Original Research Article

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Serum iron and serum ferritin levels in cholelithiasis: a randomized study

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ABSTRACT

Background: The role of iron in the pathogenesis of gallstone disease has not been well established so far. Iron deficiency has been shown to alter the activity of several hepatic enzymes, leading to increased cholesterol saturation of bile in gall bladder and hence promoting cholesterol crystallization. Serum iron, total iron binding capacity and transferrin saturation are not good indicators of iron status in individuals. In infection free situation, serum ferritin is an ideal indicator for diagnosis of iron deficiency.

Methods: The study was conducted as a prospective study in the department of Surgery. The study population was divided into two groups; Case group with 200 patients with gallstone disease and control group with 50 patients without gallstone disease. Serum iron and ferritin contents of both groups will be analyzed and compared with each

Results: In this study the gallstones are more prevalent in female population than males in ratio of 5.4:1. Serum iron in males was low in 41.93% not comparable 20.8% of control suggesting that low serum iron is not associated with Cholelithiasis in male. In males, serum ferritin was low in 64.5% of cases and 16.66% of controls. Serum ferritin levels were normal in 35.50% of cases and 66.66% of controls and above normal in 16.66% of controls suggesting that low serum ferritin is associated with gall stones in males. In this study, low serum iron was seen in 23.07% of females comparable to 23% low serum iron in control females and low ferritin was seen in 35.50% of female cases as compare to 15.38% of controls.

Conclusions: It was concluded that a low body store of serum iron is a risk factor for cholelithiasis in females and serum iron, serum ferritin may be used as marker of iron store so that low serum iron status could be diagnosed at early stage.

Keywords: Anemia, Cholelithiasis, Risk factor for gall stone, Serum iron, Serum ferritin

INTRODUCTION

Cholelithiasis is a common abdominal disorder resulting in increasing hospital admissions. About 10-12% of adults develop gallstones.1 The prevalence of common bile duct stones in patients with gallstones varies from 8 to16%.2 Pure cholesterol stones are uncommon and account for less than 10% of all stones. Whether pure or of mix nature, the common primary event in the

formation of cholesterol stone is super saturation of bile with cholesterol. Super saturation almost always is caused by cholesterol hyper secretion rather than reduced secretion of phospholipids or bile salts.3 Pigment stones contain less than 20% cholesterol and are dark because of presence of calcium bilirubinate. Black pigment stones are usually small, brittle, black and sometimes spiculated. They are formed by super saturation of calcium bilrubinate, carbonate and phosphate and occur most

often secondary to hemolytic disorder such as hereditary spherocytosis and sickle cell anemia. Like cholesterol stones they are almost always found in gall bladder. Brown pigment stones are usually less than 1 cm in diameter, brownish-yellow and soft, often mushy. They may form either in gallbladder or in bile ducts, usually secondary to bacterial infection caused by bile stasis. Precipitated calcium bilirubinate and bacterial cell bodies compose the major part of the stone.³

The role of iron in the pathogenesis of gallstone disease has not been well established so far. Iron deficiency has been shown to alter the activity of several hepatic enzymes, leading to increased cholesterol saturation of bile in gall bladder and hence promoting cholesterol crystallization.⁴ Serum iron, total iron binding capacity and transferrin saturation are not good indicators of iron status in individuals. In infection free situation, serum ferritin is an ideal indicator for diagnosis of iron deficiency and response to iron therapy in a community.⁵ If the prevalence of iron deficiency in a population must be described with a single number, serum ferritin should be used and complemented with haemoglobin in all programmed evaluations.⁶

The serum iron concentration is found least in patients with pigment stones hence enforcing the role of iron in gall stone formation.⁷ Nutritional anaemia is a major public health problem in India and is primarily due to iron deficiency. The old axiom that a typical gall stone sufferer is a fat, fertile, flatulent, female of fifty, is only partially true, as the disease is found in women soon after their first delivery and also in underweight and thin people.⁸ While, searching for other established factors, recent studies have defined the role of trace elements such as iron in the formation of gallstones.

