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Quantitative analysis of gallstones in Libyan patients

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Gallstone disease is one of the major surgical problems in the Libyan population; it is probably related to diet, especially excessive consumption of meat. The 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. Their phospholipid content and inorganic phosphates were higher than in the other types of stones and oxalate content was significantly elevated in comparison with MS ($p = 0.0471$). In MS, the cholesterol, bile acids, and bilirubin were intermediate between cholesterol and PS, whereas triglycerides were significantly more than PS ($p = 0.0004$). Bilirubin (0.0001) and bile acids ($p = 0.0009$) were significantly higher than CS ($p = 0.0001$). However, they contained the lowest amounts of sodium, potassium, magnesium, and oxalate. In PS, bilirubin ($p = 0.0001$) was significantly higher than both groups. Bile acid content was significantly higher than CS ($p = 0.0001$) but not significantly more than MS. They showed the highest values of calcium, sodium, potassium, magnesium, and chlorides compared to the other types of stones. High levels of cholesterol in stones and dyslipidemia associated with mixed as well as cholesterol gallstones suggest an etiological association and efforts to reduce dietary fat among the Libyan population may lead to decreased cholesterol and mixed gallstones.

Keywords: *gallstones*; *chemical composition*; *Libya*; *cholesterol*

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Cholelithiasis or gallbladder stones are one of the major surgical problems in the Libyan population and account for many hospital admissions and surgical interventions. Most patients with gallstones present with severe abdominal colic requiring investigations and treatment. Many of them need surgical intervention by the time they are symptomatic. This problem is probably related to obesity, cardiovascular disorders (CVD), metabolic syndrome, and dietary habits, especially excessive consumption of meat, which is known to contain large amounts of cholesterol. Obese individuals with a BMI >30 kg/m^2 have 95% cholesterol-dominant gallstones and are at a high-risk for cholesterol stones (CS) (1). The mechanism of stone formation has been the subject of extensive research for several years. However, there are no reports on the chemical composition of gallstones in Libya though a sizeable population suffers from gallstones. Earlier studies reported only on prevalence and case reports on gallstones in Libya (2–4). Studies on gallstone composition carried out in different parts of the world indicate a close link with dietary habits and ethnicity (5–15). Studies have also shown that dietary intake of total calories in the form of carbohydrates and fats were associated with high triglyceride levels in gallstone patients (16). Gallstone formation is relatively increased with consumption of dietary fats rich in saturated fatty acids. Consequently, substitution of 18:1 with unsaturated fatty acids present in low-fat diets would reduce gallstone formation without affecting the lithogenic index (17). This study describes an extensive quantitative analysis of gallstones in Libyan patients, including cholesterol, triglycerides, phospholipids, bilirubin, bile acids, calcium, phosphorus, sodium, potassium, magnesium, oxalates, and chlorides.

Table 1. Physical properties of gallstones

		Cholesterol stones	Mixed stones	Pigment stones
Sex	Females	11	12	12
	Males	0	2	4
Shape		Round 3	Round 4	Round 6
		Irregular 8	Irregular 10	Irregular 10
Color		Yellow 9, White 2	Green 12, Greenish brown 2	10 brown, 6 black
Surface		Smooth 6	Smooth 5	Smooth 12
		Rough 5	Rough 5	Rough 4
Weight (g, mean)		5.40	1.78	3.00
Character		Soft 9, hard 2	Soft 11, hard 3	Soft 10, hard 6
Size (cm, mean)		0.8 × 0.9	0.6 × 0.7	0.9 × 0.9

