study conducted at GMC Srinagar from 2018 to 2020. One hundred patients having gall stone disease were enrolled into the study and after cholecystectomy was performed the stones chemical composition was analyzed by fourier-transform infrared (FTIR) spectroscopy. The data was collated and analysed.
Results: 54 patients had mixed stones with chemical composition of cholesterol, calcium carbonate and calcium bilirubinate; 39 had cholesterol stones and 7 patients had pigmented stone with chemical composition of cholesterol, calcium carbonate and calcium bilirubinate.
Conclusions: Our results suggest that cholesterol, either singularly or in combination with calcium carbonate or bilirubin is a common component of gall stones in our population.

Keywords: Gallstone, FTIR, Cholesterol, Bilirubinate, Chemical

INTRODUCTION

Gallstone disease remains a serious health concern for human beings, affecting millions of people throughout the world.[1,2] It is one of the most prevalent gastrointestinal diseases, with a substantial burden to health care systems.[3] In the United States, there are more than 5,00,000 cholecystectomies performed per year, the total cost of which exceeds 5 billion dollars.[4]

Gallstones (GS) are considered avoidable causes of death.[5] Three types of GS occur in human population, cholesterol stones and two kinds of pigment stones called black and brown. In the West, approximately 75% of GS are cholesterol stones, 20% are black pigment stones and 5% or less are brown pigment stones.[6]

The pathogenesis of cholesterol and black pigment stones results primarily from alterations in the lipid and lipopigment compositions of gallbladder bile.[7,8] The major components of these stones are cholesterol monohydrate crystals and calcium hydrogen bilirubinate in cholesterol and pigment stones, respectively.[7] In black stones, the pigment is chemically degraded and polymerized, presumably by free radicals during its long residence time in the gallbladder. The pathogenesis of brown pigment stones is infectious with enzymatic hydrolysis of biliary lipids by anaerobic bacterial enzymes that produce biliary supersaturation with calcium salts of unconjugated bilirubin, saturated long-chain fatty acids and deconjugated bile acids.[8,9]

Aim of the study was to assess the etiology and determine the chemical composition of gallstones in our population.

METHODS

The present prospective observational study was conducted in the postgraduate department of general surgery, Government Medical College, Srinagar, Kashmir, India. This study was conducted from December 2018 to September 2020. Ethical clearance for the study was taken from the hospital ethical committee. All patients with ultrasonography (USG) documented GS, who underwent surgery during the study period were included in the study.

All the patients were subjected to either laparoscopic or open cholecystectomy, after getting a written and informed consent. A total of 100 patients underwent surgery during the study period and the relevant data of each patient was recorded. This included demographic data and the presence of known aetiological factors for gallstone disease. The considered aetiological factors included age, gender, smoking, alcohol consumption, family history of gallstone disease, history of oral contracetive pills (OCPs) and hormone replacement therapy (HRT), comorbid conditions like type II diabetes mellitus (DM), dyslipidaemia, chronic haemolytic anaemia, chronic liver disease, ileal resection and ileal bypass surgeries.

GS were collected after cholecystectomy. The stones were divided into 3 groups depending upon their colour: pale yellow or white stones as cholesterol calculi, black or blackish brown as pigment calculi and brownish yellow or greenish with laminated features as mixed calculi. The various physical parameters of stones such as number, shape, size, texture and cross-section were also noted.

In this study, compositions of GS were analyzed by fourier transform infrared (FTIR) spectroscopy. FTIR analysis measures the range of wavelengths in the infrared region that are absorbed by materials. This is accomplished through the application of infrared radiation to a sample of material. The sample's absorbance of infrared light energy of various wavelengths is measured to determine the material's molecular structure. Unknown materials are identified by searching against database of reference spectra.

The recorded data was compiled and entered in a spreadsheet (Microsoft Excel) and then exported to data editor of statistical package for the social sciences (SPSS) version 20.0 (SPSS Inc., Chicago, Illinois, USA). Continuous variables were expressed as mean±SD and categorical variables were summarized as frequencies and percentages.

