International Journal of Medicine and Medical Sciences Vol. 5(1), pp. 19-23, January 2013
Available online at http://www.academicjournals.org/IJMMS
DOI: 10.5897/IJMMS12.031
ISSN 2006-9723 ©2013 Academic Journals

Full Length Research Paper

Descriptive study of cholelithiasis with chemical constituents' analysis of gallstones from patients living in Baghdad, Iraq

Mohammed A. Taher

Department of Clinical Laboratory Sciences, College of Pharmacy, University Of Baghdad, Iraq.
E-mail: Mohammed_taher43@yahoo.com.

Accepted 6 November, 2012

The aim of this study was to describe the main clinical features of patients with cholelithiasis, and chemical analysis of stone in relevance to development of gallstone formation. The total number of 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 multiple factors, including high percentage of multiparity (63.64%), the use 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 was the most prevalent type of stone; its percentage was 49.3 greater than mixed and pigment stones which had percentages of 33.3 and 17.3, respectively. Cholesterol stone showed significantly higher cholesterol content ($P < 0.05$) than pigment stones; though not significantly higher than mixed stones. Cholesterol content in mixed stone was significantly higher ($P < 0.05$) than pigment stones. Total bilirubin content in pigment stones was significantly higher ($P < 0.05$) than mixed and cholesterol stones. The bilirubin content in cholesterol stones was higher [but not significantly ($P > 0.05$)] than mixed stones. Calcium content in pigment stone was significantly higher ($P < 0.05$) than cholesterol stones and higher [but not significantly ($P > 0.05$)] than mixed stones. Inorganic phosphate content in cholesterol stones was significantly higher ($P < 0.05$) than mixed and pigment stones. The content of inorganic phosphate in mixed stones was insignificantly higher than pigment stones. In conclusion, cholesterol stone was the most common type of stone 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, gallstones, Baghdad.

INTRODUCTION

Cholelithiasis is a real problem in Baghdad, Iraq because increasing number of females are attending hospitals due to gallstone complications; the disease seems to be more prevalent than expected. The current study was carried out in the biggest medical center in Iraq and the patients came from different residences; so they may represent the real sample of Iraqi patients living in Baghdad. Reports about gallstone types are rare, and there are no reports 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 Body mass index (BMI) > 30 kg/m^2 have 95% cholesterol-dominant gallstones and are at higher risk of cholesterol stone (Schafmayer et al., 2006). Other risk factors include marked family history of gallstone disease, ageing, multiple parity, cholecystitis and sedentary lifestyle (Cuevas 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

Table 1. Age distribution.

Age group (years)	Female	Male	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

Table 2. Sex distribution.

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

Table 3. Dempographic 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.

factors (Bashir and Meshref, 2011). The identification of the components of gallstone is essential as it provides information that could be useful to medical practitioners to find out the underlying cause of gallstone and to decide whether to treat gallstone patients surgically or therapeutically (Kafia et al., 2009). Moreover, analysis of the chemical composition of gallstones can provide a significant reference to the treatment and prevention of their recurrence (Wang et al., 2007).

Major elements involved in the formation of human gallstones are cholesterol, bilepigment and calcium (Moosavi et al., 2006). With regard to chemical composition, majority of the human gallstones are cholesterol and pigment stones (Vivek et al., 2008). Other substances found in gallstones include calcium salts of phosphate, mucin, glycoprotein, phospholipids and some metals (Pundieir et al., 2002). It has been reported that some elements play a significant role in the formation of gallstones. It was observed that 12 elements namely, sulfur, chloride, potssium, vanadium, chromium, manganase, iron, nickel, copper, zinc, bromide and lead were present in the gallstones collected from Indian farmers (Rautary et al., 2006).

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

MATERIALS AND METHODS

A total number of 75 patients with gallstone were admitted in 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 was made on the basis of information obtained from the history, physical examination, urinalysis and radiographic studies (Mjaland 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 min. Aliquot from these samples were used for determination of total cholesterol and total bilirubin. To determine calcium, an inorganic phosphate, 30 mg of powdered stone was dissolved in 3 ml of hydrochloric acid in graduated 10 ml tube and then the 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 et al., 1974), total bilirubin by Accurex Biomedicals (Ganmino and Meiter, 1965), calcium by o-cresoiphthalein complexone (OCPC) kit, (Biocon Diagnostic) (Young et al., 1975). Inorganic phosphate was determined according to Fiske and Subbarow (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; values less than 0.05 were 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, the age ranged from 20 to 64 years. The sex distribution in this study is shown in Table 1. In Table 1, the greater number of patients was in the range of 51 to 60, which included only females, so as in the age greater than 61 years. Gallstones were predominantly seen in females (66) as compared to males (9), therefore the ratio of female: male was 7.3:1 (Table 2). Multiparity was 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 was 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). The stones were divided into

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.

