

Comparison of serum calcium, copper and iron levels in serum samples from gallstone patients and control subjects

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ABSTRACT

Objective: Present study was designed to investigate the levels of calcium, copper and iron in the serum samples of gallstone patients and control subjects with no personal or family history of gallstones.

Methodology: Sera of 109 gallstone patients and 100 control subjects were obtained during January 2010 to November 2010 from Memon Charitable Hospital, Hyderabad. The serum calcium, copper and iron concentrations were determined by Hitachi double beam atomic absorption spectrophotometer 180 - 50.

Results: Comparison of serum calcium, copper, and iron levels between gallstone patients and control subjects showed that all these variables were significantly ($p < 0.01$) higher in gallstone patients. However, no significant difference ($p > 0.01$) was found when mean serum concentrations for these variables were compared between male and female gallstone patients, between female gallstone patients of up to 45 and over 45 years age and between female gallstone patients having up to 3 and more than 3 children.

Conclusions: The higher levels of calcium, copper and iron in blood samples of gallstone patients compared to controls suggest that these metals do play a contributory role in the pathogenesis of human gallstones.

KEY WORDS: Serum calcium, Copper, Iron, Gallstone patients, Cholelithiasis.

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INTRODUCTION

There is an increasing interest in the determination of metal levels in human gallstones in order to assess their possible role in the genesis of gallstones. The results of epidemiological studies carried out in different countries show that the prevalence rate of gallstone disease is 10 - 25%, which may rise by 1% every year and can become 60% after 80 years.¹⁻³ We have also noticed a rise in the surgical incidence of cholelithiasis in Hyderabad which was 3.07% during the years 1990 - 1993,⁴ rose to 9.03% during 1999 to 2001⁵ and reached to 11.14% (95% CI, 10.57 - 11.72) during 2002 to 2005.⁶

Chemical analysis of gallstones had revealed three types of gallstones of which cholesterol stones contained cholesterol as a chief constituent,

while pigment stones had calcium bilirubinate as a main constituent.⁷ The mixed composition stones contained cholesterol and calcium bilirubinate as major constituents. The other substances found in gallstones were calcium salts of phosphates, carbonate and fatty acids; phospholipids; and some metal elements like sodium, potassium, copper, magnesium, manganese and iron.⁸⁻¹¹ Disturbances in metal contents either marginal or severe are supposed to be the risk factors for many diseases.^{12,13} Metal concentrations in gallstones are moderately correlated with serum metal concentrations by some investigators.¹⁴

Furthermore, previous studies on metal contents in gallstones had shown the involvement of sodium, potassium, calcium, copper and iron in the structure mechanism of gallstones.^{8,10} Keeping in view the above fact present study was designed to investigate the levels of calcium, copper and iron in serum samples of gallstone patients and control subjects to find out any role of these metal ions in the pathogenesis of gallstones in this area.

METHODOLOGY

Sera of 109 preoperative gallstone patients (11 males and 98 females, age range 22.5 to 65.8 years) who were admitted during January 2010 to November 2010 in Memon Charitable Hospital, Hyderabad, for cholecystectomy, and 100 control subjects (10 males and 90 females age range 22.5 to 65.8 years) who were eye patients, with no personal (confirmed by ultrasound and / or X-ray) or family history of gallstones were obtained after getting informed consent (in their native language i.e. Urdu or Sindhi) from these patients. The ethical approval of the study was given by the Institutional Ethics Committee.

The concentration for serum calcium, copper and iron were determined by Hitachi double beam atomic absorption spectrophotometer 180 - 50.

All glassware were rinsed overnight in 20% HNO₃ and washed three times with deionized double-distilled water. All the reagents such as: Ammonium ferrous sulphate, copper (II) sulphate, and calcium chloride were purchased from E. Merck (Germany). The samples were prepared as per reported method.¹⁵

Diluted 1ml serum with 8ml of water and added in it a half volume (0.5ml) of each of sodium tungstate (100g NaWO₃·2H₂O/l) and 0.33 mol/l H₂SO₄ (20 ml of concentrated acid was diluted to 1 liter, standardized against a known NaOH solution, and adjusted if necessary) to make 1:10

dilution of serum. The samples were filtered through Whatman No. 42. The acid liberated the whole of the tungstic acid from the tungstate, and practically all tungstate was taken down by the protein precipitate leaving sufficient excess acid to give a filtrate with a pH between about 3.5 and 4.9.¹⁶ The absorbance and hence the concentration for calcium, copper and iron were measured by Atomic absorption Spectrometer. A series of separate standards for above metals were also prepared in the same way.

