

## ORIGINAL ARTICLE

# Low Serum Iron as a Potential Risk Factor for Gallstone Formation: A Cross-Sectional Analysis

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## ABSTRACT

**Introduction:** Gallstone disease is a prevalent gastrointestinal issue. Traditionally, it is associated with the "four Fs": fat, fertile, flatulent, female, and forty. However, it also affects men and thin, underweight women, particularly post-partum. Cholesterol gallstone formation is influenced by bile supersaturation with cholesterol, favorable nucleation kinetics, and prolonged presence of cholesterol crystals in the gallbladder. Recent studies suggest that trace elements (iron, calcium, zinc, copper) and pH abnormalities play roles in gallstone formation. **Methods:** This prospective observational study, conducted at a tertiary care center in coastal Karnataka between 2016 and 2018, aimed to assess serum iron levels in patients with ultrasonography-confirmed cholelithiasis and to evaluate serum ferritin's role in diagnosing iron deficiency anemia. **Results:** Among 113 patients, 48% were male and 52% were female, predominantly aged 41–50 years. Serum iron levels were low in 60.2% of patients, normal in 36.3%, and high in 3.5%. Fisher's exact test assessing the overall categorical association between serum iron and ferritin was non-significant ( $p = 0.128$ ). However, Kruskal–Wallis analysis showed significant differences in median ferritin across iron categories ( $p = 0.042$ ), and post-hoc pairwise comparisons demonstrated that patients with low ferritin had significantly lower serum iron than those with normal or high ferritin. **Conclusion:** Serum iron levels were frequently low in this cohort of gallstone patients, while serum ferritin showed limited utility as a standalone diagnostic marker for iron deficiency in this group. Further prospective, controlled studies including inflammatory markers and controls are recommended to validate these findings.

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have twice the risk of men, attributed to estrogen's effect on biliary cholesterol secretion. Ethnic differences, such as a 50–75% prevalence in Native Americans, highlight genetic and environmental factors.

## INTRODUCTION

The gallbladder is a bile-storing organ beneath the right lobe of the liver; it concentrates bile and aids digestion (1). The cystic artery, often branching from the right hepatic artery within Calot's triangle, provides its blood supply, while venous drainage connects to the portal vein. Lymphatic drainage occurs through nodes in the lesser omentum, celiac nodes, and others. Gallbladder contraction is regulated by cholecystokinin and parasympathetic innervation (2). Gallstones are categorized as cholesterol or pigment stones. Cholesterol stones result from crystallized bile cholesterol, while pigment stones derive from calcium bilirubinate salts, often due to chronic hemolysis, liver disease, or biliary infections. Gallstone prevalence increases with age, reaching up to 30% in individuals over 80 years. Women

Risk factors for cholesterol stones include obesity, rapid weight loss, hormonal influences (pregnancy, oral contraceptives), and family history. Pigment stones are associated with chronic hemolysis, gastrointestinal disorders like ileal disease, and infections. Conditions like spinal cord injury and pregnancy reduce gallbladder motility, leading to bile stasis and stone formation (3).

Bilirubin, an end-product of hemoglobin degradation, plays a role in pigment gallstone formation. It is derived from aged red blood cells broken down by macrophages, processed in the liver, and excreted in bile. Bacterial activity in the intestine converts bilirubin glucuronides to urobilinogens, excreted in feces and urine. Excessive bilirubin production, such as in hemolytic anemia, increases pigment gallstone formation (4).

Iron deficiency impacts gallstone formation through several mechanisms. Iron is vital for hemoglobin synthesis and enzymatic functions, absorbed in the stomach and duodenum as ferrous iron (Fe<sup>2+</sup>). It binds to transferrin for transport and is stored as ferritin or hemosiderin in the liver, spleen, and bone marrow. Iron deficiency alters hepatic enzymes that regulate cholesterol and bile salt secretion, impairing gallbladder motility by reducing nitric oxide production. This stasis fosters cholesterol crystallization.

Transferrin, the iron-transport protein encoded on chromosome 3, binds ferric ions (Fe<sup>3+</sup>) and increases during iron deficiency to optimize transport. Hypotransferrinemia (transferrin levels <10 mg/dL) leads to iron deficiency anemia and organ iron deposition, requiring plasma transferrin replacement therapy. Ferritin, an intracellular storage protein, binds up to 4500 iron atoms and acts as an acute phase reactant during inflammation. Its ferroxidase activity facilitates iron uptake, with PCBP1 aiding cytosolic iron delivery. Iron deficiency affects hepatic cholesterol metabolism through impaired enzymatic activity and disrupted sterol metabolism, involving HMG-CoA reductase and cholesterol-7 $\alpha$ -hydroxylase. Genetic regulation by iron regulatory proteins and reduced biliary transferrin levels further influence cholesterol stone formation. Studies show iron deficiency correlates with increased pigment and cholesterol gallstone prevalence. Additionally, metabolic dysfunctions, such as decreased DNA synthesis and enzymatic activities, are linked to iron deficiency (5,6).

