**ORIGINAL RESEARCH**

# Comparative Analysis of Serum Iron Levels in Gall Bladder Stone Disease Patients and Healthy Individuals at a Tertiary Care Hospital

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## ABSTRACT

**Introduction:** Cholelithiasis is a common abdominal entity which results in increased hospital admissions. About 10-12% of adults have the incidence of developing gallstones. The prevalence of common bile duct stones in patients with gallstones varies from 8 to16%. Pure cholesterol stones are not frequent, and they account for about less than 10% of all stones. Gallstones are generally classified into pure cholesterol stones, black or brown pigmented stones and/or mixed stones. **Materials and Methodology:** The study population was basically divided into two groups. One was study group with 210 patients reported with gall stones disease and fulfilling all our inclusion criteria. The second one was control group with 55 patients without any gallstone disease admitted in surgery ward. Serum iron and ferritin contents of both groups was analysed and were compared with each other. Inclusion criteria followed in control group were age group of 15 – 70 years not suffering from gall stone disease which was confirmed by ultrasonography and for study group All patients with cholelithiasis which were confirmed by ultrasonography with age group of 15 - 70 years. Detailed history was taken from all the patients who were suffering from gall stone disease. Routine investigations were included such as ultrasound of abdomen was done in all the patients. 4 mL venous blood sample was taken for the evaluation of serum iron and serum ferritin level.**Results:** There were more females in cases group 175 (84.5%) than control group 20 (48%) and males in case were (35) 15.5% and (30) 48% in control. 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. In this study in males, the normal male reference value supplied with the kit was 60-160 µg/dl. Serum iron levels were low in 42.85% of cases and 23.3% of controls. Serum iron was normal in 57.14% of cases and in 76.6% of controls. The mean serum iron among cases 66.12±28.35 and control 66.23±19.53. P value was >0.05 which is not-significance. Serum iron level in females was low in 24% of cases and 35% of controls. Serum iron was normal in 74.28% cases and 65% of controls. The mean serum iron in female cases 58.32±26.32 and control was 69.27±19.66. P value was <0.05 which is significant statistically. **Conclusion:** From this study, it was concluded that a low storage of serum iron in the body is a potential risk factor for cholelithiasis in females. Serum iron, serum ferritin may be used as markers of iron store so that low serum iron status could be diagnosed at a much earlier stage.

**Keywords:** Cholelithiasis, Gall Bladder Stones, Cholesterol Crystals, Ferritin.



## INTRODUCTION

Cholelithiasis is a common abdominal entity which results in increased hospital admissions. About 10-12% of adults have the incidence of developing gallstones.[1] The prevalence of common bile duct stones in patients with gallstones varies from 8 to16%.[2] Pure cholesterol stones are not frequent,and they account for about less than 10% of all stones. The most common primary sequence in the formation of cholesterol stone is saturation of bile with cholesterol. Super saturation is almost always caused by increased secretion of cholesterol rather than reduced secretion of phospholipids or bile salts.[3] Pigmented stones contain less than 20% cholesterol and are dark due to the presence of calcium bilirubinate. Black pigment stones are usually small, brittle, black and sometimes spiculated. They are usually formed by the super saturation of calcium

bilrubinate, carbonate and phosphate and occur most often secondary to haemolytic disorder such as hereditary spherocytosis and sickle cell anemia. Same like other cholesterol stones, they are almost always found in gall bladder. Brown pigment stones are usually less than 1 cm in diameter which are brownish-yellow and soft and often mushy. These may form mostly in gallbladder or in bile ducts, usually secondary to superimposed bacterial infection caused by bile stasis. Precipitated calcium bilirubinate and bacterial cell bodies maycontributethe major part of the stone.[3]

The first reported gallstones dated back to the 21st Egyptian dynasty which was discovered in the mummified priestess of Amenen (1085–945 BC). Gallstones are generally classified into pure cholesterol stones, black or brown pigmented stones and/or mixed stones. Situations that favour the formation of cholesterol gallstones are super saturation of bile with cholesterol, presence of cholesterol crystals in the gall bladder long enough to aggregate into stone. Recent studies have predetermined the role of trace elements (Fe, Ca, Zn and Cu) and defective pH in the formation of gallstones.[4,5]The role of iron in the pathogenesis of gallstone disease has not been well documented so far. Deficiency of iron has been proven to alter the activity of several hepatic enzymes, leading to increased cholesterol saturation of bile in gall bladder and resulting in the cholesterol crystallization.[4] Serum iron, total iron binding capacity (TIBC) and transferrin saturation are not the good indicators of status of iron in an individual. In infection free situation, serum ferritin is the ideal indicator for diagnosis of iron deficiency and response to iron supplementation in a community.[5]Serum ferritin level along with the level of haemoglobin directly correlate with the prevalence of iron deficiency in a population or in a community.[6] Hence this study was planned to study the correlation between serum iron and serum ferritin in patients suffering from gallstone disease.

