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Role of Trace Elements in the Formation of Gall Stones

¹P.K. Misra, ¹S. Dalal, ²M. Kumawat and ²S. Kharb

¹Department of Biochemistry,

²Department of Surgery, Pt. B.D. Sharma University of Health Sciences, Rohtak (Haryana), India

Corresponding Author: Simmi Kharb, Department of Surgery, Pt. B.D. Sharma University of Health Sciences, Rohtak (Haryana), 1396, Sector-1, Haryana, India Tel: 91-9812016036

ABSTRACT

Recent studies have defined the role of trace elements such as iron, calcium, zinc and copper in the formation of gallstones and deficiencies of serum iron and serum calcium can lead to increased risk of gallstone disease. The present study was planned to analyse the exact role of serum iron and calcium in the pathogenesis of gallstone disease and to assess the relationship of biliary cholesterol supersaturation with levels of serum iron and calcium. Total 100 patients suffering from cholelithiasis were included in the study and were divided into following groups based on their serum iron and calcium levels; group A included patients with normal serum iron (the controls), group B included patients with iron deficiency (the cases), group A1 included patients with normal serum calcium (the controls) and group B1 included patients with calcium deficiency (the cases). The 5 mL of blood sample was drawn intravenously before cholecystectomy, serum was analysed for the parameters like serum iron, calcium, cholesterol and biliary cholesterol (lipid extract from bile). Bile cholesterol levels were raised in group B and group B1 (the cases) as compared to group A and group A1 (the controls). Low serum iron, causes defective hepatic cholesterol metabolism and more stasis of bile because of decreased motility of gallbladder and leads to increased precipitation of cholesterol and hence gallstone formation. Also, deficiency of both iron and calcium is associated with increased chances of super-saturation of bile in gallbladder followed by increase in incidence of gallstone formation.

Key words: Gall stones, iron, calcium, bile cholesterol, cholecystitis, cholelithiasis

INTRODUCTION

Gallstone disease has troubled human lives since time immemorial. Cholecystitis and cholelithiasis are the most common disorders affecting the biliary system. There are variations in incidence of gallstones according to geographical distribution. The incidence varies from country to country and even in different parts of the same country (Sarin *et al.*, 1986).

The old axiom, a typical gallstone sufferer, is a fatty, fertile, female of forty, is only partially true as the disease has also been found in women of young ages and underweight as well as in thin individuals. But the fact is that gallstone disease is much more common in females, with a female: male ratio of 3:1 up to age 50 years (Nahrwold, 1997). Gallstone disease has also been reported in infants, thus no age is immune. Gallstones may produce symptoms or remain asymptomatic which are usually detected by abdominal ultrasound done for some other purpose. The presentation may range from flatulent dyspepsia and acute cholecystitis to its complications like empyema, chronic cholecystitis, gangrene, fistula and gallbladder carcinoma (Everhart, 1994).

Today, the incidence of gallstone disease has increased considerably due to frequent use of ultrasonography (Shehadi, 1979).

Despite the efficacy and safety of open and laparoscopic cholecystectomy, medical fraternity has long opted and practiced for other less invasive options. To effect the prevention or to attempt the medical dissolution of gallstones, it is essential to clearly understand the pathogenesis of gallstones. Depending on their composition, gallstones can be pure cholesterol, pure pigment or more frequently the mixed type. Pigment stones can further be of two types as; brown and black stones (Pauletzki *et al.*, 1966).

Many years ago, the prevailing opinion suggested that the formation of cholesterol calculi was the result of specific alterations in hepatic metabolism while the gallbladder was “a harmless spectator”. Recent added reports indicate that neither the liver nor gallbladder alone has an exclusive etiological role in the formation of calculi. It appears now that the calculi are formed as a result of dynamic interaction between the liver and the gallbladder (LaMorte *et al.*, 1979). Though the exact etiology of cholelithiasis is still not well understood, various factors held responsible are infection, metabolic changes and gallbladder stasis.

Three conditions must be met to permit the formation of cholesterol gallstones; super-saturation of bile with cholesterol, kinetically favourable nucleation and cholesterol crystals must remain in the gallbladder long enough to agglomerate into stones (Strasberg *et al.*, 1991).

