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Extensive Quantitative Analysis of Gallstones

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ABSTRACT

Background: The chemical composition of gallstones is essential to study aetiopathogenesis of gallstone disease. Objective: To determine the composition of gallstones in a Sudanese population. Patients and methods: It describes an extensive quantitative analysis of gallstones from patients presented with symptomatic gall stone disease and treated by cholecystectomy after the acceptance of the pre-given informed consent in Khartoum teaching hospital in the period between Jan 2010 and Dec 2010. Using a pretested questionnaire data collected from and analyzed statistically by SPSS computer program version 21. Results: Data are analyzed from 94 patients (six males and 88 females). Cholesterol stones showed a significantly higher cholesterol content than pigment stones ($p = 0.0042$), though not significantly higher than mixed stones. Their phospholipids content and inorganic phosphates were higher than in the other types of stones and oxalate content was significantly elevated in comparison with mixed stones ($p = 0.0402$). In mixed stones, the cholesterol, bile acids, and bilirubin were intermediate between cholesterol and pigment stones, whereas triglycerides were significantly more than pigment stones ($p = 0.0007$). Bilirubin ($p = 0.0021$) and bile acids ($p = 0.0016$) were significantly higher than cholesterol stones ($p = 0.0001$) and ($p = 0.0001$) respectively. However, they contained the lowest amounts of sodium, potassium, magnesium, and oxalate. In pigment stones, bilirubin was significantly higher ($p = 0.0001$) than both groups. Conclusion: Collaborations between surgeons, nutritionists, biochemists, and physicians should be stimulated in future studies to define the different types of gall stones in different areas in Sudan and the relevancy of such types with diets' traditions.

KEYWORDS

Cholesterol Stone (CS); Gallstone (GS); Mixed Stone (MS); Pigment Stone (PS)

1. Introduction

Gallstones, complex biomineralized deposits formed in the gallbladder, are still a major health problem all over the world [1]. When symptoms arise, cholecystectomy is recommended [2-4].

Cholelithiasis is common with the incidence ranging from 10% to 20% of the world population, 11% of the general population of the United States (US) [5]. The incidence is four times higher in women than in men with the high prevalence among younger age group [6].

On the basis of their composition, gallstones can be divided into the three types: Cholesterol stones (CS) that vary in colour from light-yellow to dark-green or brown and are oval, and they must have at least 80% cholesterol by weight (or 70%, according to the Japanese classification system); Pigment stones (PS) which are small, dark stones made of bilirubin and calcium salts that are found in bile, and they contain less than 20% of cholesterol (or 30%, according to the Japanese classification system); and Mixed stones (MS) which typically contain 20% - 80% cholesterol (or 30% - 70%, according to the Japanese classification system) [7].

By defining the pattern and type of the gallstone, we will open new windows for further investigations in the

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future helping in implementing the non-surgical interventions measures. The aim of the current study is to determine the prevalence of different types of gallstone, and to explore the composition of the stones in symptomatic gallstone disease (GSD) in Sudan.

2. Patients and Methods

A cross-sectional study was conducted in Khartoum teaching hospital, Sudan (January 2010 and December 2011). Khartoum is a capital city of the Sudan with more than six million inhabitants [8]. All patients with symptomatic calcular cholecystitis that confirmed ultrasonographically and treated by cholecystectomy were involved into our study after acceptance of the informed consent. Before commencing the study ethical approval was obtained from ethical committee Sudan Medical Specialization Board. Using predesigned questionnaire, general physical examination was performed to participants and then enrolled into an appropriate investigation.

The stones were divided into 3 groups depending upon their colors: pale yellow and whitish stones as cholesterol calculi, black and blackish brown as pigment calculi and brownish yellow or greenish with laminated features as mixed calculi. Then, they were powdered in a pestle and mortar and dissolved in different solvents depending upon the type of ionic constituents that would be analyzed. To determine calcium, oxalate, inorganic phosphate, magnesium, chloride, soluble proteins, triglycerides, iron, copper, sodium and potassium, 30 mg stone powder was dissolved in 3 ml I N HCl (one normal HCl) in a graduated 10 ml tube and its final volume was made up to 10 ml with distilled water. The tube was kept in boiling water bath for 1 hour. Total oxalate, inorganic phosphate, magnesium, and chloride all were estimated by a suitable colorimetric method, while iron and copper were estimated by atomic absorption spectrophotometer, sodium and potassium by flame photometer. The dissolved stone solutions were stored at 2˚C - 8˚C, when not in use.

