

CORRELATION BETWEEN THE CHEMICAL COMPONENTS OF GALLSTONES AND SERA OF STONE FORMERS

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ABSTRACT

Background: Major elements involved in the formation of human gallstones are cholesterol, bile pigment and calcium. These substances are normally found in the blood. This study was aimed to find out the frequency of different type of gallstones and correlation between the components of gallstones and sera of stone formers.

Material & Methods: Fifty gallstones and blood samples were collected and analyzed from patients admitted for cholecystectomy to Rizgary Teaching Hospital, Erbil, Iraq, from July 2008 to December 2008.

Results: In 50 gallstone, 27(54%) were cholesterol stones, 20(40%) mixed and 3(6%) pigment stones. Female to male ratio was 8:1 and the predominant age of stone formers was 31-60 years. There was significantly negative correlation between serum cholesterol and that of cholesterol and pigment gall stones ($r=-0.730$ and -0.999). There was significant positive correlation between serum bilirubin and pigment gallstones ($r=0.812$). Inorganic phosphate in serum was moderately correlated to that in cholesterol and mixed gallstones ($r=0.377$ and 0.178) with significant negative correlation in case of pigment stones ($r=-0.845$). Moderate positive and negative correlation was found for calcium in case of three stones ($r=0.202$, $r=-0.213$ and -0.210).

Conclusion: In Erbil the occurrence of cholesterol gallstones is high as compared to mixed and pigment gallstones. Our results indicated low, moderate and high positive or negative correlation between the chemical constituents of gallstones and sera of stone formers suggesting the different aetiology of the cholesterol, mixed and pigment stones.

Key words: Gallstone, Cholesterol, Bilirubin, Calcium.

INTRODUCTION

Increased pathophysiological interest in the formation of gallstones made the knowledge of their exact chemical composition very important especially of cholesterol gallstones, the only stone which can be treated with cholelitholytic agents.^{1,2} Analysis of chemical composition of gallstones can provide a significant reference to the treatment and prevention of their reoccurrence.³ Major elements involved in the formation of human gallstones are cholesterol, bile pigment and calcium.⁴ With regard to chemical composition the majority of human gallstones are cholesterol and pigment stones.^{5,6} Cholesterol gallstones consist of more than 70% cholesterol and also a small amount of pigment residues and trace elements.⁷ Other substances found in gallstones include calcium salts of phosphate, mucin, glycoprotein, phospholipids and some metals. All these substances are normally found in the sera of stone formers.⁸

It has been reported that some elements play a significant role in the formation of gallstones. It was observed that 14 minor or trace elements namely Sulfur, Chloride, Potassium, Vanadium, Chromium, Manganese, Iron, Nickel, Copper, Zinc, Bromide and Lead were present in the gallstones collected from Indian stone formers.⁹ However there is no report available in the literature to indicate any direct correlation between the various substances found in the gallstones and sera of stone patients in Erbil.

The present study aimed to find out the frequency of different type of gallstones and correlation between the components of gall stones and sera of stone formers, in order to help identify patients for future innovative non-surgical intervention.

MATERIAL AND METHODS

Gallstones and blood samples (5 ml) from 50 patients of cholelithiasis were collected after

cholecystectomy at Rizgary Teaching Hospital, Erbil, Iraq, from July 2008 to December 2008.

The stones were powdered in a pestle and mortar. To determine the total cholesterol and bilirubin, 30 mg of stone powder was dissolved in 3 ml chloroform in a test tube.^{8,10} The tube was kept in boiling water bath for 2 minutes. The stone solution thus obtained was used for determination of cholesterol and bilirubin. The stone solution was stored at 2-8 °C when not in use. To determine phosphate, calcium and iron, 30 mg stone powder was dissolved in 3 ml of 1N HCl in a graduated 10 ml tube and its final volume was made 10 ml with distilled water. The tube was kept in boiling water bath for one hour. The solution thus obtained was stored at 2-8 °C.