RESULTS

In our study, the age ranged from 10-70 years (Table 1) with majority, i.e. 51 patients (51%) being 21-40 years of age. The mean age of the patients in our study was 42.5±14.83 years. There were 74 females and 26 males, with a female to male ratio of 2.8:1, respectively. The mean body mass index (BMI) of our study subjects was 25.4±3.97 kg/m^2. Family history of gallstone disease was seen in 14% of patients. Underlying comorbidities were present in 46 patients, with hypertension and diabetes mellitus being the commonest comorbidities. There were only 7 (7%) patients in our study with history of dyslipidemia.

Table 1: Demographic parameters.

Demographic parameters	No. of patients (n)	Percentage (%)
Age (years)		
≤20	2	2
21-40	51	51
41-60	35	35
>60	12	12
Mean±SD (range)=35.9±12.54		
Gender		
Male	26	26
Female	74	74
Female to male ratio: 2.8:1		
BMI (kg/m^2)		
<18.5	3	3
18.5-24.9	54	54
25.0-29.9	29	29
≥30	14	14
Mean±SD=25.4±3.97		
Family history of GSD		
Present	14	14
Absent	86	86
Underlying comorbidity		
Hypertension and diabetes mellitus	17	17
Hypertension	12	12
Hypothyroidism	9	9
Diabetes melitus	5	5
Diabetes melitus and hypothyroidism	3	3
Dyslipidemia		
Present	7	7
Absent	93	93
Chemical composition of gall stones [FTIR]		
Mixed stone	54	54
Cholesterol stone	39	39
Pigmented stone	7	7

GS were categorized into three types based on their composition (and content) of cholesterol or other materials as cholesterol stones (having 80-100% cholesterol), mixed stones (20-79% cholesterol) and pigment stones (<20% cholesterol stones) on FTIR spectroscopy. Accordingly, 54 patients had mixed stones with chemical composition of cholesterol, calcium carbonate and calcium bilirubinate. 39 had cholesterol stones and 7 patients had pigmented stone with chemical composition of cholesterol, calcium carbonate and calcium bilirubinate. The co-relation of age and gender with the chemical composition of GS (as assessed with FTIR spectroscopy) is shown in Table 2.

Table 2: Co-relation of age and gender with type of gallstone in study patients.

Correlation	Mixed stone		Cholesterol stone		Pigmented stone	
	No.	%	No.	%	No.	%
Correlation of age with type of gallstone						
≤ 20	1	1.9	0	0.0	1	14.2
21-40	25	46.3	20	51.3	6	85.8
41-60	24	44.4	11	28.2	0	0.0
> 60	4	7.4	8	20.5	0	0.0
Total	54	100	39	100	7	100
Chi-square=16.443; p value=0.012 (statistically significant)						
Correlation of gender with type of gallstone						
Male	14	25.9%	12	30.8%	0	0.0
Female	40	74.1%	27	69.2%	7	100
Total	54	100%	39	100%	7	100
Chi-square=2.921; p value=0.232 (not significant)						

DISCUSSION

Gallstone disease is responsible for about 95% of biliary tract abnormalities. Over half of the cases are asymptomatic, usually detected incidentally by an abdominal ultrasound.[10] Recently, owing to the widespread use of ultrasonography, the prevalence of gall stone disease has increased considerably.[5]

Owing to their multi-factorial pathogenesis, the analysis of chemical composition of GS is primarily important to identify their mechanism of formation. Recent changes in diet and improved environmental hygiene are suggested to be responsible for the compositional change of GS.[11,12]

In our study, the age ranged from 10-70 years with the mean age of our study group being 35.9±12.54 years. Our findings are in concordance with those of Gupta AM et al and Singh KK et al, wherein the age of the patients varied from 21-73 years, and the mean age ranged from 43 years to 46 years, respectively.[13,14]