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

Chemical constituents (mg/l)	Cholesterol stones (n=30)	Mixed stones (n=25)	Pigment stones (n=13)
Cholesterol	593.66±3.1[a]	545.39±3.7[a]	507.16±18.76[b]
Total bilirubin	8.35±0.46[a]	4.09±0.16[a]	26.93±0.83[b]
Calcium	2.91±0.02[a]	10.52±0.02[b]	17.01±0.02[b]
Inorganic phosphate	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 ($P > 0.05$).

3 groups, depending upon their color; variations from yellow and white stones were identified as cholesterol stones, dark brown and black as pigment stones, and brownish yellow or green as mixed stones (Table 4).

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 in-significantly 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 insignificantly higher 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 in 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

In this study, with a 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 the range of 51 to 60 (Table 1). This may be attributed to the fact that the patients in this age group were females who were 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 et al., 1999). Of the 75 females, 42 (63.64%) were 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 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 by 3-folds to the risk of gallstone disease (Ko 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 did not explain the reasons for this association (Erick 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, 2006). Obesity is recognized as a major gallstone risk factor associated with gallbladder dysmotility (Vezina 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 3). In accordance with the findings of previous studies (Hui et al., 2009), 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 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 (drivers, office workers and housewives), 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 (Pacchinoni et al., 2002).

Results in Table 5 showed that in 75 gallstone, 37 (49.3%) were cholesterol stones, 25 (33.3%) mixed stone and 13 (17.3%) pigment stone. Cholesterol stone was the predominant type in this study which is in agreement with other studies (Bashir and Meshref, 2011) but is different from a Libyan study which revealed that most stones were pigment (Abdulla 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 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 a 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 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 partly in agreement with other studies carried out in Haryana (Pundir et al., 2011).

The color of the pigment stones could be attributed to color of bilirubin which forms salt with calcium to form calcium bilirubinate (Ostrow, 1984). It is known that β-glucuronidase of bacterial origin hydrolyses conjugated bilirubin into free bilirubin which forms salt with calcium as calcium bilirubinate (Swidsinski and Lee, 2001). 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 et al., 2010). Other study also found that calcium carbonate was identified as the most frequently occurring compound in pigment stones. This could be attributed to the suggestion that 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 et al., 2002).

The inorganic phosphate content was highest in cholesterol calculi and lowest in pigment calculi. There was no significant difference in 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 is in conflict with the findings from North India (Pundir 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 (Mjaland et al., 1998).

Conclusion

An interesting finding in this study is that the females are at higher risk of cholelithiasis development than males, in a ratio of female: male (7.3:1). This finding is attributed to high percentage of patients with high risk factors such as multiparity, use 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 cholesterol seemed to be the major component in all types of stones, so it is regarded as a major key in controlling cholelithiasis.

ACKNOWLEDGMENTS

My deepest thanks to all the surgeons in the Medical City Hospital particularly Prof. Dr. Monder Al-Aubaidy, Prof.

Dr. Azzam K. Agha and Dr. Muayad A. Fadhel for their help in providing me patient stones and information.

REFRENCES

Abdalla MJ, Peela J, Trushakant NP, Abdul H, Hayam AA, Saeid OE, Ezedin BA, Maisoon NE, Mahmood FT (2010). Quantitative analysis of gallstones in Libyan patients. Libyan J. Med. 5:4627.

Allian CC, Poon LS, Cham CS, Richmond W, Fu PC (1974). Eznymatic determination of total serum cholestrol. Clin. Chem. 20:470-5.

Bashir J, Meshref AA (2011). Chemical composition of gallstones from Al-Jouf province of Saudi Arabia. Malays. J. Med. Sci. 18(2):47-52.

Caroli-Bosc FX, Puglies P, Peteen EP, Demarquay JF, Montet JC, Hastier P, Staccini P, Delmont JP (1999). Gallbladder volume in adults and its relation to age, sex, body mass index, body surface area and gallstones. An epidemiologic study in a non-selected population in France. Digestion 60:344-348.

Cuevas A, Miquel JF, Reyes MS, Zanlungo S, Nervi F (2004). Diets as a risk factor for cholesterol gallstone disease. J. Am. Coll. Nutr. 23(3):187-196.