The collected data was spread on master sheet, entered computer and managed statistically using SPSS computer program version 21. Numerical data was expressed as a mean ± SD. The stone-types were compared between groups using the Chi-square test. The confidence level was set at 95% CI and p values less than 0.05 were statistically considered significant.

3. Results

3.1. Demography

The study included 94 patients (six (6.4%) males and 88 (93.6%) females) with female to male ratio of 14.7:1. Their age ranged between 27 and 80 years, mean age ± SD was 45.95 ± 10.253.

3.2. Stones Properties

In 61 (64.9%) were multiple, while the rest 33 (35.1%) were solitary. While on preoperative ultrasound scan multiple and solitary stones detection were as 56 (59.6%) and 38 (40.4%) respectively. this observational difference it was statistically significant ($p = 0.0001$).

According to stone classification, 48 (51.07%) were pigment stones (PS), 30 (31.91%) were mixed stones (MS) and 16 (17.02%) were Cholesterol stones (CS) indicating the incidence of gallstones in the studied population from Khartoum as follows: Pigment calculi (51.06%) more than Mixed calculi (31.91%) more than cholesterol calculi (17.02%).

Cholesterol stones (CS) were bigger in size as compared to mixed stones (MS) and pigment stones (PS) **Table 1**. The incidence of gallstones was highest in age group 41 - 50 years (43.6%), **Table 2**.

3.3. Determination of Metabolites

The cholesterol, bilirubin, bile acids, fatty acids, triglycerides, phospholipids and soluble protein were found in all 94 gallstones.

Cholesterol stones (CS) had the highest composition of cholesterol, while mixed stones (MS) had a high content of triglycerides and pigment stones (PS) were composed mostly of bilirubin (**Tables 3** and **4**).

The mean total cholesterol concentration was significantly higher in cholesterol calculi (713 mg/gm), compared to pigment (421 mg/gm) ($p = 0.0042$) and in mixed stones (637 mg/gm) as compared to pigment stones ($p = 0.0261$). However, there was an insignificant difference between total cholesterol content of cholesterol

Table 1. Physical properties of gallstones in patients with symptomatic calcular cholecystitis in the study group (n = 94).

	Cholesterol stones	Mixed stones	Pigment stones
Sex (Female/Male)	16/0	27/3	45/3
Shape	Rounded 14, Faceted 2	Rounded 12, Irregular 11, Faceted 3, Laminated 4	Irregular 35, Faceted 9, Laminated 4
Colour	Pale Yellow 9, White 7	Greenish 4, Red 8, Brownish yellow 18	Blackish brown 18, Black 30
Surface	Smooth 16	Smooth 19, rough 11	Smooth 7, rough 41
Weight (g, mean)	5.7	1.9	3.2
Character	Soft 13, hard 3	Soft 24, hard 6	Soft 32, hard 16
Size (cm, mean)	0.9 × 0.9	0.5 × 0.6	0.7 × 0.8

stones and mixed stones (p = 0.1032).

The mean triglyceride content was highest in cholesterol stones (69.3 mg/gm) and lowest in pigment stones (32.8 mg/gm). The triglyceride content was significantly higher in cholesterol calculi (p = 0.0371) and mixed calculi (p = 0.0183) as compared to pigment calculi. However, the difference was insignificant between cholesterol calculi and mixed calculi (p = 0.1303).

The mean fatty acid content was highest in cholesterol calculi (21.71 mg/gm) and lowest in mixed calculi (13.82 mg/gm). The difference of fatty acid content was significant between cholesterol calculi and mixed calculi (p = 0.0216) and between cholesterol calculi and pigment calculi (p = 0.0093) but insignificant between mixed and pigment calculi (p = 0.1017).