Materials and methods

Gallstones from 41 patients with cholelithiasis were collected after cholecystectomy at the Department of Surgery, Seventh October Hospital, Benghazi, Libya, between March and September 2008. This study was approved by the ethics committee of the Al-Arab Medical University, Benghazi, Libya. The stones were classified into three types depending on their color and degree of hardness. Yellow and whitish stones were identified as CS, black and dark brown as pigment stones (PS), and brownish yellow or green as mixed stones (MS). Demographic data on age and sex was also collected. The stones were powdered using a mortar and dissolved in different solvents depending on the type of chemical constituent to be analyzed. To determine total cholesterol and total bilirubin, 30 mg stone powder was dissolved in 3 ml chloroform in a test tube. The tube was kept in boiling water bath for 2 min. Aliquots from these samples were used for determination of total cholesterol and total bilirubin. To determine calcium, oxalate, inorganic phosphate, magnesium, chloride, triglycerides, sodium, and potassium, 30 mg of the powdered stone was dissolved in 3 ml of HCl in a graduated 10-ml tube and the volume was made up to 10 ml with distilled water. The tubes were kept in a boiling water bath for one hour. To analyze phospholipids, 20 mg of powdered stone was dissolved in 15 ml of a 2:1 mixture of chloroform and methanol containing 1 N HCl. To measure bile acids, the stones were dissolved in chloroform–methanol (2:1) mixture. The solutions were preserved at 4°C until they were used.

Total cholesterol was estimated by a colorimetric enzymatic method (Biocon Diagnostics, Germany) (18), total bilirubin by Accurex Biomedicals (19), triglycerides by an enzymatic colorimetric method of Biocon Diagnostics (20), oxalate by the method described by Satyapal and Pundir based on colorimetric enzymatic method (21), calcium by an o-cresolphthalein complexone (OCPC) kit (Biocon Diagnostics) (22). Phospholipid and inorganic phosphate were determined according to Fiske and Subba Rao (23), magnesium by xylidyl blue (Biocon Diagnostics) (24), chloride by the method of Schoenfeld (25), sodium and potassium by flame photometry (Clinical flame photometer, Evano Electro Selenium Halsted, Essex, UK), and bile acids by the colorimetric method of Carey (26). For colorimetric procedures, we used reagents from Biocon Diagnostics, Germany, and a spectrophotometer from Labomed Inc., USA (UV-Vis-1179, RS Spectrophotometer). Statistical analyses were performed with GraphPad software (GraphPad Software Inc., USA). The unpaired *t*-test was used for comparison of group means. A *p*-value of <0.05 was considered significant.

Results

All 41 patients (six males and 35 females) had multiple gallstones. CS were bigger than MS and PS. Fourteen of the patients (34%) had MS, 16 (39%) had PS, and 11 (27%) had CS (Table 1). The incidence of gallstones was highest in age group of 41–50 years (Table 2).

CS had the highest composition of cholesterol, while MS had a high content of triglycerides and PS were comprised mostly of bilirubin (Tables 3 and 4).

Table 2. Frequency of various types of gallstones according to age

Age group (years)	CS	MS	PS	Total
≤20	0	0	1	1
21–30	2	2	5	9
31–40	5	5	2	11
41–50	2	4	6	12
51–60	1	3	1	5
≥61	1	0	1	2
	11	14	16	41

Note: CS, cholesterol stones; MS, mixed stones; PS, pigment stones.

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Table 3. Concentrations of metabolites in the different types of biliary calculi

Stone type	Cholesterol (mg/gm)	Triglycerides (mg/gm)	Bilirubin (mg/gm)	Bile acids (mg/gm)	PL (mg/gm)
CS	608±173	51±14	0.5±0.30	16.5±2.60	8.0±2.70
MS	518±125	56±10	2.0±0.30	20.0±2.00	5.7±0.90
PS	466±87	40.5±11	4.0±0.90	22.0±3.60	6.0±1.40

Note: CS, cholesterol stones; MS, mixed stones; PS, pigment stones; PL, phospholipids. Values are the mean and standard deviation.

The concentrations of different ions also varied with the type of stone. PS had the highest concentrations of most of the ions, including calcium, sodium, potassium, magnesium, and chloride, whereas CS contained higher concentrations of phosphates and oxalates (Tables 5 and 6).

Discussion

Cholesterol

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 (27). 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 pathogenesis of cholesterol gallstones is rooted in altered lipid metabolism, e.g. hyperlipidemia type IIa and type IV give rise to a greater proportion of cholesterol relative to other bile lipids secreted from the liver into bile (28, 29). The co-existence of nucleating factors, gallbladder hypomotility (28), and mucus hyper secretion also contribute to cholesterol precipitation leading to the development of gallstones (30, 31).

