Study of Some Metal Elements in Four Chemically Different Types of Human Gallstones

[1]N. A. CHANNA, [2]F.D. KHAND, [3]M. Y. KHUHAWAR, [4]M. I. BHANGER
[1]*Institute of Biochemistry, University of Sindh, Jamshoro*
[2]*Department of Biochemistry, Isra University, Hyderabad*
[3]*M. A. Kazi Institute of Chemistry, University of Sindh, Jamshoro*
[4]*Center of Excellence in Analytical Chemistry*
University of Sindh, Jamshoro, Pakistan

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Summary: To assess the role of metal elements in the pathogenesis of human gallstones, the concentrations of some metal elements (Na, K, Ca, Mg, Mn, Cu, Fe, Pb, Ni, and Zn) in four chemically different types of human gallstones (pure cholesterol, cholesterol +calcium carbonate, cholesterol + bilirubin and calcium bilirubinate) were measured by atomic absorption spectrometry. It was observed that the concentrations of the metal elements varied greatly with the chemical nature of stone. Calcium, magnesium and manganese were found in high range of concentrations in gallstones composed of cholesterol +calcium carbonate, whereas, high concentrations for sodium, potassium, copper, iron, lead, nickel and zinc were seen in calcium bilirubinate stones. Sexwise comparison of the data for cholesterol gallstones, showed that the levels for calcium, sodium, magnesium, manganese, iron, lead and nickel were higher in gallstones recovered from females as compared to that of males, whereas reverse was true for the concentrations of potassium and zinc. Comparison of the data for cholesterol gallstones from females of above and below 45 years age revealed no significant differences between the subjects of the two age groups. The findings of the present study may suggest that the metal elements calcium, sodium, potassium, copper, and iron do contribute towards the formation mechanism of human gallstones.

Introduction

Gallstones are composed of a limited number of chemical constituents, including cholesterol, bilirubin, calcium, carbonate and phosphate. These constituents are usually arranged into three main types of gallstones, namely pure cholesterol stones, pure pigment (bilirubin) stones and mixed or multifaced stones.

In Pakistan the prevalence of pure cholesterol stones is far more common than the pigment and mixed component stones [1 – 3].

The identification of the components of gallstones is essential as it provides information that could be useful for practitioners to decide whether to treat gallstone patients therapeutically or surgically [4, 5].

Metal imbalances either marginal or severe are considered as risk factors for several diseases of public health importance [6, 7]. The role of calcium and iron in cholesterol gallstone formation has been under discussion for some time [8, 9]. Yamamoto *et al* [10] estimated the levels of eight metal elements in three chemically different types of gallstones and reported that, metal content in these gallstones varied according to the type of gallstone.

In the present study, one main objective of analysis of metal contents in four chemically different types of gallstones was to investigate the role of metals in the formation of each type of stone and further to assess whether diet or environment do play any role in the genesis of these stones.

Results And Discussion

Of the 50 gallstones analyzed (Table 1), 34(68%) were pure cholesterol stones, 5(10%) were composed of cholesterol + calcium carbonate, 8(16%) of cholesterol + bilirubin and 3(6%) of calcium bilirubinate. The means, standard deviations and the percentage of frequency of occurrence of each of ten metal elements estimated in the present study are summarized in Table 2. Interestingly, magnesium and lead were not detectable in that metals especially when present in higher amounts play a significant role in stone formation either in association with structure of conglomerate crystals or in combination with organic molecules [6].

Table 1. Classification of gallstones as analyzed by infrared spectroscopy (n = 50)

S. No.	Gallstone Type	No. of gallstones	Percentage of total gallstones
1	Pure Cholesterol	34	68
2	Cholesterol and calcium carbonate	5	10
3	Cholesterol and bilirubin	8	16
4	Calcium bilirubinate	3	6

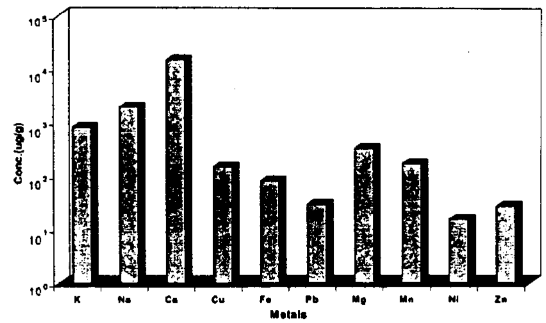

Fig. 1: Mean Concentrations of Metal Elements in Fifty Human allstones.