## MATERIALS AND METHODOLOGY

The study population was basically divided into two groups. One was study group with 210 patients reported with gall stones diseaseand fulfilling all our inclusion criteria that we followed for the study. The second one was control group with 55 patients without any gallstone disease admitted in surgery ward. Serum iron and ferritin contents of both groups wasanalysed and were compared with each other. Inclusion criteria followedin control group were age group of 15 – 70 years not suffering from gall stone disease which was confirmed by ultrasonography and for study group All patients with cholelithiasis which were confirmed by ultrasonography with age group of 15 - 70 years. Exclusion criteria includepatients taking iron supplementation for anaemia, Previous case of biliary tract surgery. Detailed history was taken from all the patients who were suffering from gall stone disease. Routine investigations were included such as ultrasound of abdomen was done in all the patients. 4 mL venous blood sample was taken for the evaluation of serum iron and serum ferritin level.

## RESULTS

As planned, the study population was divided into two groups. Group A consisted of 210 patients with reported gallstone disease fulfilling the inclusion criteria. Group B consisted of 55 patients with age group ranged between 15–70 years who were admitted in in-patient ward and did not have gallstone when examined on ultrasonography. There were more females in cases group 175 (84.5%) than control group 20 (48%) and males in case were (35) 15.5% and (30) 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.

In this study in males, the normal male reference value supplied with the kit was 60-160 µg/dl. Serum iron levels were low in 42.85% of cases and 23.3% of controls. Serum iron was normal in 57.14% of cases and in 76.6% of controls. The mean serum iron among cases 66.12±28.35 and control 66.23±19.53. P value was >0.05 which is not-significance which was depicted in Table – 1.

In our study, the normal reference values were supplied with the kit for females was taken as 35-145 µg/dl. Table – 2 shows that the serum iron level in females was low in 24% of cases and 35% of controls. Serum iron was normal in 74.28% cases and 65% of controls. The mean serum iron in female cases 58.32±26.32 and control was 69.27±19.66. P value was <0.05 which is significant statistically. (Table - 2)

In this study, the normal reference range for males 23-336 ng/ml. In males, serum ferritin was low in 62.8% of cases and 16.66% of controls. Serum ferritin levels were normal in 37.14% of cases and 66.66% of controls. Serum ferritin was above normal in 16.66% in control group. The mean serum ferritin among cases 49.21±23.22 and control 78.12±42.53. P value was <0.05 which is also statistically significant as shown in Table – 3.

In this study, the normal reference range in females was 11-306 ng/ml. In female, serum ferritin was low in 35.4% of cases and 20% of controls. Serum ferritin was normal in 64.57% of cases and 65% of controls. Serum ferritin was above normal in 15% among control group. The mean serum ferritin among cases 29.33±25.82 and control 51.13±36.33. P value<0.05 and the result shows statistically significant data as tabulated in Table – 4.

**Table 1: Serum iron distribution in males.**

| Serum iron level (µg/dl) | Study group (n=35) | Control group (n=30) | P - value |
|---|---|---|---|
| **Low** | 15(42.85%) | 7 (23.3%) | 0.762 |
| **Normal** | 20 (57.14%) | 23 (76.6%) | |
| **Above normal** | 0 | 0 | |
| **Mean±SD** | 66.12±28.35 | 66.23±19.53 | 0.982 |

**Table 2: Serum iron distribution in females.**

| Serum iron level (µg/dl) | Study group (n=175) | Control group (n=20) | P - value |
|---|---|---|---|
| **Low** | 42(24%) | 7 (35%) | <0.001 |
| **Normal** | 133 (74.28%) | 13 (65%) | |
| **Above normal** | 0 | 0 | |
| **Mean±SD** | 58.32±26.32 | 69.27±19.66 | 0.052 |

**Table 3: Serum ferritin distribution in males.**

| Serum ferritin level (µg/dl) | Study group (n=35) | Control group (n=30) | P - value |
|---|---|---|---|
| **Low** | 22(62.8%) | 5 (16.6%) | 0.001 |
| **Normal** | 13 (37.14%) | 20 (66.66%) | |
| **Above normal** | 0 | 5 (16.6%) | |
| **Mean±SD** | 49.21±23.22 | 78.12±42.53 | <0.001 |