Bile salts

Bile salts were significantly lower in CS than in MS and PS, while bile acids were significantly higher in PS. Supersaturation of bile with calcium bilirubinate is inhibited by bile salts, which bind calcium, reducing the activity of free calcium ions. When supersaturation occurs, usually due to increased concentrations of bilirubinate anion, nucleation may be initiated by binding of calcium bilirubinate to mucin glycoproteins in bile (31, 32). Similarly, the phospholipid content was marginally higher in cholesterol than PS and MS. Cholesterol has to be in relative proportion with the bile salts and phosphatidyl choline to remain soluble in the bile and thereby avoid stone formation (33, 34). About 5% of cholesterol is barely soluble in 20% phosphatidyl choline and this should require approximately 60% of bile salts. Any concentration above this level would cause precipitation of cholesterol, as shown by the presence of the highest amount of cholesterol in CS is an indicator of this fact (35). PS contained less cholesterol than the other two types of stone. Cholesterol in PS is mostly because of co-precipitation with bilirubin and other compounds but not due to its supersaturation in the bile (36). In contrast to this, MS have less cholesterol than CS but more than PS. This might be due to the presence of both cholesterol and bile pigment in these stones.

Triglycerides

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 (37).

Phospholipids

Phospholipid content was highest in CS and lowest in MS. There were significantly more phospholipids in CS than in MS ($p = 0.0080$) and PS ($p = 0.0170$).This might be due to accumulation of phospholipids along with cholesterol during CS formation.

Table 4. *P*-values for differences in chemical composition of biliary calculi shown in Table 3

Groups	Cholesterol	Triglycerides	Bilirubin	Bile acids	Phospholipids
CS vs. MS	0.1472	0.3186	0.0001	0.0009	0.0080
CS vs. PS	0.0085	0.0263	0.0001	0.0001	0.0170
MS vs. PS	0.1983	0.0004	0.0001	0.8521	0.5101

Note: CS, cholesterol stones; MS, mixed stones; PS, pigment stones.

Table 5. Concentrations of different ions in gallstones (mg/gm powder)

Type	Ca^{2+}	PO_4^-	Na^+	K^+	Mg^{2+}	Cl^-	Oxalate
CS	11.4±2.40	14.0±1.60	1.4±0.50	0.5±0.17	8.5±1.80	19.5±1.50	7.0±1.18
MS	18.0±4.70	10.5±1.90	1.15±0.18	0.32±0.06	7.75±0.90	21.5±1.90	6.0±1.20
PS	21.75±3.60	9.0±1.50	3.5±0.73	0.65±0.17	10.8±1.25	33.5±3.60	6.5±0.90

Note: CS, cholesterol stones; MS, mixed stones; PS, pigment stones. Values are the mean and standard deviation.

Bilirubin

As expected, the bilirubin content was highest in PS. This has been demonstrated in many earlier studies (32). The bilirubin in these stones is mostly unconjugated because the conjugated form is water soluble and excreted through bile (38). Decreased secretion of biliary acids, increased secretion of unconjugated bilirubin into the bile, and infection of the biliary tract are the most important causative factors (39, 40).

Calcium

Calcium content was highest in PS, while phosphorus was lowest. It is known that bilirubin combines with calcium to form a precipitate of calcium bilirubinate (41). Since PS have excess bilirubin, calcium forms calcium bilirubinate (42).

Phosphorus

Phosphorus content was low in PS. The reason for this is not clear but may be related to the availability of calcium, which is more in these stones in the form of calcium bilirubinate. Phosphorus content was significantly higher in CS and MS than PS. It is likely that phosphorus may play a more important role than calcium in CS formation by forming a salt with calcium, which might be responsible for the hardness of the CS (6).

Sodium and potassium

Sodium content was higher in PS than in the other types. It was not surprising that bile acids were more in PS because an increase in sodium content facilitates excessive formation of bile salts. Potassium was also higher in PS. It is presumed that the sodium to potassium ratio will be maintained in the bile. Hence, higher sodium content is associated with higher potassium content, although the increase in the latter was not as much as that of the sodium content.

