



DESCRIPTIVE STUDY OF CHOLELITHIASIS WITH CHEMICAL CONSTITUENTS ANALYSIS OF GALLSTONES FROM PATIENTS LIVING IN BAGHDAD, IRAQ

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ABSTRACT

Cholelithiasis is a healthy problem in Iraqi females due to apparent high rate of women relative to males complaining of gallstone. The aim of this study is to describe the main clinical features of patients with cholelithiasis and chemical analysis of stone in relevant to develop of gallstone formation. The total number of the patients with gallstone in this study was 75 (66 females and 9 males). The ratio of females : males was (7.3:1), this high ratio may be due to multifactorial including high percentage of multiparity (63.64%), using of contraceptives (46.97%), family history (44%), obesity (53.3%), and sedentary life style (non-workers) (72%). The stones were classified into cholesterol, pigment and mixed stones. Cholesterol stone is the most prevalent type of stones, the percentage was (49.3%) greater than mixed and pigment stone where the percentages were (33.3%) and (17.3%) respectively. Cholesterol stone showed significantly higher cholesterol content ($P < 0.05$) than pigment stones though insignificantly higher than mixed stones. Cholesterol content in mixed stone is significantly higher ($P < 0.05$) than pigment stones. Total bilirubin content in pigment stones is higher significantly ($P < 0.05$) than mixed and cholesterol stones. The bilirubin content in cholesterol stones is higher but insignificantly ($P > 0.05$) than mixed stones. Calcium content in pigment stone was higher significantly ($P < 0.05$) than cholesterol stones and higher but insignificantly ($P > 0.05$) than mixed stones. Inorganic phosphate content in cholesterol stones was higher significantly ($P < 0.05$) than mixed stones and pigment stones. The content of inorganic phosphate in mixed was higher insignificantly than pigment stones. In conclusion cholesterol stone was the most common type of stones but interestingly there was a high ratio of females: males suggesting efforts to reduce all variable risk factors which lead to cholelithiasis especially among females.

KEY WORDS: cholelithiasis, chemical constituents, gallstone.

INTRODUCTION:

Cholelithiasis is a real problem in Baghdad, Iraq, because increasing number of females attending the hospital due to gallstone complications; the disease seems to be more prevalent than expected. The current study was carried out in biggest medical center in Iraq and the patients come from different residence so they may represent real sample of Iraqi patients living in Baghdad. Reports about gallstone types are rare and no report concerning the analysis of chemical composition of gall stone in Iraq. This problem is probably related to obesity, cardiovascular disorders, metabolic syndrome and dietary habits, especially consumption of diet which is known to contain large amounts of meat. Obese individuals with $BMI > 30 \text{ kg/M}^2$ have 95% cholesterol- dominant gallstones and are at higher risk of cholesterol stone (Shafmyer C. *et al.*, 2006). Other risk factors are marked family history of gallstone disease, aging, multiple parity, cholecystitis, and sedentary lifestyle (Murshid KR., 1998; Marscall HU and Einarsson C., 2007; Cuevas A. *et al.*, 2004).

Gallstone chemical analysis gives important evidence for origin, etiology, and the metabolic basis of its formation, and helps in the identification of risk factors that predispose certain individuals to the calculi formation,

chemical classification and location of biliary calculi differ in various parts of the world and change over time because of nutritional, socio-economic, and demographic factors (Bashir Jarrar and Meshref A., 2011). The identification of the components of gallstone is essential as it provides information that could be useful for medical practitioner to find out the underlying cause of gallstone and to decide whether to treat gallstone patients surgically or therapeutically (Kafia M. *et al.*, 2009). Moreover, analysis of chemical composition of gallstones can provide a significant reference to the treatment and prevention of their recurrence (Wang Q. *et al.*, 2007). Major elements involved in the formation of human gallstones are cholesterol, bilepigment and calcium (Moosavi K. *et al.*, 2006). With regard to chemical composition the majority of human gallstone are cholesterol and pigment stones (Vivek K. *et al.*, 2008; Sahuquillo A. *et al.*, 2008). Other substances found in gallstones include calcium salts of phosphate, mucin, glycoprotein, phospholipids and some metals (Pundieir CS. *et al.*, 2011). It has been reported that some elements play a significant role in the formation of gallstones. It was observed that 14 elements namely Sulfur chloride, Potassium, Vanadium, Chromium, Manganese, Iron, Nickel, Copper, Zinc, Bromide and Lead were

present in the gallstones collected from Indian formers (Rautary *et al.*, 2006).