Gallstone disease highlights the complex interplay of nutritional, hormonal, genetic, and metabolic factors. Addressing iron deficiency through improved dietary intake and supplementation is critical, especially in regions where nutritional anemia remains a public health concern. Further research on serum ferritin and transferrin regulation may enhance understanding and management of gallstone disease.

**MATERIALS AND METHODS**

This study aimed to evaluate serum iron and ferritin levels as diagnostic tools in patients with iron deficiency anaemia. It was a prospective observational study conducted at a tertiary care centre in coastal Karnataka. The study period was 2016–2018. The calculated minimum sample size was 100 (95% confidence interval, 80% power, OR = 2); 113 eligible patients were enrolled during the study period, exceeding the minimum calculated sample size to improve study power. The objectives of the study were to estimate serum iron levels in gallstone disease and to estimate serum ferritin levels as a diagnostic tool for iron deficiency anaemia in patients with gallstone disease. Patients who gave consent for the study and those diagnosed as having cholelithiasis on ultrasonography

were included in this study. Those patients who had liver cirrhosis, Crohn’s disease, long-term NSAID use, pregnancy, or drugs linked to gallstone formation were excluded from the study. Serum iron was measured by the colorimetric method (reported as  $\mu\text{g/dL}$ ; normal range 60–150  $\mu\text{g/dL}$ ), while ferritin levels were measured by electrochemiluminescence immunoassay (reported as ng/mL; normal range 15–240 ng/mL). Statistical analysis used SPSS v22 after initial data organization in Excel. Ethical clearance was obtained from the Institutional Ethics Committee (INST.EC/EC/151/2016-17). A healthy control group was not included due to the time-bound, hospital-based nature of the study and logistical/ethical constraints on sampling community volunteers within the approved protocol. We recommend that future studies include community or hospital-based controls to enable comparative inference.

**RESULTS**

A total of 113 patients were included. Age distribution: 16 patients (14.2%) <30 years; 17 (15.0%) 31–40 years; 30 (26.5%) 41–50 years; 21 (18.6%) 51–60 years; 21 (18.6%) 61–70 years; 8 (7.1%) >70 years (Table I). Thus, the highest prevalence was in the 41–50-year group. Among the 113 patients, 54 (48%) were males and 59 (52%) were females.

Serum iron levels were low in 27/54 males and 41/59 females. They were normal in 23/54 males and 18/59 females, and high in 4/54 males and 0/59 females (Fig. 1).

Overall serum iron levels were: low 68/113 (60.2%), normal 41/113 (36.3%), and high 4/113 (3.5%). Serum ferritin levels: normal 78/113 (69.0%), low 13/113 (11.5%), and high 22/113 (19.5%) (Fig. 2).

Among patients with low ferritin, 92.3% had low iron and 7.7% had normal iron. Among those with normal ferritin, 56.4% had low iron, 39.7% normal iron, and 3.8% high iron. Among those with high ferritin, 60.2% had low iron, 36.3% normal iron, and 3.5% high iron (Table I).

**Table I: Comparison of Serum Ferritin and Serum Iron levels**

		Iron ( $\mu\text{g/dl}$ )			Total
		Low	Normal	High	
Ferritin (ng/ml)	Low	12 92.3%	1 7.7%	0 0%	13 100%
	Normal	44 56.4%	31 39.7%	3 3.8%	78 100%
	High	12 54.5%	9 40.9%	1 4.5%	22 100%
Total		68 60.2%	41 36.3%	4 3.5%	113 100%

Fisher’s exact test p = 0.128, NS

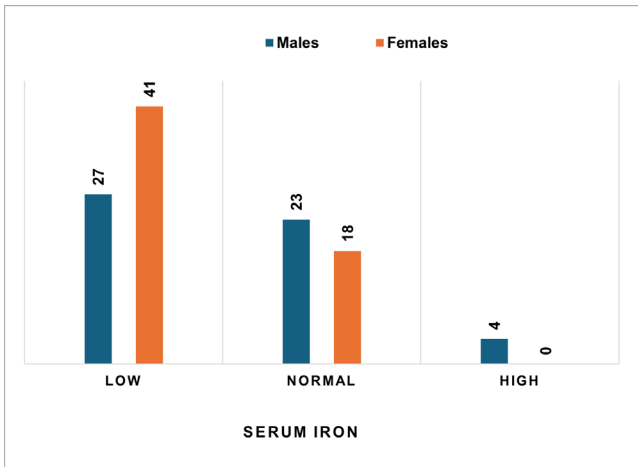


Figure 1: Serum iron levels among Males and Females (Low/Normal/High categories)