Haemoglobin concentration alone cannot be used to diagnose iron deficiency. However, the concentration of haemoglobin should be measured, even though not all anaemia is caused by iron deficiency. The prevalence of anaemia is an important health indicator and when it is used with other measurements of iron status, the haemoglobin concentration can provide information about the severity of iron deficiency. Serum ferritin will act as more specific indicator for iron deficiency anaemia.

If we can predict which factors contribute to the development of gall stone disease, then its prevention could be affected by modifying these factors. This study was planned to study the correlation between serum iron and serum ferritin in patients suffering from gallstone disease.

METHODS

The study was conducted as a prospective study in the department of Surgery in collaboration with department of Biochemistry in this institute.

The study population was divided into two groups.

- Case group with 200 patients with gallstone disease admitted in surgery ward fulfilling the inclusion criteria.
- Control group with 50 patients without gallstone disease admitted in surgery ward.

Serum iron and ferritin contents of both groups will be analyzed and compared with each other.

Inclusion criteria

For control group

Case group with age group of 15 - 70 years not suffering from gall stone confirmed by ultrasonography

For case group

All patients with Cholelithiasis confirmed by ultrasonography with age group of 15 - 70 years.

Exclusion criteria

- Patients taking iron for anemia
- Previous case of biliary tract surgery.

Procedure

In this study, a detailed history was taken from all the patients suffering from with and without gall stone disease. Routine investigations such as ultrasound of abdomen were done in all the patients. Venous blood sample of 4 ml was taken in red vacutainer for evaluation of serum iron and serum ferritin level. Serum ferritin level was assessed by chemiluminescence. Serum iron was estimated by the ferrozine kit method.

RESULTS

The study population was divided into two groups. Group A consisted of 200 patients with gallstone disease admitted in surgery ward fulfilling the inclusion criteria. Group B consisted of 50 patients with age group of 15–70 years who were admitted in ward and did not have gallstone on ultrasonography. There were more females in cases group 169 (84.5%) than control group 24 (48%) and males in case were (31) 15.5% and (26) 48% in control. The age distribution of these patients in group A having gall stone; 3% were in age group of 15-19 years, 15.5% were in the age group of 20-29, 27.5% in the age group of 30-39, 22.5% in the age group of 40-49, 19.5% in the age group 50-59, and 12% in age group of 60-70 years. The mean of age in case group was 41.78±12.81 and in control group was 40.02±12.94. There was low prevalence of gallstones in young population and highest prevalence of gallstones in 30-39 years.

Table 1: Serum iron distribution in males.

Serum iron level (µg/dl)	Case group (n=31)	Control group (n=24)	P value
Low	13 (41.93%)	5 (20.8%)	
Normal	18 (58.06%)	19 (79.16%)	0.749
Above normal	0 (0%)	0 (0%)	
Mean±SD	65.45±27.55	65.86±18.55	0.950

P value >0.05 (not significant).

In this study in males, the normal reference value was supplied with the kit, for male normal value was 60-160 μ g/dl. Serum iron levels were low in 41.93% of cases and 20.8% of controls. Serum iron was normal in 58.06% of cases and in 79.16% of controls. The mean serum iron among cases 65.45 \pm 27.55 and control 65.86 \pm 18.55. P value was >0.05 which is not-significance.

Table 2: Serum iron distribution in females.

Serum iron level (µg/dl)	Case group (n=169)	Control group (n=26)	P value
Low	39 (23.07%)	6 (23%)	
Normal	130 (76.92%)	17 (65.38%)	< 0.001
Above normal	0 (0%)	3 (11.53%)	
Mean±SD	56.84±25.75	69.43±45.82	0.042

P value <0.05 (not significant).