In our study, majority of patients were females 74 (74%) and 26 (26%) were males, with a female to male ratio of 2.8:1. Similar results were observed by Weerakoon et al in their study of 102 patients.[15] Of them 77 (76%) were females and 25 (24%) were males, with a female to male ratio of 3:1. Angwafo et al in their study also observed female predominance with 16 females and 10 males.[16] Singh et al in their study had 69% females and 31% males.[14]

In our study, 30 (30%) patients were smokers. Protective effects of cigarette smoke on the pathogenesis of GS and absence of such relationship, identified in some previous studies indicate controversial role of effect of smoking on GS formation.[17-20] As the exact mechanisms by which smoking affects the pathogenesis of GS have not been identified, further studies are required to identify the effect of smoking on GS pathogenesis.

Family history of gallstone disease was seen in 14 (14%) patients of our study. Our findings are at variance with those of Weerakoon et al where positive family history of gallstone disease was present in only 6 (7%) patients, however, our findings were similar to those observed by Shaffer.[15,21]

In our study, normal BMI (18.5-24.9 kg/m^2) was observed in 54 (54%) followed by 29 (29%) patients who had BMI of 25-29.9 kg/m^2, 14 (14%) patients had ≥30 kg/m^2 and 3 (3%) patients had BMI of <18.5 kg/m^2 with the mean BMI of 25.4±3.97 kg/m^2. Our findings are in concordance with that of Singh et al wherein majority of their study patients, i.e. 68% had normal BMI and BMI of 25-29.9 kg/m^2 was seen in 17% of patients of their study population.[14] In a large prospective cohort study by Liu et al, increase in BMI was significantly associated with higher risks of gallstone disease.[22]

Underlying comorbidities were seen in 46 (46%) patients in our study among which 17 (17%) patients had hypertension and diabetes mellitus, 12 (12%) patients had hypertension and 9 (9%) patients had hypothyroidism. Diabetes mellitus alone was seen in 5 (5%) patients while as 3 (3%) patients were observed to have diabetes mellitus and hypothyroidism both. Diabetes and hypertension together being the most common associated comorbidities in our patients is probably due to the increased adoption of westernized diet and sedentary lifestyle in our population. Diabetes is identified as known risk factor in the presence of other identifiable risk factors like obesity and family history of gallstone disease.[23] Moreover in Western population, obesity is the strongest risk factor for GS even in patients with DM.[24]

In our study, out of the 74 female patients, 19 (27.1%) patients had history of consumption of oral contraceptives pills (OCP). Weerakoon et al have reported similar results, wherein GS were found in 21 (32%) patients taking oral contraceptives.[15] A possible increased risk of cholecystectomy in women with four or more children and

in those taking oral contraceptives has been reported in various other studies.[25,26]

Many investigators have classified gallstones into two groups, cholesterol and pigment, based on their major composition. GS containing cholesterol as the main constituent are classified as cholesterol stones, whereas those predominantly composed of bile pigments are called pigment stones.[27,28] However, in the Japanese and NIH-classification, cholesterol stones are defined as stones with cholesterol content of more than 70% of the stone dry weight.[27,29]

We classified GS as cholesterol, pigment and mixed by using FTIR method and stones having >80% of cholesterol were classified as pure cholesterol stone. 54 (54%) patients were found to have mixed stone, 39 (39%) patients had cholesterol stone and 7 (7%) patients had pigmented stone in our study group. Our results were consistent with the findings of Gupta et al and Mohan et al wherein mixed type of GS were the most common form of GS.[13,30]

Our study demonstrated that FTIR spectroscopy for chemical analysis of GS is a quick method and tool of choice for classifying a large sample size of GS, however interpretation of FTIR spectrum of GS should be done carefully as the absorption bands of GS constituents often overlap with each other and the absorption of bound water makes interpretation of FTIR spectrum of calcium bilirubinate gallstone difficult. Similar conclusions were drawn from other studies by Suo et al and Ha and Park while analyzing the chemical composition of GS.[31,32]

CONCLUSION

We conclude that cholesterol is a significant component of GS and mixed stones are the commonest GS in our part of the world, however, further studies should be conducted to analyse and evaluate our findings.

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