The aim of the present article is to study the clinical features of gallstone patients with identification of the constituents of different types of gallstones obtained from patients living at Baghdad city, Iraq.

MATERIALS AND METHODS

The total number of 75 patients with gallstone was admitted to Medical City Hospital in Baghdad (surgery unit) from October 2010 to May 2011. There were 66 female and 9 male patients with mean age (44.53±1.39). All patients were with symptomatic gallstone having a history of pain in the upper quadrant, and epigastric regions from the past 3 to 6 months. An abdominal ultrasonography is the standard diagnostic test for gallstone detection (Trowbridge *et al.*, 2003). Gallstones were obtained from all the patients. Diagnosis of renal stones is made on the basis of information obtained from the history, physical examination, urinalysis, and radiographic studies. (Mjalando *et al.*, 1998). In this study the stones were analyzed by classical chemical method in which stones were collected and powdered in pestle and mortar. To determine the total cholesterol and bilirubin, 30 mg of the powder was dissolved in 3 ml of chloroform in a test tube. The tube was kept in boiling water bath for 2 minutes. Aliquot from these samples were used for determination of total cholesterol and total bilirubin. To determine calcium, inorganic phosphate, 30 mg of powdered stone was dissolved in 3 ml of hydrochloric acid in graduated 10 ml tube and then volume made up to 10 ml with distilled water. The tubes were kept in a boiling water bath for one hour.

Total cholesterol was estimated by a colorimetric enzymatic method (Biocon Diagnostic, Germany) (Allian CC. and Poon LS., 1974), total bilirubin by Accurex Biomedicals (Ganmino, 1965). Calcium by o-cresolphthalein complexone (OCPC) kit, (Biocon Diagnostic) (Young *et al.*, 1975). Inorganic phosphate was determined according to Fiske and Subba Reo (Fiske CH. And Subba R., 1925).

Statistical Analysis

The results were expressed as mean± standard error of mean (SEM). Student's t-test was used to examine the degree of significance value less than 0.05 was considered significant. To compare between stone types analysis of variance (ANOVA) was also used. The statistical analysis was performed using the statistical Package for Social Sciences, version 17 (SPSS 17).

RESULTS

The total number of cholecystectomy specimen (stones) studied were 75. In this study age ranges from 20-64 years. The age distribution in this study is shown in table (1).

Table 1. Age distribution

Age group(years)	Females	Males	Total
20-30	14	0	14
31-40	11	6	17
41-50	12	3	15
51-60	24	0	24
>61	5	0	5

In table-1- the greater number of patients was in range of 51-60 including only females, so as in the age greater than 61 years.

Table 2. Sex distribution

Gender	Number of the patients
Female	66
Male	9
Female: Male	7.3:1

Gallstones are predominantly seen in females (66) as compared to males (9), therefore the ratio of female: male was 7.3:1 (table-2).

Table 3. Demographic data of the patients.

Characters	Number of the patients
Multiparity*	42(63.64%)
Oral contraceptives*	31(46.97%)
Family history	33(44%)
Renal stone	11(14.6%)
Obese	40(53.3%)
Non-workers	54(72%)

*only for the females.

Multiparity seen in 42(63%) cases, oral contraceptives were used by 31(46.97%) female patients longer than 6 months, and the number of the patients with family history was 33 (44%). The gallstone is associated with renal stone in 11 (14.6%) patients. Most patients in this study were obese 40(53%), and non-workers 54(72%) (table-3).

Table 4. Percentage of each type of gallstone in stone former.

Stone types	Gallstone carriers (n=75)
Cholesterol stone	37(49.3%)
Mixed stone	25(33.3%)
Pigment stone	13(17.3%)

n=number of Gallstone carriers.

The stones were divided into 3 groups depending upon their color, varied from yellow and white stones identified as cholesterol stones, dark brown and black as pigment stones and brownish yellow or green as mixed stones (table-4).

Table 5. Chemical constituents changes among different types of gallstones.