The mean total bilirubin concentration was highest in pigment stones (4 mg/gm) and lowest in cholesterol stones (0.3 mg/gm). It was significantly higher in pigment stones compared to cholesterol stones and mixed stones (p = 0.0001) and insignificantly higher in mixed stones as compared to cholesterol stones (p = 0.0021).

The mean bile acid content in pigment stones (25 mg/gm) was comparatively higher than that in cholesterol calculi (7.2 mg/gm) and mixed calculi (23 mg/gm). There was a significant difference between bile acid content of pigment calculi and cholesterol calculi (p = 0.0001), and between mixed calculi and cholesterol calculi (p = 0.0012). However, the difference was insignificant between pigment calculi and mixed calculi (p = 0.7359).

The mean phospholipids content was highest in cholesterol stones (6.9 mg/gm) and lowest in mixed stones (5.8 mg/gm). There was a significant difference between phospholipid content of cholesterol calculi and mixed calculi (p = 0.0095), and between cholesterol calculi and pigment calculi (p = 0.0201). However, the difference was insignificant between mixed calculi and pigment calculi (p = 0.3978).

The soluble protein content was highest in pigment calculi (98.62 mg/gm) and lowest in mixed calculi (10.03 mg/gm). The protein content was significantly higher in pigment calculi as compared to mixed calculi (p = 0.0001) and cholesterol calculi (p = 0.0091). However, the difference was insignificant between cholesterol calculi and mixed calculi (p = 0.0983).

3.4. Determination of Cations and Anions

The sodium, potassium, calcium, magnesium, copper, iron, inorganic phosphate, oxalate and chloride were found in all 94 gallstones Tables 5 and 6.

The concentrations of different ions also varied with the type of stone. The mean calcium content was highest in pigment calculi (22.8 mg/gm) and lowest in cholesterol calculi (9.6 mg/gm). It was significantly higher in pigment calculi as compared to cholesterol calculi (p = 0.0001) and in mixed calculi as compared to cholesterol calculi (p = 0.0018). However, there was an insignificant

Table 2. Incidence of different types of gallstones in relation to age in patients with symptomatic chalcular cholecystitis in the study group (n + 94).

Age group (years)	Cholesterol stones (CS)	Mixed stones (MS)	Pigment stones (PS)	Total (%)
<30	0	1	5	06 (6.4)
31 - 40	3	10	10	23 (24.5)
41 - 50	9	10	22	41 (43.6)
51 - 60	2	6	6	14 (14.9)
61 - 70	2	1	5	08 (8.5)
71 - 80	0	2	0	2 (2.1)
Total	16	30	48	94 (100)

Table 3. Concentrations of metabolites in the different types of biliary calculi in patients with symptomatic calcular cholecystitis in the study group (n = 94).

Stone type	Cholesterol (mg/gm)	Triglycerides (mg/gm)	Fatty acids (mg/gm)	Bilirubin (mg/gm)	Bile acids (mg/gm)	PL[a] (mg/gm)	Soluble Protein (mg/gm)
CS[b]	713 ± 109	69.3 ± 17	21.71 ± 8.73	0.3 ± 0.10	7.2 ± 0.9	6.9 ± 2.98	27.37 ± 9.93
MS[c]	637 ± 131	51 ± 11	13.82 ± 4.97	2.4 ± 0.70	23 ± 1.8	5.8 ± 0.8	10.03 ± 4.27
PS[d]	421 ± 79	32.8 ± 13	15.03 ± 5.13	4 ± 0.90	25 ± 3.9	6.1 ± 1.7	98.62 ± 19.93

[a]PL, phospholipids; [b]CS, cholesterol stones; [c]MS, mixed stones; [d]PS, pigment stones. Values are the mean and standard deviation.

Table 4. P-values for differences in chemical composition of biliary calculi shown in Table 3 of patients with symptomatic calcular cholecystitis in the study group (n = 94).

Groups	Cholesterol	Triglycerides	Fatty acids	Bilirubin	Bile acids	Phospholipids	Soluble Protein
CS vs. MS	0.1032	0.1303	0.0216	0.0021	0.0012	0.0095	0.0983
CS vs. PS	0.0042	0.0371	0.0093	0.0001	0.0001	0.0201	0.0091
MS vs. PS	0.0261	0.0183	0.1017	0.0093	0.7359	0.3978	0.0001

Table 5. Quantitative analysis of cations and anions in different types of biliary calculi expressed as mg/gm dry stone powder (Data are mean ± SE) of patients with symptomatic calcular cholecystitis in the study group (n = 94). Values are the mean and standard deviation.