Eric NT, Meir JS, Gary CC (2005). Obesity, weight gain and the risk of kidney stones. JAMA 293(4):455-462.

Fiske CH, Subbarow Y (1925). The colorimetric determination of phosphorus. Biol. Chem. 66:375-400.

Ganmino SR, Meiter SE (1965). standard methods of clinical chemistry.Meiter S, editor.vol.5.New York, NY: Academic press 1965. P.55.

Henry V, Sebastian EB, Dietrich A, Wolfgang H, Christian S, Peter S, Ulrich J, Markus ML (2005). Independent risk factors for gallstone formation in a region with high cholelithiasis prevalence. Digestion 71(2):97-105.

Hui S, Hong T, Shan J (2009). Gender and metabolic difference of gallstone disease. World J. Gasteroenterol. 15(15):1886-1891.

Kafia MS, Lazeeza SO, Sirwan AG (2009). Correlation between the chemical composition of gallstones and sera of stone formers. Gomal J. Med. Sci. 7(1):2-6.

Ko CW, Beresford SA, Schulte SJ, Matsumoto AM, Lee SP (2005). Incidence, natural history, and risk factors for biliary sludge and stones during pregnancy. Hepatology 41:359-365.

Mjaland O, Adamsen S, Hjelmquist B, Ovaska J, Buuanes T (1998). Cholecystectomy rates, gallstone prevalence, and handling of bile duct injuries in Scandinavia. Surg. Endosc. 12:1386-1389.

Moosavi K, Vantankhah S, Salimi J (2006). Relative measurement of heavy elements in the bile, gallbladder and gallstones. Iran. J. Radiat. Res. 3(4):195-1198.

Ostrow JD (1984). The etiology of pigment gallstones. Hepatology 4(5):215s-22s.

Pacchinoni M, Nicoletti C, Caminiti M (2002). Association of obesity and type 2 diabetes mellitus as a risk factor for gallstones. Dig. Dis. Sci. 45(10):2002-6.

Pundir CS, Chaudhary R, Rani K, Chandran P, Kumari M, Carg P (2011). Chemical analysis of biliary calculi in Haryana. Ind. J. Surg. 63:370-73.

Rautary TR, Tapash R, Venkatathri (2006). Analysis of Indian cholesterol gallstones by particle induced x-ray emission and thermogravimetry-derivative. Eur. J. Gastroenterol. Hepatol. 18:999-1003.

Schafmayer C, Hartleb J, Tepel J, Albers S, Freitag S, Völzke H, Buch S, Seeger M, Timm B, Kremer B, Folsch UR, Fandrich F, Krawczak M, Schreiber S, Hampe J (2006). Predictors of gallstone composition in 1025 symptomatic gallstones from Northern Germany. BMC Gastroenterol. 6:36.

Shaffer EA (2006). Gallstone disease: Epidemiology of gallbladder stone disease. Best Pract. Res. Clin. Gastroenterol. 20:981-996.

Swidsinski A, Lee S (2001). The role of bacteria in gallstone pathogenesis. Front. Biosci. 6:93-103.

Trowbridge RL, Rutkowski NK, Shojania KG (2003). Does this patient have acute cholecystitis? JAMA 289:80-6.

Valdivieso V, Covarrubias C, Siegel F, Cruz F (1993). Pregnancy and cholelithiasis: Pathogenesis and natural course of gallstones diagnosed in early puerperium. Hepatology 17:1-4.

Verma GR, Randey AK, Bose SM, Prasad R (2002). Study of serum calcium and trace elements in chronic cholelithiasis. ANZ J. Surg. 72:596-9.

Vezina WC, Paradis, Grace DM (1990). Increased volume and decreased emptying of the gallbladder in large (morbidity obese, tall normal, and muscular normal) people. Gastroenterology 98:1000-1007.

Vivek KS, Vinita S, Awadhesh KR, Surya NT, Pradeep KR, Jagdish PS (2008). Quantitative analysis of gallstones using laser-induced breakdown spectroscopy. Appl. Opt. 47(31):38-47.

Wang Q, Zhang H, Ouyang J (2007). Progress in study of application of modern instruments to investigation of gallstone. Guang Pu Xue Yu Guang Pu Fen Xi. 27:202-6.

Young DS, Pestaner LC, Gibberman V (1975). Effect of drugs on clinical laboratory tests. Clin. Chem. 21(5):1D-432D.