Chemical constituents	Cholesterol stones (n=30)	Mixed stones (n=25)	Pigment stones (n=13)
Cholesterol(mg/g)	593.66±3.1 ^a	545.39±3.7 ^a	507.16±18.76 ^b
Total bilirubin (mg/g)	8.35±0.46 ^a	4.09±0.16 ^a	26.93±0.83 ^b
Calcium (mg/g)	2.91±0.02 ^a	10.52±0.02 ^b	17.01±0.02 ^b
Inorganic phosphate (mg/g)	26.3±0.28 ^a	11.45±0.27 ^b	1.68±0.06 ^b

-n=number of stones.

-Different letters refer to significant difference between means±SE of different type of stones (P<0.05).

-Similar letters refer to non- significant difference between means±SE of different type of stones (P>0.05).

The total cholesterol was significantly higher in cholesterol stone compared to pigment stone (P<0.05) and in mixed stones as compared to pigment stones (P<0.05) (table-5). However, there was an insignificant difference between total cholesterol content of cholesterol stones and mixed stones (P>0.05). The total bilirubin concentration was highest in pigment calculi and lowest in mixed calculi. It was significantly higher in pigment calculi compared to mixed calculi (P<0.05) and insignificantly higher in cholesterol stones as compared to mixed stones (P>0.05). The mean calcium content was highest in pigment stones and lowest in cholesterol stones. The calcium content was significantly higher in pigment calculi and mixed stones as compared to cholesterol calculi (P<0.05) but the content was higher insignificantly in pigment calculi than mixed calculi (P>0.05). The calcium content in various gallstones was in the following order: pigment calculi>mixed calculi>cholesterol calculi (table-5). The inorganic phosphate content was highest in cholesterol calculi and lowest in pigment calculi. There was no significant difference of inorganic phosphorous content between mixed and pigment calculi (P>0.05). However, there was a significant difference between cholesterol calculi and pigment calculi and between cholesterol and mixed calculi (P<0.05).

DISCUSSION

The prevalence of gallstone varies considerably between and within populations; the highest known prevalence is among the American Indians up to 60-70% in females and 10-15% in white adults of development countries (James S. *et al.*, 2011). In this study, total of about 75 cases, the mean age at presentation was 44.53±1.39. Also the study found that maximum number of the patients was within range of 51-60 (table-1). This may be attributed to that the patients in this age group were females who are more liable to develop cholelithiasis, also increasing age may contribute to the development of gallstone by increase in dilatation and therefore volume of gallbladder (Caroli-Bosc FX. *et al.*, 1999) of the 75 females, 42 (63.64%) were of multiparous having 4 or more pregnancies and 31 (46.97%) of the females used oral contraceptive longer than 6 months. Parity appears to be a factor in the development of gallstone women with more pregnancies and longer lengths of fertility periods appear to have a higher likelihood of developing gallstones than those who remain nulliparous (Valdivieso V. *et al.*, 1993).

The number of the patients with family history in present study was 33(44%) out of 75. In family studies, history of gallstones in first-degree relatives increases 3-folds the

risk of gallstone disease (Ko CW. *et al.*, 2005). Eleven patients (41.6%) have both renal stone and gallstone in the present study. A recent study found that patients with cholelithiasis were at a risk of developing nephrolithiasis and the opposite is true and that both types of calculi may be associated, the study not explain the reasons of this association (Erick N. *et al.*, 2005).

Most of the patients in this study were obese (40 out of 75) (53.3%). The reason for increased risk of gallstones in obese patients is due to an increased hepatic secretion of cholesterol (Shaffer EA., 2007; Shaffer EA. and Small DM., 1977). Obesity is recognized as a major gallstone risk factor, it has been associated with gallbladder dysmotility (Vezina WC. *et al.*, 1990). In this study, gallstone disease was predominantly seen in females (88%) as compared to males (12%); the ratio of female :male was 7.3:1 (table-2). In accordance with the findings of previous studies (Hui Sun *et al.*, 2009; Henry V. *et al.*, 2005) the present study may suggest that female sex is a major risk factor for gallstone disease. It had been reported that women had two fold higher risks for cholelithiasis compared to men (Henry V. *et al.*, 2005). The high ratio in Iraqi patients may be due to many factors, which act together to elevate the ratio. About (46.97%) of the female patients were taking contraceptives for longer than 6 months and 72% of the patients were non-workers, in addition to high percentage of obesity in the patients included in this study, all these factors may participate to deviate the present ratio from most studies (Everhart JE. 1994; Pachinoni M. *et al.*, 2002).