The blood samples were centrifuged at 2,000 rpm for 20 minutes and the supernatant serum was collected and stored at 4 °C until used. All kits used in this study were manufactured by Biolabo 02160, Maizy, France. The total cholesterol and bilirubin^{11,12} were determined by enzyme assay using kit version AT-80106 and AT-80403 respectively. Determination of inorganic phosphate was done by the method described by Gamst et al.¹³ spectrophotometrically (without deproteinisation) and absorbance was measured at 340 nm. Iron was also determined spectrophotometrically, with absorbance at 600 nm using kit version AT-92108.

Two samples from each gallstone and serum of the stone formers were analyzed for each constituent.

RESULTS

Among 50 patients with cholelithiasis, the female to male ratio was 8:1. The incidence of different type of gall stones in relation to the age of stone formers is shown in Table-1.

Figure-1 represents the morphological features of some studied stones, such as size, shape and color. Out of 15 patients, some were single stone formers while others were multiple stone formers. The collected gallstone specimens were of variable sizes, their size varied from as small as less than one cm³ to 13.5 cm.³ The weights of collected gallstones were 0.9-20.9 g. The color of the studied gallstones were; white, yellowish white, brown, green and black.



Fig. 1: Morphological features of some studied stones.

The content of cholesterol, bilirubin, inorganic phosphate, calcium and iron in gallstones and sera of stone formers are summarized in Table-2 and their correlation coefficients in Table-3.

There was a significantly negative correlation between the total cholesterol of serum and that of cholesterol and pigment gallstones ($r = -0.730$ and -0.999 respectively).

Non-significant correlation was found between the total cholesterol in serum and mixed gall stone ($r = -0.059$).

Table-1: Incidence of different type of gallstones in relation to age.

Age group (years)	Type of Gallstone			Total
	Cholesterol	Mixed	Pigment	
11-20	1	1	—	2
21-30	1	2	—	3
31-40	6	3	3	12
41-50	8	6	—	14
51-60	9	6	—	15
>60	2	2	—	4
Total	27	20	3	50

Table-2: Content of various chemical components of different types of gallstones and sera of gallstone patients

Type of gallstone	Total Cholesterol		Total Bilirubin		Inorganic Phosphate		Calcium		Iron	
	Serum mg/dl	Stone mg/g	Serum mg/dl	Stone mg/g	Serum mg/dl	Stone mg/g	Serum mg/dl	Stone mg/g	Serum mg/dl	Stone mg/g
Cholesterol	200.50	525.10	0.65	1.69	6.00	12.29	9.16	5.72	0.87	1.13
Calculi	±33.86	±24.63	±0.33	±0.64	±1.47	±0.76	±0.58	±2.09	±0.24	±0.34
Pigment	285.36	378.00	0.87	1.82	6.21	7.47	8.63	11.00	0.73	1.76
Calculi	±36.01	±63.89	±0.10	±0.83	±0.83	±0.01	±0.44	±0.23	±0.09	±0.09
Mixed	205.76	432.85	1.39	1.7	4.87	7.71	8.16	4.19	0.72	1.26
Calculi	±15.66	±5.01	±0.22	±0.31	±0.39	±0.12	±0.38	±0.83	±0.09	±0.01

Stones data is on dry weight basis. (Mean ± SEM)

Table-3: Correlation co-efficient of various chemical components of different types of gallstones and sera of stone formers

Type of gall stone	Total Cholesterol	Total Bilirubin	Inorganic Phosphate	Calcium	Iron
Cholesterol Calculi	-0.730	-0.057	0.377	0.202	0.609
Pigment Calculi	-0.999	0.812	-0.845	-0.213	-0.011
Mixed Calculi	-0.059	0.076	0.178	-0.210	0.043

In pigment gall stone patients there was a significant positive correlation between the total bilirubin content of the serum and pigment gallstone ($r= 0.812$), while no significant correlation was seen in total bilirubin content of the serum and cholesterol & mixed gall stones ($r= -0.057$ and -0.076 respectively).

Inorganic phosphate in serum was moderately correlated to that in cholesterol and mixed gall stones ($r=0.377$ and 0.178 respectively). While a significant negative correlation was observed between inorganic phosphate in serum and pigment gall stones ($r= -0.845$).