In our study, the normal reference values were supplied with the kit for females 35-145 $\mu g/dl$. In females' serum iron was low in 23.07% of cases and 23% of controls. Serum iron was normal in 76.92% cases and 65.28% of controls. Serum iron was above normal value in 11.53% of control. The mean serum iron in female cases 56.84 \pm 25.75 and control was 69.43 \pm 45.82. P value was <0.05 which is significant.

Table 3: Serum ferritin distribution in males.

Serum ferritin level (ng/ml)	Case group (n=31)	Control group (n=24)	P value
Low	20 (64.5%)	4 (16.66%)	
Normal	11 (35.50%)	16 (66.66%)	0.001
Above normal	0 (0%)	4 (16.66%)	0.001
Mean±SD	39.91±24.53	77.12±41.91	< 0.001

P value <0.05 (not significant).

In this study, the normal reference range for males 23-336 ng/ml. In males serum ferritin was low in 64.5% of cases and 16.66% of controls. Serum ferritin levels were normal in 35.50% of cases and 66.66% of controls. Serum ferritin was above normal in 16.66% of control. The mean serum ferritin among cases 39.91±24.53and control 77.12±41.91. P value was <0.05 which is statistically significant.

Table 4: Serum ferritin distribution in females.

Serum ferritin level (ng/ml)	Case group (n=169)	Control group (n=26)	P value
Low	60 (35.50%)	4 (15.38%)	
Normal	109 (64.50%)	16 (61.53%)	0.001
Above normal	0 (0%)	· · · · · · · · · · · · · · · · · · ·	
Mean±SD	29.68±25.52	50.79±35.86	< 0.001

P value < 0.05 (not significant).

In this study, the normal reference range in females was 11-306 ng/ml. In female, serum ferritin was low in 35.5% of cases and 15.38% of controls. Serum ferritin was normal in 64.50% of cases and 61.53% of controls. Serum ferritin was above normal in 23.07% of control. The mean serum ferritin among cases 29.68 ± 25.52 and control 50.79 ± 35.86 . P value <0.05 The result is significant.

Table 5: Distribution of low serum iron and low serum ferritin among cases.

Parameter	Iron low	Iron normal	P value
Ferritin low	30 (15%)	37 (18.50%)	< 0.001
Ferritin	22 (110/)	111	<0.001
normal	22 (11%)	(55.50%)	
Total	52 (26%)	148 (74%)	

P value <0.05 (not significant).

In this study, there were total 67 (33.50%) patient with low serum ferritin and 133 (66.50%) patient with normal serum ferritin. There were 30 (15%) patient who had low serum iron and low serum ferritin and 37 (18.50%) patient with normal serum iron but with low serum ferritin.

Out of 133 (66.50%) patient 22 (11%) patient had low serum iron and 111 had both serum iron and serum ferritin normal. The co-relation coefficient- 0.202. P value <0.05 (significant).

In this study, there were total 8 (16%) patient with low serum ferritin and 32 (64%) patient with normal serum ferritin and 10 (20%) patient with serum ferritin above normal level. There were 1 (2%) patient who had low serum iron and low serum ferritin and 6 (12%) patient with normal serum iron but with low serum ferritin and 1 (2%) patient with low serum ferritin and serum iron above the normal value.

Out of 30 (60%) patient 0 (0%) patient had low serum iron and 30 (60%) had both serum iron and serum ferritin normal and 2 (4%) patient with serum iron above the normal value with normal serum ferritin. The correlation coefficient- 0.038. P value >0.05 (not significant).

Table 6: Distribution of low serum iron and low serum ferritin among control.

Parameter	Iron low	Iron normal	Iron above	P value
Ferritin low	1 (2%)	6 (12%)	1 (2%)	
Ferritin normal	0 (0%)	30 (60%)	2 (4%)	0.876
Ferritin above	10 (20%)	0 (0%)	0 (0%)	
Total	11 (22%)	36 (72%)	3 (6%)	

P value >0.05 (not significant).