Stone	Ca^{2+}	PO_4^-	Na^+	K^+	Mg^{2+}	Cl^-	Copper	Iron	Oxalate
CS	9.6 ± 2.7	16 ± 1.04	1.5 ± 0.4	0.61 ± 0.19	9.7 ± 1.7	17.8 ± 1.4	0.720 ± 0.8	0.64 ± 0.63	7.2 ± 1.3
MS	19.9 ± 4.2	11.4 ± 1.6	1.3 ± 0.2	0.38 ± 0.08	9.1 ± 0.9	22.3 ± 2.2	0.354 ± 0.73	1.09 ± 0.71	5.9 ± 1.4
PS	22.8 ± 2.9	10.2 ± 1.9	3.8 ± 0.7	0.73 ± 0.18	10.2 ± 1.3	36.1 ± 3.2	0.348 ± 0.62	0.78 ± 0.64	6.8 ± 0.9

Table 6. P-values for differences in composition of various ions of gallstones shown in (Table 5) of patients with symptomatic calcular cholecystitis in the study group (n = 94).

Comparison	Ca^{2+}	PO_4^-	Na^+	K^+	Mg^{2+}	Cl^-	Copper	Iron	Oxalate
CS vs. MS	0.0018	0.0081	0.9101	0.0520	0.7312	0.0847	0.3021	0.0164	0.0902
CS vs. PS	0.0001	0.0001	0.0027	0.0975	0.8014	0.0012	0.2903	0.5718	0.2410
MS vs. PS	0.0805	0.1073	0.0010	0.0391	0.8771	0.0424	0.9790	0.0403	0.3015

difference between calcium content of pigment calculi as compared to mixed calculi (p = 0.0805).

The inorganic phosphate content was highest in cholesterol calculi (16 mg/gm) and lowest in pigment calculi (10.2 mg/gm).

There was a significant difference between cholesterol calculi and pigment calculi (p = 0.0001) and cholesterol calculi and mixed calculi (p = 0.0081) but no significant difference between mixed and pigment calculi (p = 0.1073).

The mean sodium content was highest in pigment calculi (3.8 mg/gm) and lowest in mixed calculi (1.3 mg/gm). The difference in sodium content was highly significant in pigment calculi as compared to mixed calculi (p = 0.0010) and cholesterol calculi (p = 0.0027) but insignificant between cholesterol calculi and mixed calculi (p = 0.9101).

The mean potassium content was highest in pigment calculi (0.73 mg/gm) and lowest in mixed calculi (0.38 mg/gm). The potassium content was significantly higher in pigment calculi as compared to mixed (p = 0.0391). The difference of potassium content of cholesterol calculi and mixed calculi (p = 0.0520) and between pigment and cholesterol calculi (p = 0 .0975) was however insignificant.

The magnesium content was highest in pigment calculi (10.2 mg/gm) and lowest in mixed calculi (9.1 mg/gm). However, there was no significant difference between magnesium content of three calculi (p > 0.05).

The mean chloride content was highest in pigment calculi (36.1 mg/gm) and lowest in cholesterol calculi (17.8 mg/gm). The chloride content was significantly higher in pigment calculi as compared to cholesterol (p = 0.0012) and mixed calculi (p = 0.0424) but insignificant between cholesterol calculi and mixed calculi (p = 0.0847).

The copper content was highest in cholesterol calculi (0.72 mg/gm) and lowest in pigment calculi (0.348 mg/gm). However, there was no significant difference between copper content of three calculi (p > 0.05).

The iron content was highest in mixed calculi (1.09 mg/gm) and lowest in cholesterol calculi (0.64 mg/gm). The content of iron was significantly higher in mixed calculi as compared to cholesterol calculi (p = 0.0164) and pigment calculi (p = 0.0403). However, there was an insignificant difference (p = 0.5718) between iron content of cholesterol calculi and pigment calculi.