The established risk factors for causing this super-saturation of cholesterol are elderly aged females, obesity with rapid weight loss, cirrhosis and diet related factors.

While, searching for other established factors, recent studies have defined the role of trace elements such as iron, calcium, zinc and copper in the formation of gallstones (Verma *et al.*, 2002). Out of these trace elements, iron deficiency has been shown to alter the activity of several hepatic enzymes leading to increased bile cholesterol saturation and promotion of crystal formation (Johnston *et al.*, 1997). Iron acts as a cofactor for Nitric Oxide Synthase (NOS), which synthesizes Nitric Oxide (NO) and is important for the maintenance of basal gallbladder tone and normal relaxation (Swartz-Basile *et al.*, 2000). The NOS acts as a calcium-calmodulin dependent enzyme. Hence, a deficiency in serum calcium causes deranged function of NOS resulting in altered gallbladder motility, leading to biliary stasis and subsequently increased crystal formation in bile (Verma *et al.*, 2002). Thus, deficiencies of serum iron and serum calcium can lead to increased risk of gallstone disease. Hence, the present study was planned to analyze the exact role of serum iron and serum calcium in the pathogenesis of gallstone disease. To study the role of trace elements, iron (Fe) and calcium (Ca) in the formation of gallstones to assess the relationship of biliary cholesterol super-saturation with levels of serum iron and calcium.

MATERIALS AND METHODS

The present prospective study was conducted in the Departments of Surgery and Biochemistry, Pt. BD Sharma Postgraduate Institute of Medical Sciences, Rohtak. 100 consecutive patients suffering from cholelithiasis admitted in various surgical wards of PGIMS, confirmed by ultrasonography were included in the study irrespective of their age, sex and parity. Both open and laparoscopic cholecystectomies were included in the study, whose serum and bile specimens were procured for analysis.

Inclusion criteria: All patients suffering from cholelithiasis confirmed by ultrasonography were included in the study.

Exclusion criteria: Patients suffering from empyema of gallbladder, mucocele of gallbladder and those patients of laparoscopic cholecystectomy whose bile sample were not available were not included in the study. Based on serum iron content, patients of cholelithiasis were divided into two groups:

Group A: Patients with normal serum iron (the controls)

Group B: Patients with iron deficiency (the cases)

Similarly, based on serum calcium levels, patients were divided into two groups:

Group A1: Patients with normal serum calcium (the controls)

Group B1: Patients with calcium deficiency (the cases)

Five milliliter of blood sample was drawn intravenously before cholecystectomy, centrifuged at 2000 rpm for 2 min, serum was collected and stored at 4°C.

During the operation for cholecystectomy, bile was aspirated from gallbladder. In case of laparoscopic cholecystectomy, bile was aspirated from one of the 5 mm port. The bile was kept in a sterile labelled container and analysed. Serum iron was estimated by ferrozine kit method (Siedel *et al.*, 1984), serum calcium was estimated by Arsenazo III colorimetric method (Young and Pestaner, 1975), serum cholesterol was estimated by Enzopak kit (Allain *et al.*, 1974) and biliary cholesterol was estimated after extraction of lipids from bile (Folch *et al.*, 1957).

Statistical analysis: Data obtained was computed as mean±SD and student's t-test was used.

RESULTS

In the present prospective study, all the 100 patients of cholelithiasis were divided into groups A and B based on serum iron levels and again the same patients were divided into groups A1 and B1 based on the serum calcium levels. The 88 patients fell in group A and 12 patients to group B, where as 89 patients were present in group A1 and 11 in group B1. Various interesting observations were made from the study which were analyzed and compared with the available literature.