Results in table (4) showed that in 75 gallstone, 37(49.3%) cholesterol stones, 25(33.3%) mixed stone and 13(17.3%) pigment stone. Cholesterol stone was the predominant type in this study in agreement with other studies (Bashir jarrar and Meshref A., 2011; Kafia M. *et al.*, 2009), but different from a Libyan study which revealed that most stones were pigment (Abdulla M. *et al.*, 2010). The differences may be attributed to different dietary conditions and habits and different socio-economic status of the people in these areas. This study also explained that the mean of chemical constituents of stones was different in the three types as shown in table (Table-5). Total cholesterol was significantly higher in cholesterol calculi compared to pigment stone (P<0.05) and in mixed stones as compared to pigment stones (P<0.05). However, there was an insignificant difference between total cholesterol content of cholesterol stones and mixed stones (P>0.05). Gallstones are believed to form, when the concentration of cholesterol exceeded that which can be held in mixed miceller solution with bile acids and phospholipids.

Supersaturation of cholesterol is believed to be due to abnormal production of bile from liver. The concept of cholesterol supersaturation as basis for gallstone formation has been emphasized for cholesterol stones, which are composed of mainly cholesterol. The high level of cholesterol in cholesterol calculi has been related to high carbohydrate diet (Cuevas A. *et al.*, 2004). The total bilirubin concentration was highest in pigment calculi and lowest in mixed calculi. It was significantly higher in pigment calculi compared to mixed calculi and cholesterol calculi ($P < 0.05$) and insignificantly higher in cholesterol calculi as compared to mixed calculi ($P > 0.05$). These observations are in agreement partly with other study carried out in Haryana (Pundir CS., *et al.*, 2011). The color of the pigment stones could be attributed to color of bilirubin, which form salt with calcium to form calcium bilirubinate (Ostrow JD., 1984). It is known that β -glucuronidase of bacterial origin hydrolyses conjugated bilirubin into free bilirubin, which form salt with calcium as calcium bilirubinate (Swidsinski A. and Lee S., 2002). The mean calcium content was highest in pigment calculi and lowest in cholesterol calculi. It was significantly higher in pigment calculi and mixed calculi as compared to cholesterol calculi ($P < 0.05$). However, there was an insignificant difference between calcium content of pigment calculi as compared to mixed calculi ($P > 0.05$). The calcium content in various gallstones was in the following order: pigment calculi > mixed calculi > cholesterol calculi. It is in agreement with Libyan article where the calcium was highest in pigment stones (Abdulla M. *et al.*, 2010). Other study also found that calcium carbonate was identified as the most frequently occurring compound in pigment stones (Amin AM. *et al.*, 2002). This could be attributed to the suggestion that the copper and iron may act as chelating agents for calcium bilirubinate. The central aggregates of calcium salts constitute hard foreign bodies which may lead to ulceration of gallbladder mucosa and microscopic hemorrhage. The iron released by this process may be another source of its deposition in gallstones. Injury to gallbladder mucosa also provides an opportunity for release of epithelium β -glucuronidase, an additional enzyme contributing towards precipitation of calcium bilirubinate (Verma GR. *et al.*, 2002). The inorganic phosphate content was highest in cholesterol calculi and lowest in pigment calculi. There was no significant difference of inorganic phosphorus content between mixed calculi and pigment calculi ($P > 0.05$). However, there was a significant difference between cholesterol calculi and mixed calculi ($P < 0.05$) and cholesterol calculi and pigment calculi ($P < 0.05$). This in confliction to the findings from North India (Pundir CS., *et al.*, 2011), where mixed calculi had highest inorganic content. Inorganic phosphate might be playing an important role in the formation of cholesterol gallstones by forming salt with calcium. Reports from different parts of the world indicate that the incidence of gallstones is correlated with socio-economic conditions and dietary factors (Jensen KH and Jorgensen T., 1991; Mjaland O. *et al.*, 1998).

CONCLUSION

An interesting finding in this study that the females at higher risk of cholelithiasis development than males at a ratio of female: male (7.3:1). This finding is attributed to high percentage of patients with high risk factors such as multiparity, using of oral contraceptives, obesity, family history and sedentary lifestyle (non-workers). However this observation needs further randomized studies to confirm it. Cholesterol stone was the most common type of stones and the cholesterol seemed to be the major component in all types of stones so it is regarded as a major key in controlling cholelithiasis.

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