The oxalate content was higher in cholesterol stone (7.2 mg/gm) and lowest in mixed stone (5.9 mg/gm). However, there was no significant difference between oxalate content of the three calculi (p > 0.05).

The chemical composition of gallstones in this study were as: Cholesterol 1771 mg/gm (73.1%), Triglycerides 153.1 mg/gm (6.3%), Fatty acids 50.56 mg/gm (2.1%), Bilirubin 58.5 mg/gm (2.4%), Bile acids 6.7 mg/gm (0.3%), Phospholipids 18.8 mg/gm (0.8%), Soluble Protein 136.02 mg/gm (5.6%), 52.3 mg/gm (2.16%), 37.6 mg/gm (1.55%), Na^+ 6.6 mg/gm (0.27%), K^+ 1.72 mg/gm (0.07%), 29 mg/gm (1.2%), Cl^- 76.2 mg/gm (3.15%), Copper 1.422 mg/gm (0.06%), Iron 2.51 mg/gm (0.1%), Oxalates 19.9 mg/gm (0.8%), Table 7.

4. Discussion

Gallstone disease known as cholelithiasis is the most common digestive surgical disorder and account for an important part of health care expenditure. The most commonly involved age group for gallstone disease (43.6%) is found to be 41 - 50 years with a female predominance (F:M = 14.7:1), this is in concordance with Jaraari *et al*. [9] in Libya and P. Chandran *et al*. [10] in India, and differ from others [6,11,12].

In this study Pigment stone was found to be the commonest comprising 51.06%, followed by mixed stone 31.91% and cholesterol stone 17.02%. The findings are

Table 7. Percentage of chemical composition of gallstones of patients with symptomatic calcular cholecystitis in the study group (n = 94).

	mg/gm	Percent
Cholesterol	1771	73.1
Triglycerides	153.1	6.3
Fatty acids	50.56	2.1
Bilirubin	58.5	2.4
Bile acids	6.7	0.3
Phospholipids	18.8	0.8
Soluble Protein	136.02	5.6
Ca^{2+}	52.3	2.16
PO_4^-	37.6	1.55
Na^+	6.6	0.27
K^+	1.72	0.07
Mg^{2+}	29	1.2
Cl^-	76.2	3.15
Copper	1.422	0.06
Iron	2.51	0.1
Oxalates	19.9	0.8
Total	2421.932	99.96

similar with the findings in the study done in Libya and San Francisco [9,13]. Where as in the study in Nepal (India) [14] mixed stone was found to be the commonest comprising 78.75%, followed by cholesterol 12.5%, pigment 8.75% and black pigment stone 1.25%. In other study in Haryana, India mixed calculi (38%) more than pigment calculi (36%) more than cholesterol calculi (26%) [10].

Study showed that gallstones were multiple in 64.9% patients; this is in agreement with P. Chandran *et al.* and Banchob Sripa *et al.* [10,12]. Whereas, Jaraari *et al.* [9] in Libya studied 41 patients all had multiple gallstones. Study showed that there is difference in the number of stone detected by ultrasound and that found intraoperatively ($p < 0.0001$), this might be due to the experience ultrasonographists.

CSs were bigger in size as compared to mixed and pigment stones. The size of cholesterol calculi was in the range 0.3 - 2.7 cm and 0.3 - 3.1 cm with an average of 0.9 cm and 0.9 cm, this is in agreement with others [9,10].

Determination of metabolites showed that the mean total cholesterol was higher in cholesterol calculi (713 mg/gm), compared to mixed stones (637 mg/gm) and pigment stone (421 mg/gm).

There was an insignificant difference between total cholesterol content of cholesterol and mixed stones ($p > 0.05$), others found similar result [9,10]. Gallstones are believed to form when the concentration of cholesterol exceeded that which can be held in mixed micellar 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 mainly of cholesterol [12]. The co-existence of nucleating factors, gallbladder hypomobility, and mucus hypersecretion also contribute to cholesterol precipitation leading to the development of gallstones [9].