Table 2. Comparison of metal contents in different types of human gallstones*

Types of stones	K	Na	Ca	Mg	Cu	Mn	Fe	Pb	Zn	Ni
No. of samples analyzed	34	34	30	32	32	33	34	23	34	34
Pure Cholesterol Mean±S.D	0.88±0.48	1.83±1.00	6.46±6.94	139±104.05	34.82±13.33	82.14±80.57	72.12±35.03	22.00±7.07	18.64±14.16	16.37±6.46
Relative frequency(%)	100	100	100	41	100	85	100	9	76	94
No. of samples analyzed	4	4	4	4	5	4	5	3	5	5
Cholesterol+calcium carbonate Mean±S.D	0.89±0.29	3.33±3.08	84.14±62.98	7235.33±12135.59	484.2±624.8	1145.5±1872.7	262.6±355.9	ND	46.20±38.29	12.20± 9.65
Relative frequency(%)	100	100	100	75	100	100	100		100	100
No. of samples analyzed	7	7	7	7	7	7	8	5	8	8
Cholesterol+bilirubin Mean±S.D	0.72±0.15	1.20±9.97	6.32±5.9	ND	70.13±90.66	76.00±76.50	58.88±6.43	20.0±48.99	18.29±8.65	15.38±6.69
Relative frequency(%)	100	100	100		100	100	100	33	88	100
No. of samples analyzed	3	3	3	3	3	3	2	3	3	3
Calcium bilirubinate Mean±S.D	2.03±0.60	8.06±3.93	31.13±1.09	950.30±37.00	3308.0±2616.7	653.0±496.8	1116.0±282.8	66.00±72.7	181.0±97.50	18.00±15.13
Relative frequency(%)	100	100	100	100	100	100	100	33	100	100

*Values are expressed in µg/g except for K, Na and Ca which are expressed in mg/g.
ND = Not detectable

Figure 1 shows the mean concentrations of ten metal elements measured in fifty human gallstones. The order of metal content in these stones is calcium > sodium > potassium > magnesium > copper > manganese > iron > zinc > nickle > lead. The presence of calcium in highest amounts in all the gallstones confirms that calcium is a contributor factor in the formation of human gallstones as suggested by earlier workers [11 – 13].

Table 3 represents the differences resulting from the statistical treatment of the data presented in Table 2. The F and p values given in the Table indicate the significant difference among the four types of human gallstones. As is evident, among the stone types, the concentrations of sodium, potassium, calcium, manganese, copper and zinc differed significantly ($p < 0.01$). Calcium, magnesium and manganese were found in highest concentrations in gallstones composed of cholesterol + calcium carbonate whereas highest concentrations for sodium, potassium, copper, iron, lead, nickle and zinc were seen in calcium bilirubinate stones. The lowest metal contents were noted for cholesterol + bilirubin stones.

Sexwise comparison of the data for cholesterol gallstones (Fig. 2) disclosed that the levels for calcium, sodium, magnesium, manganese, iron, lead and nickel were higher in gallstones recovered from females than males whereas reverse was true for potassium and zinc levels. We offer no explanation for this variation among the two sexes.

Table 3: Statistical comparison of metal content in different types of gallstones

METAL	GALLSTONE TYPES	F & P Values
K	Ca-bilirubinate > Cholesterol + Ca-carbonate > Cholesterol > Cholesterol + bilirubin	F=5.00 P< 0.01
Na	Ca-bilirubinate > Cholesterol + Ca-carbonate > Cholesterol > Cholesterol + bilirubin	F=30.69 P<0.01
Ca	Cholesterol+Ca-carbonate > Ca-bilirubinate > Cholesterol > Cholesterol + bilirubin	F=25.10 P< 0.01
Cu	Ca-bilirubinate > Cholesterol + Ca-carbonate > Cholesterol + bilirubin > Cholesterol	F=30.14 P<0.01
Fe	Ca-bilirubinate > Cholesterol + Ca-carbonate > Cholesterol > Cholesterol + bilirubin	F=0.89 P< N.S.
Pb	Ca-bilirubinate > Cholesterol > Cholesterol + bilirubin > Cholesterol + Ca-carbonate	F=0.87 P< N.S.
Mg	Cholesterol + Ca-carbonate > Ca-bilirubinate > Cholesterol > Cholesterol + bilirubin	F=2.09 P< N.S.
Mn	Cholesterol + Ca-carbonate > Ca-bilirubinate > Cholesterol > Cholesterol + bilirubin	F=5.46 P< 0.01
Ni	Ca-bilirubinate > Cholesterol > Cholesterol + bilirubin > Cholesterol + Ca-carbonate	F=0.17 P< N.S.
Zn	Ca-bilirubinate > Cholesterol + Ca-carbonate > Cholesterol > Cholesterol + bilirubin	F=28.83 P<0.01