Moderate (positive and negative) correlation was found between the calcium content of the serum and the cholesterol, pigment and mixed gall stones ($r=0.202$, $r= -0.213$ and -0.210 respectively).

Regarding the total iron, a significant positive correlation was found between the iron content of serum and cholesterol gall stones ($r=0.609$).

No significant correlation was found between the iron content of the serum and the pigment and mixed gall stones ($r=0.011$ and 0.043 respectively).

DISCUSSION

Gallstones remain a serious health concern affecting millions of people throughout the world. It exhibits prevalence rates of about 25% in industrialized societies but are uncommon in underdeveloped or developing societies. The present investigation shows that in Erbil, the cholesterol stones are predominant as compared to mixed and pigment stones. Our results are in good agreement with the studies carried out in Riyadh, Korea, Germany, United State and Singapore.¹² A high proportion of cholesterol gallstone in Erbil may be as a result of longer life expectancy and altered nutritional habit. High incidence of cholelithiasis in female is in accordance with literature, Rains¹⁴ reported that females had a greater risk of

gallstone diseases, especially if they had used oral contraceptives. Moreover, the concentration of bile salts in bile reduced by estrogen and thereby making it lithogenic. Under the influence of female sex hormone, the muscle may relax, biliary passage dilates and duodenal content of pancreatic secretion regurgitate into gallbladder and promote conditions which favor the formation of gallstones.

Our data revealed different correlations between the chemical composition of sera and stones in different type of gallstones, suggesting the different etiology of cholesterol, mixed and pigment stones. Cholesterol plays significant role in the cholesterol stone formation which is evident from comparatively high content of cholesterol in cholesterol stones than that in pigment stones and mixed ones.

In cholesterol gallstone patients moderate and significant positive correlation between inorganic phosphate, calcium and iron of the sera and gallstones indicate that increase in inorganic phosphate, calcium and iron in gallstone is followed by their increase in sera of cholesterol stone patients. This is evident from the presence of inorganic phosphate, calcium and iron salts as a major components of cholesterol stones.

In pigment stone patients, a significant positive correlation was found between total bilirubin of serum and the stone of those patients, which reveals that the increase in bilirubin in serum leads to its precipitation in the stone to form a pigment stone; the same observation was concluded by other researchers.^{15,16}

In mixed stone patients, moderate positive correlation between inorganic phosphate of the serum and the stone indicates that the increase in level of inorganic phosphate in the serum leads to its deposition into stone. High iron content of the mixed stone (Table-1) as compared to cholesterol or pigment stones, indicates the role of iron in the formation of mixed stone.

Cholesterol is not water soluble, so in order to remain suspended in fluid it must be transported within clusters of bile salts called micelles.¹⁷ If there is an imbalance between these bile salts and cholesterol, then the bile fluid turns to sludge. This thickened fluid consists of a mucus gel containing cholesterol and calcium bilirubinate. If the imbalance worsens, cholesterol crystals form due to the super-saturation which can eventually form gallstones. Super-saturation and cholelithiasis can occur as a result of various abnormalities, although the cause is not entirely clear. Black stones are more likely to develop in people with hemolytic anemia or cirrhosis. In case of brown stones, infection plays a role in the development of these stones.

People who may be at risk for developing gallstones may want to try modifying their diet to decrease their risk.^{18,19} For example, the amount of dietary fiber consumed is a therapy for gallstones that is often overlooked. Gallstones may be prevented by increasing consumption of both soluble and insoluble fiber which reduces the absorption of deoxycholic acid by producing a favorable shift in the triad of factors that control cholesterol's solubility in bile. The benefit of fiber is seen in the low incidence of gallstones in vegetarians. Coffee drinking has been associated with a decreased risk of symptomatic gallstones. Regular and vigorous exercise may also decrease the risk of gallstones.²⁰

CONCLUSION

In Erbil the occurrence of cholesterol gallstones is high as compared to mixed and pigment gallstones. Our results indicated low, moderate and high positive or negative correlation between the chemical constituents of gallstones and the sera of stone formers suggesting the different aetiology of the cholesterol, mixed and pigment stones.

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