The triglyceride content was higher in cholesterol calculi (69.3 mg/gm) and mixed calculi (51 mg/gm) as compared to pigment calculi (32.8 mg/gm). The triglyceride content was significantly higher ($p < 0.05$) in cholesterol and mixed calculi as compared to pigment calculi. However, the difference was insignificant between cholesterol and mixed calculi ($p > 0.05$). The result is similar to study by Chandran *et al.* [10] but is comparable to study by Jaraari *et al.* [9] in Libya where triglyceride content was higher in mixed stones than in the other two types of stones. 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 [15].

The mean fatty acid content was highest in cholesterol calculi (21.71 mg/gm) and lowest in mixed calculi (13.82 mg/gm). The difference of fatty acid content was significant between cholesterol and mixed calculi ($p < 0.05$) and between cholesterol and pigment calculi ($p < 0.05$) but insignificant between mixed and pigment calculi ($p > 0.05$).

The esterified fatty acid content was highest in cholesterol and lowest in mixed calculi. Similar result also found by Chandran *et al.* [10]. The high content of esterified fatty acids in cholesterol stones might be due to interaction between excessive cholesterol and fatty acids.

The mean total bilirubin concentration was highest in pigment stones (4 mg/gm) and lowest in cholesterol stones (0.3 mg/gm).

The total bilirubin concentration was highest in pigment and lowest in mixed calculi. It was significantly higher in pigment calculi compared to mixed and cholesterol calculi ($p < 0.009$) and in cholesterol calculi as compared to mixed calculi ($p < 0.02$).

These observations are in agreement with other reports by Chandran *et al.* [10], Kumar D *et al.* [16] and Pundir CS *et al.* [17]. The colour of the pigment stones could be attributed to colour of bilirubin, which form salt with calcium to form calcium bilirubinate. It is known that β-glucuronidase of bacterial origin hydrolyses conjugated bilirubin into free bilirubin, which form salt with calcium as calcium bilirubinate [18].

The mean bile acid content in pigment stones (25 mg/gm) was comparatively higher than that in cholesterol calculi (7.2 mg/gm) and mixed calculi (23 mg/gm). The bile acid content in pigment stone was comparatively higher than that in cholesterol and mixed calculi. The difference was significant between PS and CS and between MS and CS ($p < 0.05$), but the difference was insignificant between PS and MS ($p > 0.05$). The result was similar to that achieved by both Jaraari *et al*. [9] and Chandran *et al*. [10]. But it was five times higher than that reported in Japan [19].

Bile acids were significantly lower in CS than MS and PS, while it 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 [20].

Similarly, the phospholipid content was marginally higher in CS than PS and MS. As the mean phospholipids content was highest in CS (6.9 mg/gm) and lowest in MS (5.8 mg/gm).

Cholesterol has to be in relative proportion with the bile salts and phosphatidyl choline to remain soluble in the bile and thereby stone formation [21].

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 [22]. PS contained less cholesterol than the other two types of stones. Cholesterol in PS is mostly because of co-precipitation with bilirubin and other compounds but not due to its supersaturation in the bile [23]. 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 stone.

The protein content was significantly higher in PS (98.62) as compared to MS (10.03) and CS (27.37). Similarly Chandran *et al*. [10] was achieved nearer results.

Binette *et al*. [24] suggested that the proteins to be the candidates either to facilitate or hinder the formation of stones. Maki [25] proposed the ability of bacterial β-glucuronidase enzymes (protein) to hydrolyse the bilirubin glucuronide complex thus releasing a poorly soluble bilirubin to explain the formation of PS as well as CS.

Determination of cations and anions showed that the mean calcium content was highest in pigment calculi (22.8 mg/gm) and lowest in cholesterol calculi (9.6 mg/gm). The inorganic phosphate content was highest in cholesterol calculi (16 mg/gm) and lowest in pigment calculi (10.2 mg/gm).

So in this study calcium content was highest in PS, while phosphorus was lowest. This is in agrees with studies by Jaraari *et al*. [9] and Pundir CS *et al*. [17]. It is known that bilirubin combines with calcium to form a precipitate of calcium bilirubinate [26]. Since PS has excess bilirubin, calcium forms calcium bilirubinate [27].

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. Jaraari *et al*. [9] in their study were concluded similar results. 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.

The magnesium content was highest in PC (10.2 mg/gm) and lowest in MS (9.1 mg/gm). This is in conformity with observations made by others [9,10].