Serum iron levels of all 100 patients were estimated quantitatively. Male patients had serum iron in the range of 13.8-28.2 $\mu\text{g dL}^{-1}$ with mean±SD of 19.1±5.6 $\mu\text{g dL}^{-1}$ whereas, the female patients had serum iron in the range of 3.4-26.4 $\mu\text{g dL}^{-1}$ with mean±SD of 13.0±4.9 $\mu\text{g dL}^{-1}$. The difference in serum iron contents of male and female gall stones patients was found to be statistically significant with male patients having more serum iron levels ($p < 0.005$). Serum iron levels of all 100 patients were estimated quantitatively. Male patients had serum iron in the range of 13.8-28.2 $\mu\text{g dL}^{-1}$ with mean±SD of 19.1±5.6 $\mu\text{g dL}^{-1}$ whereas, the female patients had serum iron in the range of 3.4-26.4 $\mu\text{g dL}^{-1}$ with mean±SD of 13.0±4.9 $\mu\text{g dL}^{-1}$. The difference in serum iron contents of male and female gall stones patients was found to be statistically significant with male patients having more serum iron levels ($p < 0.005$).

Based on their iron content, all patients were divided into group A and group B with normal and low iron levels, respectively. The range of serum iron of all the patients was 3.5-30 $\mu\text{g dL}^{-1}$. This range was 9-30 $\mu\text{g dL}^{-1}$ in patients of group A with a mean±SD of 14.7±4.0 $\mu\text{g dL}^{-1}$ whereas it was 3.5-5.5 $\mu\text{g dL}^{-1}$ in group B patients with a mean±SD of 4.7±0.7 $\mu\text{g dL}^{-1}$. The serum iron content of group B patients was significantly lower than group A patients ($p < 0.005$).

DISCUSSION

Gall stone disease is a very common gastrointestinal problem in day today practice. There was definite clustering of cases around 4th and 5th decade as evident from data. The 18 (18%) patients out of the whole study group presented in their 6th decade where as 15 (15%) belonged to 3rd decade. There were 12 (12%) patients who had age more than 60 years at the time of presentation with eldest being 83 years and only 1 (1%) patient falling into 2nd decade with age of 20 years. These findings were similar to those observed in epidemiological study of cholelithiasis (Jayanthi, 1996).

The prevalence of gallstone disease was much more in females as compared to males with 82 (82%) patients out of the total 100 being females and the rest 18 (18%) were male with a male: Female ratio was of 1:4 as evident from Table 1. This is in accordance with the previous studies which stated a high prevalence of cholelithiasis in females which may be due to the effects of estrogen and progesterone on the biliary tract. Estrogenic influences increase the effect of hepatic lipoprotein receptors and stimulate hepatic hydroxyl methyl glutaryl coenzyme A (HMG Co-A) reductase activity. Consequently, together cholesterol uptake and biosynthesis are increased leading to super-saturation of bile with cholesterol and helping in formation of gallstones. Progesterone alters the sphincter of Oddi and gallbladder function ultimately causing a derangement in bile flow dynamics. Even though, the effects of progesterone on the biliary tract have been implicated in the increased incidence of gallstones among the women, the specific effects of prolonged elevated levels of progesterone on the sphincter of Oddi and bile flow dynamics are still incompletely understood (Crawford, 1999; Tierney *et al.*, 1999). There was a great predilection for stone formation in multiparous females (90.3%) as compared to that of primipara (8.5%) and nullipara (1.2%). Pregnancy favours the formation of gallstones through the hormonal influence on bile composition (increased biliary cholesterol secretion, diminished and disturbed bile acid pool). Estrogen induces an increased input to the hepatic free cholesterol pool by up regulating the low density lipoprotein. Decreased gallbladder motility during third trimester of pregnancy and an altered function of gallbladder mucosa that may favour nucleation and growth of stones (Acalovschi, 2001).

Pain which was colicky in nature, intermittent and felt in the right hypochondrium was the most consistent symptom. This pain increased on taking fatty food and relieved on taking antispasmodic medications. Sometimes, a constant dull ache was felt in epigastric region which decreased on taking analgesics. Flatulent dyspepsia was the second most common presenting symptom, followed by vomiting. Only 15% of the patients had all the three symptoms. Various authors have described similar symptomatology in their studies with pain being the most common presentation in gallstone disease (Vauthey and Saldinger, 1992).