Chlorides

Chlorides were higher in PS than MS and CS. Chlorides are among the important constituents of bile, and they may form salts with sodium, thereby contributing to stone formation.

Magnesium

Magnesium was higher in PS and CS than MS. This is in conformity with observations made by Chandran et al. (13).

Oxalates

Since the higher oxalate content in CS is likely to be associated with higher magnesium content, the formation of magnesium oxalate may be responsible for the hardness of PS.

The Libyan population seems to be more susceptible to cholelithiasis when compared with other countries according to earlier studies in Libya (2, 3). The observation that CS are the most prevalent type of gallstones is in agreement with studies performed in northern India (6, 7, 11), Japan (14), and Singapore (15), but differs from sub-Saharan countries and southern India, where PS are more prevalent, with a higher bilirubin and calcium content and lower cholesterol content when compared with stone composition in Libya (43, 44).

The potential reasons for stone formation may be attributed to (a) dietary factors, (b) multiple pregnancies with less spacing between pregnancies, (c) metabolic syndrome, (d) familial, and (e) ethnic (45–47). It is known that foods that are rich in lipids result in development of hyperlipidemias and subsequent increase in all lipid constituents. There is no corresponding

Table 6. *P*-values for differences in composition of various ions of gallstones shown in Table 5

Comparison	Ca^{2+}	PO_4^-	Na^+	K^+	Mg^{2+}	Cl^-	Oxalate
CS vs. MS	0.0002	0.0001	0.1044	0.0016	0.1952	0.0080	0.0471
CS vs. PS	0.0001	0.0001	0.0001	0.0291	0.0005	0.0001	0.2140
MS vs. PS	0.0218	0.0248	0.0001	0.0001	0.0001	0.0001	0.2105

Note: CS, cholesterol stones; MS, mixed stones; PS, pigment stones.

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Table 7. Percentage of chemical composition of gallstones from various countries

Country and reference	No. of stones	Cholesterol (%)	Triglycerides (%)	Bilirubin (%)	Calcium (%)	PO_4^- Mg, Oxalates, Na^+, K^+, and Cl^- (%)
Australia (49)	5	93			3.5	3.5
England (51)	11	66			17	17
Germany (1)	1,025	93.3		5.5	4.8	
India (13)	200	56.6	5.7	0.3	2.2	35.2
Kuwait (51)	10	60			37	3
South Africa (51)	11	66			29	5
Sweden (51)	27	94			4	2
USA (51)	42	88			9	3
Bolivia (50)	66	93.3			4.1	
Libya (present study)	41	53	4.9	2.16	1.70	38.4

increase in substances such as bile salts and phospholipids, which are responsible for solublization of cholesterol and other biliary constituents. Consequently, there is a precipitation of cholesterol and bilirubin leading to stone formation (40).

Comparison of the chemical composition of gallstones in Libyan individuals with those in other countries is summarized in Table 7. Though cholesterol is a major component of gallstones, the composition of gallstones varies from country to country and region to region. One study in sub-Saharan Africa showed that majority of stones were of the pigment type, in which the contribution of cholesterol is less than in other stones (43). Similarly, a study in southern India reported predominance of pigment and mixed gallstones with reduced cholesterol and increased bilirubin and calcium concentrations in the stones (44).

CS are more prevalent than PS in north India, Australia, Bolivia, Germany, England, Kuwait, USA, Sweden, and South Africa (48–51). Although all types of gallstones are prevalent among the Libyan population, it is interesting to note that cholesterol is the major component of all stones.

Conclusion

An interesting finding of our study is that although PS was the most common type of gallstones, cholesterol seemed to be the major component in all types of stones. High cholesterol content in CS especially suggests supersaturation of cholesterol in bile consequent to dyslipidemia (excessive cholesterol and altered lipid metabolism) is an etiological factor. Higher triglyceride content in MS also suggests that dyslipidemic changes contribute to etiology. Our findings suggest that dyslipidemia consequent to high intake of fats by Libyan population may be responsible for gallstones, and dietary modification might reduce the incidence of gallstones. 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.

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