**Table 4: Serum ferritin distribution in females.**

| Serum iron level (µg/dl) | Study group (n=175) | Control group (n=20) | P - value |
|---|---|---|---|
| **Low** | 62(35.4%) | 4 (20%) | 0.001 |
| **Normal** | 113 (64.57%) | 13 (65%) | |
| **Above normal** | 0 | 3(15%) | |
| **Mean±SD** | 29.33±25.82 | 51.13±36.33 | <0.001 |

**Table 5: Distribution of low serum iron and low serum ferritin among study group.**

| Parameters | Iron low | Iron normal | P - value |
|---|---|---|---|
| **Ferritin low** | 35(63.6%) | 41 (26.4%) | <0.001 |
| **Ferritin normal** | 20 (36.3%) | 114 (73.54%) | |
| **Total** | 55 (26.19%) | 155(73.80%) | |

P value <0.05 (not significant)

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

| Parameters | Iron low | Iron normal | P - value |
|---|---|---|---|
| **Ferritin low** | 1 | 7 | 0.882 |
| **Ferritin normal** | 0 | 30 | |
| **Ferritin high** | 12 | 0 | |
| **Total** | 13 | 37 | |

## DISCUSSION

Iron deficiency has been proved to change the activity of several hepatic enzymes which leads to increased saturation of gall bladder's bile cholesterol and in turn high chances of promoting the cholesterol crystal formation. Iron acts as a coenzyme for nitric oxide synthetase (NOS) and that is necessary for the maintenance of gall bladder tone and its normal relaxation.[7] It was found that iron deficiency resulted in the changed motility of gall bladder and sphincter of Oddi which leads to biliary stasis and thus increased cholesterol crystal formation in the gall bladder bile.[8]Therefore, iron seems to play a major role in the pathogenesis of gallstones. So, the regulation of serum iron by ferritin needs to be further studied as ferritin is the most specific marker for iron levels in the body.

Considering at the genetic level, the expression of genes controlling ferritin levels viz Iron regulatory protein (IRP-1) was thought to play a major role in pathogenesis of cholelithiasis. Body ferritin levels, in contrast to haemoglobin, are not affected by elevation above sea level or chronic smoking behavior.[9] Therefore, ferritin can more intimately reflect the relationship with iron deficiency and can be considered as the specific indicator thus enabling to assess the relation between gall bladder stone and iron deficiency anaemia. A study was conducted to correlate iron deficiency anaemia with gall stone disease and to evaluate the serum ferritin level as a diagnostic tool in iron deficiency anaemia in patients

with gall stone disease.[10] Fifty patients reported with cholelithiasis which was 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 in the study.[11] Thusthe authors have concluded that the iron deficiency plays a significant role in the supersaturation of bile whichresulted in thegall stone formation.[12] Iron deficiency was thoughtto alter the hepatic enzyme metabolism, causing super saturation of cholesterol in gall bladder bile irrespective of serum cholesterol levels; hence promoting the crystalline formation of cholesterol.[13]

In this study, the mean age in the case group (gallstones present) was 41.78±12.81 years (ranging from 15-70 years). The mean age in the participants in control group (healthy volunteers) was ranged 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 0.05 which is not significant. The normal range of serum iron levels in females 35-145 µg/dl.24% female patients with gallstone disease had serum iron levels below the normal value. The control group with 35% females had serum iron levels which were below normal. There were only 74.28% female patients with gallstones whose serum iron levels were normal which was 65% in the healthy control group. The mean serum iron in female cases was 58.32±26.32 and controls was 69.27±19.66 (P value <0.052).

In 2012, Prasad et al evaluated in his study that, there were 62% of female patients with gallstone disease identified with lower serum iron levels when compared to the normal value which ranges from 59-158 µg/dl. There were 38% females reported 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 normalwhich was observed with 38% females in the healthy control group. Most of the patients with gallstone disease whose serum iron levels were subnormal were females.[14] In 2015 a study conducted by Halgaonkar et al, 93 out of 100 patients had reported to have decreased serum iron, whereas only 7 had normal serum iron. None of their patients had increased serum iron levels.15 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).[16]

## CONCLUSION

From this study, it was concluded that a low storage of serum iron in the body is a potential risk factor for cholelithiasis in females.Serum iron, serum ferritin may be used as markers of iron store so that low serum iron status could be diagnosed at a much earlier stage.

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