Table 1: Distribution of patients according to age, sex, parity and presenting symptoms

Distribution of patients	No. of patients	Percentage
Male	18	18
Female	82	82
Nullipara	1	1.2
Primipara	7	8.5
Multipara	74	90.3
Pain	100	100
Dyspepsia	54	54
Vomiting	45	45
All the above symptoms	15	15
Jaundice	0	0

While studying the pathogenesis of gallstone formation, certain known risk factors can be enlisted like elderly age, female sex, obesity and rapid weight loss, cirrhosis and different diet linked issues. On seeking for other risk factors, latest studies have defined the role of trace elements like iron and calcium which might play a definitive role in the formation of gallstones.

Iron deficiency alters the activity of several hepatic enzymes, leading to increased gallbladder bile cholesterol saturation and promotion of cholesterol crystal formation (Kumar *et al.*, 2006). It is also suggested that iron deficiency alters the activity of several hepatic enzymes (Roslyn *et al.*, 1987). They concluded that consumption of diet rich in carbohydrates but deficient in iron altering hepatic metabolism of cholesterol that might be important in gallstone formation. Researchers concluded that iron deficient diet altered hepatic enzyme metabolism which in turn increased gallbladder bile cholesterol and promoted cholesterol crystal formation (Johnston *et al.*, 1997). Researchers have observed the same parameters in their study (Salomons *et al.*, 1997). Researchers have demonstrated that diminished gallbladder neuronal nitric oxide synthase contributed to the gallbladder stasis that occurred with iron deficiency (Swartz-Basile *et al.*, 2000). Iron, a cofactor for nitric oxide synthase, plays a key role in normal relaxation of gallbladder. It has been reported that iron deficiency resulted in altered motility of gallbladder and sphincter of Oddi and thus increased cholesterol crystal formation in the gallbladder bile (Swartz-Basile *et al.*, 2000; Goldblatt *et al.*, 2001). Thus, iron deficiency was found to have a major role in gallstone formation.

The difference in serum iron contents of male and female gall stones patients was found to be statistically significant with male patients having more serum iron levels ($p < 0.005$). Thus, subclinical anaemia was more prevalent in females in this study. It was probably related to nutritional status in females and iron losses occurring due to multiple pregnancies.

On a close examination of group A and group B patients, it can be concluded that the occurrence of gall stones was more common in females (79.5 and 100% in group A and group B, respectively) than males (20.5 and 0% in group A and group B, respectively). In females, cholelithiasis had greater predilection for iron deficient patients (100%) as compared to patients with normal serum iron (79.5%). These findings were in accordance to literature (Kumar *et al.*, 2006; Roslyn *et al.*, 1987; Johnston *et al.*, 1997). Researchers have concluded that iron deficiency led to gall bladder stasis thus, causing increased cholesterol crystal formation in bile within the gall bladder (Swartz-Basile *et al.*, 2000; Goldblatt *et al.*, 2001).

The serum cholesterol level of all the patients included in the study were determined. The range of serum cholesterol in all the patients was 66-202 mg dL⁻¹. The range for male patients was 87-179 mg dL⁻¹ whereas in females, it was 66-202 mg dL⁻¹. Thus, there was no difference in serum cholesterol based on sex in all the patients. These findings were contradictory to the report where young women with cholesterol gallstones were slightly hypercholesterolemic. But women with gallstones have higher saturation index of the bile than women without gallstones with the same levels of serum cholesterol (Cavallini *et al.*, 1987). A study has found a positive correlation between serum and biliary cholesterol in patients of cholesterol gallstones (Kanwar *et al.*, 1996).

The serum cholesterol levels in group A and B is shown in Table 2 were not significantly different in groups A and B and the mean serum cholesterol level was found to be within the normal range of non-stone formers suggesting that serum cholesterol is not a significant criterion in the supersaturation of bile with respect to cholesterol.