In this study, It is observed from above readings that patients who had gallstones in age group of 15-19 years had low serum iron in 1.9% and low serum ferritin in 4.5%, those were in the age group of 20-29 had low serum iron in 17.3% and low serum ferritin in 14.9%, in the age group of 30-39 had low serum iron in 32.7% and low serum ferritin in 26.9%, in the age group of 40-49 had low serum iron in 15.4% and low serum ferritin in 22.4%, in the age group 50-59 had low serum iron in 21.2% and low serum ferritin in 14.9%, and in age group of 60-70 years had low serum iron in 11.5% and low serum ferritin in 16.4%. Maximum number of low serum iron and low serum ferritin case in the age group of 30-39 years. The p value is <0.05 which is significant, indicating a significant prevalence of low serum iron and low serum ferritin in cases in 30-39 years age group.

DISCUSSION

Iron deficiency has been shown to alter the activity of several hepatic enzymes, leading to increased gall bladder's bile cholesterol saturation and promotion of cholesterol crystal formation. Iron acts as a coenzyme for nitric oxide synthetase (NOS), and that is important for the maintenance of basal gall bladder tone and normal relaxation. It was found that iron deficiency resulted in altered motility of gall bladder and sphincter of Oddi, leading to biliary stasis and thus increased cholesterol crystal formation in the gall bladder bile. Hence, iron seems to play a significant role in gallstone pathogenesis. Therefore, regulation of serum iron by ferritin needs to be studied; as ferritin is the most specific marker for iron levels in the body.

At the genetic level the expression of genes controlling ferritin levels viz Iron regulatory protein (IRP-1) might play a significant role in pathogenesis of cholelithiasis. Body ferritin levels, in contrast to haemoglobin, are not affected by residential elevation above sea level or smoking behavior. Therefore, ferritin can more closely reflect relationship with iron deficiency and can be its more specific indicator, thus enabling to assess the relation between gall bladder stone and iron deficiency anaemia.

A study to correlated iron deficiency anemia with gall

stone disease and to estimate the serum ferritin level as a diagnostic tool of iron deficiency anemia in patients with gall stone disease. ¹³ Fifty patients suffering from cholelithiasis, confirmed by ultrasonography were included in the study. 50 healthy volunteers were taken as control group. Serum iron was estimated by the Ferrozine kit method. ¹⁴ The authors concluded that iron deficiency plays a significant role in supersaturation of bile, leading to stone formation. ¹⁵ Iron deficiency probably alters the hepatic enzyme metabolism, causing super saturation of cholesterol in gall bladder bile irrespective of serum cholesterol levels; hence promoting the cholesterol crystal formation. ¹⁶

In this study mean age in the case group (gallstones present) was 41.78±12.81 years (ranging from 15-70 years). In this study to the control group (healthy volunteers) the mean age was 40.02±12.94 years (ranging from 17-60 years). In this study, female prevalence was higher (84.5%) against male (15.5%) population (p value <.05). The peak incidence of gallstone in our study was seen in 30-39 years age group.

In this study, gallstones were more common in female between 30-39 years and there was low prevalence of gallstones in young population. This is because of early symptomatology of gallstones in females. This sex related difference showing more prevalence of cholelithiasis in females could be linked to pregnancy and female sex hormones and also to iron deficiency. In our study, in males the normal reference value was supplied with the kit, for males' normal value was 60-160 µg/dl. Serum iron levels were low in 41.93% of cases and 20.8% of controls. Serum iron was normal in 58.06% of cases and in 79.16% of controls. The mean serum iron among cases 65.45±27.55 and control 65.86±18.55 P value was >0.05 which is not-significant. In female normal reference values was supplied with the kit for females 35-145 µg/dl.23.07% female patients with gallstone disease had serum iron levels below the normal value. There were 23% females in the healthy volunteer group whose serum iron levels were below normal. There were only 76.92% female patients with gallstones whose serum iron levels were normal which was 65.38% in the healthy control group and serum level was above normal in 11.53% of healthy control group. The mean serum iron in female cases was 56.84±25.75 and controls was 69.43±45.82 (P value <0.05).