Statistical Analysis: MS Excel, 2003 was used for statistical analysis. Results were expressed as mean \pm SEM. Student's *t* test was used to compare the mean values between gallstone patients and control subjects, between the female gallstone patients of upto 45 and above 45 years age and between female gallstone patients having upto three and more than three children, A p value less than 0.01 was considered statistically significant.

RESULTS

Comparison of serum calcium, copper, and iron levels between gallstone patients and control subjects showed significantly ($p < 0.01$) higher concentrations for these metals in gallstone patients as compared to control subjects (Table-I). We also compared serum calcium, copper and iron levels between male and female gallstone patients, between female gallstone patients of upto 45 and above 45 years age, and between female gallstone patients having three and more than three children. The purpose of this comparison was to see the effects of gender, menopause (age and female hormones) and parity on the levels of metals under study, as it is well documented that females are more prone to develop gallstones especially those who are of child bearing age and have multiple pregnancies.

No significant difference ($p > 0.01$) for studied metals was found between male and female gallstone patients, between female gallstone patients of upto 45 and above 45 years age, and also between female gallstone patients having three and more than three children. Similarly, no significant difference in serum levels of these metals was found in patients with different types of gallstones.

When we compared serum metal contents with type of gallstones, we could not find any significant variation (Table-II). We failed to find any strong or moderate correlation of serum metal contents with serum lipid profile. However, a very weak positive correlation of serum calcium with total, bound and free cholesterol and total lipids and a very weak

Table-I: Comparison of serum calcium, copper and iron levels between gall stone patients and age and gender matched controls.

Groups	Calcium (Mean±SEM) (mg/dl)	Copper (Mean±SEM) (mg/dl)	Iron (Mean±SEM) (mg/dl)
Gallstone patients (n=109)	13.1 ± 4.63*	0.09 ± 0.03*	0.20 ± 0.03*
Control subjects (n=100)	9.96 ± 4.51	0.06 ± 0.005	0.14 ± 0.05
Females (n=98)	12.97 ± 4.62*	0.086 ± 0.04*	0.20 ± 0.06*
Control subjects	10.34 ± 4.21	0.06 ± 0.01	0.136 ± 0.01
Males (n=11)	14.74 ± 4.81*	0.094 ± 0.03*	0.21 ± 0.06*
Control subjects	7.63 ± 3.91	0.051 ± 0.02	0.143 ± 0.02
Females of Up to 45 years age (n=71)	13.28 ± 4.60*	0.093 ± 0.04*	0.19 ± 0.07*
Control subjects	10.85 ± 4.21	0.066 ± 0.01	0.132 ± 0.03
Females of above 45 years age (n=27)	12.17 ± 4.57*	0.096 ± 0.04*	0.20 ± 0.06*
Control subjects	9.18 ± 4.55	0.048 ± 0.03	0.144 ± 0.02
Females having up to 3 children (n=81)	12.63 ± 4.47*	0.19 ± 0.06*	0.21 ± 0.04*
Control subjects	9.87 ± 4.51	0.047 ± 0.05	0.149 ± 0.05
Females having more than 3 children (n=17)	15.18 ± 5.02*	0.21 ± 0.07*	0.20 ± 0.03*
Control subjects	10.6 ± 3.98	0.067 ± 0.02	0.129 ± 0.01

*P< 0.01

Note: A comparison of metal levels between male patients and female patients, between female patients upto 45 years and female patients with age above 45 years, and between female patients having upto 3 children and patients with more than 3 children revealed no statistically significant difference.

negative correlation of copper with bound and LDL- cholesterol was seen in present study.