Cholesterol levels of gallbladder bile were determined in all the 100 patients in the study. It was observed that the range of bile cholesterol in males was 79-270 mg dL⁻¹ with a mean±SD of 158.4±58.0 mg dL⁻¹ and in females range was 72-1050 mg dL⁻¹ with mean±SD of 302.9±282.0 mg dL⁻¹. These findings were in accordance to those of Tierney *et al.* (1999) who

Table 2: Comparison between the levels of serum iron, serum cholesterol and biliary cholesterol in group A and group B patients

Groups	No. of patients	Serum cholesterol (mg dL ⁻¹)		Serum iron (µg dL ⁻¹)		Bile cholesterol (mg dL ⁻¹)	
		Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
A	88	66-201	133.0±32.0	9-30	14.7±4.0	72-1050	212.8±204.7
B	12	92-202	129.7±38.0	3.5-5.5	4.7±0.7	572-903	747.1±115.0

Table 3: Comparison between the levels of serum calcium, serum cholesterol and biliary cholesterol in group A1 and group B1 patients

Groups	No. of patients	Serum calcium (mg dL ⁻¹)		Serum cholesterol (mg dL ⁻¹)		Bile cholesterol (mg dL ⁻¹)	
		Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
A1	89	8.6-11.3	9.8±0.7	87-202	132.5±32.3	72-903	230.4±218.0
B1	11	7.5-8.5	8.0±0.3	66-201	142.6±48.5	172-1050	652.8±296.1

contributed increased levels of biliary cholesterol to the effects of hormones like progesteron. The range of bile cholesterol levels in group A and B is shown in Table 2. The difference in values was statistically significant indicating that the mean bile cholesterol of patients with iron deficiency was higher than patients with normal serum iron ($p < 0.05$). These findings were similar to those that observed that iron deficient diet altered hepatic enzyme metabolism which in turn increase gallbladder bile cholesterol and promoted cholesterol crystal formation (Johnston *et al.*, 1997). As shown in Table 2, the comparison between the levels of serum iron and bile cholesterol in group A and group B patients showed statistically significant difference in values of both the groups showing that group B patients had low serum iron and high bile cholesterol levels indicating supersaturation of bile in iron deficient patients. These findings were in accordance with those of Kumar *et al.* (2006) who also observed in their study that iron deficiency altered the activity of several hepatic enzymes leading to increased gallbladder bile supersaturation and promotion of cholesterol crystals.

A deficiency in serum calcium causes deranged function of Nitric Oxide Synthase (NOS) resulting in altered gallbladder motility, leading to biliary stasis and subsequently increased crystal formation in bile (Verma *et al.*, 2002). Nitric Oxide (NO) is important in maintenance of basal gallbladder tone and normal relaxation.

The serum calcium levels in group A1 and group B1 showed statistically significant difference as shown in Table 3. The values were lower in group B1 patients as compared to the patients of group A1 ($p < 0.05$).

There was statistically significant difference in the range of bile cholesterol in group A1 and group B1 patients indicating that the levels of bile cholesterol were higher in group B1 patients ($p < 0.05$).

The comparison of serum calcium and bile cholesterol in group A1 and group B1 patients showed statistically significant difference meaning that group B1 patients have low serum calcium values and high bile cholesterol levels. Verma *et al.* (2002) also observed that deficiency in serum calcium and higher biliary calcium played a role as one of the underline factors for cholelithiasis.

When the levels of serum and bile cholesterol were compared between male and female patients and the ratio between the two entities was calculated, it was observed that ratio of serum to bile cholesterol was low in females indicating higher levels of biliary cholesterol in females as compared to males.

CONCLUSION

In this study, it was observed that there were high bile cholesterol levels in patients with iron and calcium deficiency along with low serum to bile cholesterol ratio and raised bile cholesterol levels in female patients. Thus, it can be concluded that the deficiency of serum iron and serum calcium along with increased cholesterol in bile results in supersaturation of bile resulting in increased crystal formation in gallbladder bile. Low serum iron, causing defective hepatic cholesterol metabolism and more stasis of bile because of decreased motility of gallbladder leads to increased precipitation of cholesterol and hence gallstone formation. Also, calcium deficiency causes deranged function of nitric oxide synthase which acts as a calcium calmodulin dependent enzyme producing relaxation of gallbladder. Hence, gallbladder stasis occurs which subsequently increases crystal formation in bile so deficiency in both iron and calcium is associated with increased chances of supersaturation of bile in gallbladder with subsequent increased incidence of gallstone formation.

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