The oxalate content was higher in CS (7.2 mg/gm) and lowest in MS (5.9 mg/gm). This disagrees with others [9,10], where it was higher in PS. Whereas, Raha *et al*. [28] were found calcium oxalate to be one of the major component of MS. Both magnesium and oxalate form insoluble salt which might be crystallized to strengthen the texture of PS [16].

The mean chloride content was highest in PS (36.1 mg/gm) and lowest in CS (17.8 mg/gm). Similar result also obtained by others in their series [9,10]. Same authors proposed that the chloride ions are always present in biological fluids in human beings including bile, which might get deposited in the form of sodium chloride salt along with major salts of the gallstones.

The copper content was highest in CS (0.72 mg/gm) and lowest in PS (0.348 mg/gm). This is also observed in another one study [10].

The role of copper in pathogenesis of calcium bilirubinate in gallstones was suggested in literature [29].

The iron content in this study was highest in MS (1.09 mg/gm) and lowest in CS (0.64 mg/gm). These observations are in agreement with others [10,17]. Been *et al*. [29] reported the presence of small amount of iron in a thick black shell around the central dark inclusion of gallstones.

The high iron content in MS might be promoting aggregation of calcium bilirubinate particles which are the major constituents especially due to the polyelectrolytic nature of iron [7,30].

Verma *et al*. [31] suggested that the central aggregates of calcium salts may lead to ulceration of gallbladder mucosa and microscopic haemorrhage. The iron released by this process might be another source of its deposition in gallstones.

Comparison of the chemical composition of gallstones in Sudanese individuals with those in other countries is summarized in **Table 8**. Though cholesterol is a major component of gallstones, the composition of gallstones varies from country to country and region to region.

A major limitation of the present study is the potential

Table 8. Percentage of chemical composition of gallstones from various countries.

Country	No. of patients	Cholesterol (%)	Triglycerides (%)	Bilirubin (%)	Calcium (%)	PO_4^-, Mg^{2+} Oxalates, Na^+, K^+, and Cl^- (%)
Kuwait [31]	10	60			37	3
Libya [9]	41	53	4.9	2.16	1.7	38.4
India [32]	200	56.6	5.7	0.3	2.2	35.2
USA [31]	42	88			9	3
England [31]	11	66			17	17
South Africa [31]	11	66			29	5
Sweden [31]	27	94			4	2
Germany [2]	1025	93.3		5.5	4.8	
Present study	94	73.1	5.3	2.4	2.16	7.04

self-selection bias due to the hospital-based study design, which is not being exactly representative of the whole general population. Second, it is well established that a large proportion of GSD patients remain asymptomatic and they are therefore often ignored for many years. Thus the present study may be representative of the clinical and not of the true prevalence. The solution to such study is to conduct a number of prospective longitudinal analogous studies, the results of which would be expected to complement the cross-sectional findings of this study.

5. Conclusions

In conclusion, CS showed significantly higher cholesterol content than pigment stones though not significantly higher than MS. In MS, the cholesterol, bile acids, and bilirubin were intermediate between CS and PS, whereas triglycerides were significantly higher than PS. Bilirubin and bile acids were significantly higher than CS. In PS, bilirubin was significantly higher than both groups. Bile acid content was significantly higher than CS but not significantly more than MS.

Collaborations between surgeons, nutritionists, biochemists, and physicians should be stimulated in future studies to define the different types of gall stones in different areas in Sudan and the relevancy of such types with diets' traditions.

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Abbreviations

B	Beta
˚C	Centigrade
Ca^{2+}	Calcium
Cl^-	Chloride
Cm	Centimeter
CS	Cholesterol stones
F	Female
GS	Gallstone
GSD	Gallstone disease
I N HCl	One normal hydrochloric acid
K^+	Potassium
M	Male
Mg^{2+}	Magnesium
mg/gm	Milligram per gram
MS	Mixed stones
N	Number
Na^+	Sodium
P	Probability value
PL	Phospholipids
PO_4^-	Phosphate
PS	Pigment stones
SD	Standard deviation
SE	Standard error
SPSS	Statistical Package for the Social Sciences
US	United states