In 2012, a study conducted by Prasad et al, there were 62% female patients with gallstone disease who had serum iron levels below the normal value (59-158µg/dl). There were 38% females in the healthy volunteer group whose serum iron levels were below normal. There were only 12% female patients with gallstones whose serum iron levels were normal, which was 38% females in the healthy control group. Most of the patients with gallstone disease whose serum iron levels were subnormal were females. ¹⁷ In 2015 a study conducted by Halgaonkar et al, out of 100 patients About 93 patients had decreased

serum iron, whereas only 7 had normal serum iron. None of their patients had increased serum iron levels.¹⁸ In 2014, PK Misra et al conducted a prospective study, all the 100 patients of cholelithiasis were divided into groups A and B based on serum iron levels. The 88 patients fell in group A and 12 patients to group B. The serum iron content of group B patients was significantly lower than group A patients (p<0.005).¹⁹

In this study, in males, serum ferritin was low in 64.5% of cases and 16.66% of controls. Serum ferritin levels were normal in 35.50% of cases and 66.66% of controls and above normal in 16.66% of controls. P value was <0.05 which is statistically significant. The mean serum ferritin levels among male was 39.91±24.53 in cases as compare to 77.12±41.91 in control. In this study, in females, serum ferritin was low in 35.5% of cases and 15.38% of controls. Serum ferritin was normal in 64.50% of cases and 61.53% of controls and above normal in 23.07% of controls. The mean serum ferritin among female cases 29.68±25.52 as compare to 50.79±35.86 in control. P value was <0.05 which is statistically significant

In 1987, Farida Agha et al conducted a study to estimate the serum ferritin levels in normal individuals using radioimmunoassay techniques. They concluded that serum iron levels are low in gall stone patients it is expected that serum ferritin levels will also be on lower side. Ferritin levels are lower in female population and the demography of gall stone disease suggests that it is more prevalent in female population. So, serum ferritin and its regulation by iron regulator protein, understandably, will affect gall stone formation.²⁰

In this study, the mean serum iron among cases was 58.17±26.15 as compared to 67.72±35.15 in control (P value <0.05) And mean serum ferritin among cases was 31.26±25.58 as compared to 63.96±40.74 in control. This study suggests that iron deficiency anemia plays a significant role in the super- saturation of gallbladder bile with cholesterol and may act as an independent factor in formation of cholesterol gallstones. Defective hepatic cholesterol metabolism and stasis of bile because of decreased motility of the gall bladder can lead to more precipitation of cholesterol and hence formation of stone. The increased prevalence of gall stone formation in females could be attributed to the fact, that anemia is more common in females than in males.

The study was concluded that gallstones are more prevalent in female population than males in ratio of 5.4:1. Serum iron in males was low in 41.93% not comparable 20.8% of control suggesting that low serum iron is not associated with Cholelithiasis in male. In males, serum ferritin was low in 64.5% of cases and 16.66% of controls. Serum ferritin levels were normal in 35.50% of cases and 66.66% of controls and above normal in 16.66% of controls suggesting that low serum ferritin is associated with gall stones in males. In this

study, low serum iron was seen in 23.07% of females comparable to 23% low serum iron in control females and low ferritin was seen in 35.50% of female cases as compare to 15.38% of control suggesting that a low body store of serum is a risk factor for Cholelithiasis in females. The age group most commonly involved with low serum iron was 30-39 years showing low serum iron in 32.7% and low serum ferritin in 26.9% suggesting that females with age group 30-50 are most commonly involved and they are also associated with low total body iron store suggesting increased risk of cholelithiasis in this age group because of low body stores of iron. It is that every patient with gallstones above 30 years should be screened for serum iron, serum ferritin may be used as marker of iron store so that low serum iron status could be diagnosed at early stage and progression to severe iron deficiency can be prevented.

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