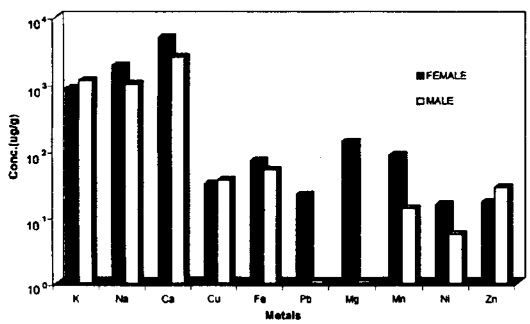

Fig. 2: Sexwise Comparison of metal Contents in Cholesterol Gallstones.

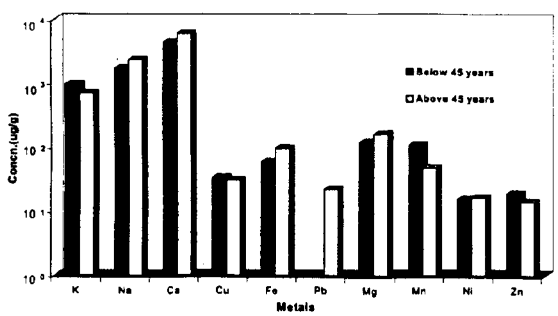

Fig. 3: Agewise Comparison of Metal Contents in Cholesterol Gallstones from Females.

This finding however merits more detailed and thorough investigations to elucidate the factors responsible for the variations.

Figure 3 shows the comparison of metal contents in cholesterol gallstones from females of below and above 45 years age group. No significant difference in the metal contents was seen between the two female age groups with exception to lead which was not detectable in gallstones recovered from females of under 45 years age. Similar were the observations of Yamamoto et al., from Japan [10]. When compared the data of metal contents of our series of human gallstones with that of Yamamoto et al from Japan [10], we noted lower concentrations of calcium and copper and higher of other metals in our series. These variations in metal concentration between the two series could be due to variations in the food composition and dietary habits of the two populations.

From this study it may be concluded that the metal elements calcium, sodium, potassium, copper and iron do contribute towards the formation mechanism of human gallstones.

Experimental

Ammonium ferrous sulphate, copper (11) sulphate, potassium chloride, sodium chloride, magnesium chloride, calcium chloride, manganese sulphate, nickel sulphate, cobalt nitrate, lead sulphate and nitric acid were purchased from E. Merck (Germany) and Hydrogen peroxide from Fluka (Switzerland). All chemicals used were of analytical grade. For the preparation of reagents and final washings of the glassware used, doubly distilled deionized water was used throughout the study.

Fifty gallstone samples recovered by cholecystectomy from as many patients (treated in

Liaquat Medical College Hospital, Jamshoro) were washed carefully with doubly distilled deionized water (to remove bile and debris) and dried over silica gel. Each gallstone sample was powdered with an agate mortar and pestle and the powder thus obtained was stored in a sample tube, kept over silica gel in dark until analyzed for chemical composition by infrared spectroscopy [1]

The digestion of the samples was carried out as follows:

Replicate 250 mg dried stone powder weighed into separate flasks was treated with 5ml nitric acid. Same amount of nitric acid was also added to an empty conical flask serving as a blank. Each flask was covered with a watch glass and their contents refluxed gently on an electric hot plate for one hour. For achieving complete digestion 5ml nitric acid and 2ml 30% hydrogen peroxide were added and the contents were again refluxed gently for one hour (some samples however, required twice or thrice addition of nitric acid and hydrogen peroxide for complete digestion). After digestion, the watch glasses were removed from the flasks and the heating continued until the volume reduced to 2 to 3ml. The contents of flasks were then cooled, diluted, and filtered through Whatman No. 42 filter paper into 25ml volumetric flasks and brought to volume with doubly distilled deionized water. The concentrations of calcium, copper, iron, lead, magnesium, manganese, nickle, potassium, sodium and zinc in these samples were measured by atomic absorption spectrometry as per reported method [14].

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