DISCUSSION

Many investigators have consistently reported that super saturation of bile with cholesterol occurs in most patients with gallstones¹⁷ however, the mechanism of initiation of gallstone formation is not clear in these patients. Normally gall bladder mucosa absorb a part of the calcium in bile.¹⁸ resulting into its low concentration level in bile compared to that in serum. In present study the mean serum concentration for calcium in gallstone formers (13.2 ± 4.6mg/dl) compared to control subjects (9.96 ± 4.5mg/dl) was higher (Table-I). This observation is in agreement with the findings of Verma et al.¹⁹

We have previously reported that majority of gallstone patients in this area use rapeseed oil as the source of cooking and frying food.^{20,21} which contains copper.²² The copper absorbed through soil by the rapeseeds can increase the chances of increased serum copper concentration in individuals who are rapeseed oil consumers. Besides calcium, other metals like copper and

zinc are also absorbed by normal gall bladder mucosa. Although the presence of copper irritates the mucous membrane and disturbs the digestive system²³, this is probably an attempt to reduce the cationic concentration in the bile, because higher cationic biliary concentration tends to promote the formation of cholesterol crystals.²⁴ The gallbladder also has a defense mechanism against precipitation of calcium by way of secreting hydrogen ions from gallbladder mucosa and keeping the pH of bile low. Bile acidification appears to be extremely important in preventing calcium precipitation.²⁵

Saito et al²⁵ reported that calcium was the major constituent of all gallstones in his series. Verma et al also reported copper, iron and calcium as the major elements present in all the gallstone samples.¹⁹ Our previous study on the analysis of metal contents in gallstones in this area showed that calcium, copper and iron were present in significantly higher amounts in Cholesterol + Calcium carbonate gallstones than in pure cholesterol gallstones.¹⁰ Both copper and iron were significantly higher in calcium bilirubinate stones as compared to any other type of gallstones. Chandran et al²⁶ suggested that

Table-II: Statistical comparison of serum metal levels in patients with different types of gallstones.

Metals	Pure Cholesterol (n= 74)	Pure calcium carbonate (n=5)	Calcium bilirubinate (n= 7)	Cholesterol + Calcium carbonate (n= 31)	Cholesterol + bilirubin (n= 10)	P Values (<0.05)
Calcium	12.21± 0.35	13.15 ± 0.65	13.21± 0.71	13.33± 1.28	11.18 ± 0.70	N.S
Copper	0.09 ± 0.031	0.09 ± 0.012	0.136 ± 0.005	0.09 ± 0.022	0.09±0.001	N.S
Iron	0.199 ± 0.008	0.198 ± 0.015	0.234 ± 0.005	0.197 ± 0.009	0.204 ± 0.013	N.S.

p is calculated by ANOVA, N.S.= Non-significant

copper and iron may act as chelating agents for calcium bilirubinate gallstones. As far as serum concentrations for copper and iron are concerned in present study, they are slightly higher in calcium bilirubinate stone formers than in other types of gallstone formers, although the mean levels of both these metals were within normal ranges.

The central aggregates of calcium salts constitute hard foreign bodies which may lead to ulceration of gallbladder mucosa and microscopic haemorrhage.¹⁹

Shareef et al in his study on the correlation between chemical components of gallstones and metal contents of sera of gallstone formers found that there was moderate (positive and negative) correlation between the calcium content of the serum and the cholesterol, pigment and mixed gallstones ($r=0.202$, -0.213 and -0.210 respectively).¹⁴ In contrast to this study our results revealed a very weak positive correlation of serum total, bound and free cholesterol and total lipids with calcium, and a very weak negative correlation of bound and LDL-cholesterol with copper.

We did not find any significant variation of serum metal contents with type of gallstones, it may be due to less number of gallstones of calcium carbonate and calcium bilirubinate composition. Copper plays a role in the pigment formation in bile,⁵ so its highest concentration in calcium bilirubinate stones suggests that it may have a contributory role in the formation of those types of gallstones. In present study, calcium was found in highest concentrations in cholesterol + calcium carbonate gallstones. Our this finding is in line with other investigators who reported that high levels of cholesterol and calcium in serum leads to increase in their level in bile followed by their co-precipitation as calcium salt of cholesterol (cholesterol + calcium carbonate in present study) to form cholesterol gallstones.²⁷

CONCLUSION

It may be concluded that increased serum calcium, copper and iron contents may have a role in the pathogenesis of different types of human gallstones in this area.

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Conflict of Interest: No conflict of interest.

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