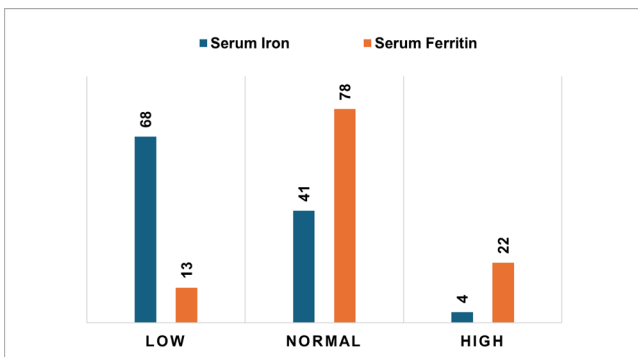


Figure 2: Distribution of serum iron and serum ferritin levels (percentage by category)

Fisher’s exact test was used to assess the overall categorical association between iron status (low/normal/high) and ferritin category (low/normal/high); this test was non-significant ( $p = 0.128$ ), indicating no overall categorical association. Because iron and ferritin values were non-normally distributed, Kruskal–Wallis analysis was performed to compare median ferritin across iron categories and showed a significant difference ( $p = 0.042$ ) (Table II). Post-hoc pairwise comparisons demonstrated that the significant difference was driven by lower median ferritin in the low-iron group compared with the normal-iron group (post-hoc  $p = 0.031$ ) (Table III). These results indicate that although the overall categorical

Table III: Post HOC analysis

Iron ( $\mu\text{g/dl}$ )	Ferritin (ng/ml)	
	Mann Whitney Test p Value	Significance
Low vs Normal	0.031	S
Low vs High	0.038	S
Normal vs High	0.984	NS

association was not significant, subset analysis revealed that low ferritin was associated with lower iron values. Median ferritin by iron category: low-iron group median 70.35 ng/mL; normal-iron 91.28 ng/mL; high-iron 99.82 ng/mL.

ROC analysis (Fig. 3) for serum iron to identify low ferritin showed AUC = 0.802, suggesting moderate discriminative ability. In this analysis a serum iron cut-off of 27.5  $\mu\text{g/dL}$  had sensitivity 69.2% and specificity 71.0% for identifying low ferritin.

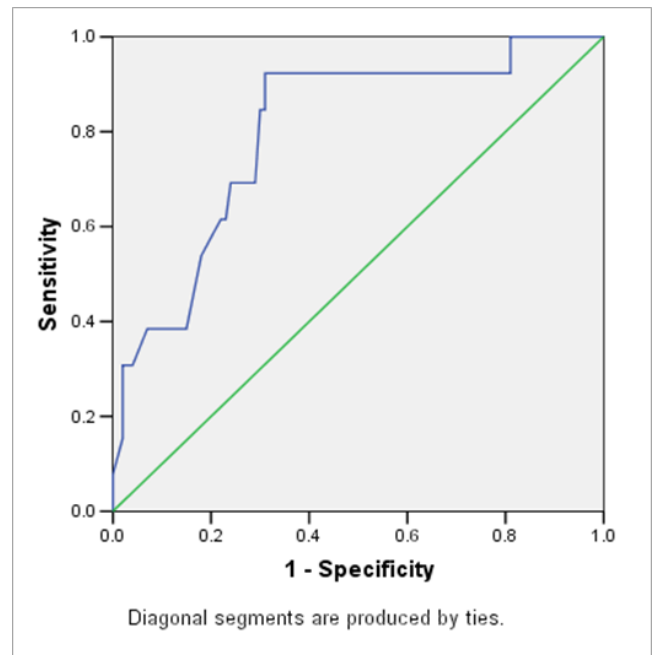


Figure 3: ROC Curve with area under the curve (AUC = 0.802, cut-off = 27.5  $\mu\text{g/dL}$ ; sensitivity 69.2%, specificity 71.0%). AUC indicates moderate discriminative ability ( $p < 0.05$ )

Table II: Kruskal-Wallis Analysis Serum ferritin and Serum iron levels

Iron ( $\mu\text{g/dl}$ )	N	Ferritin (ng/ml)					Kruskal-Wallis Test p Value
		Mean	Standard Deviation	Median	Inter Quartile Range		
Low	67	125.14	162.276	70.35	33.82-160.5	0.042 Significant	
Normal	39	154.35	144.11	91.28	65.34-200.3		
High	4	192.06	223.71	99.82	53.5-422.8		

## DISCUSSION

In this prospective (descriptive), observational study, the serum iron and serum ferritin levels of patients diagnosed with gallstones were estimated.

Gender wise distribution of the 113 patients included in the study showed 59 females and 54 males. Serum iron was found to be low in 69.5% of females and 50% of males. The serum ferritin levels were low in only 13 out of 113 patients. Among the 13 patients with low ferritin, 10 were females and 3 were males. On analysis, most of the patients were found to be between 41 and 50 years of age.

These correspond with the age-old concept that a typical gallstone sufferer is a fat, fertile, flatulent female of forty. This is, however, not true, as studies indicate that the incidence of acute cholecystitis is three to four times greater in young patients (7).

60.2% of patients had low serum iron; only 11.5% had low ferritin. Fisher's exact test was non-significant overall ( $p = 0.128$ ), but non-parametric and pairwise analyses indicated that patients with low ferritin had significantly lower median serum iron.

Fisher's test addresses overall categorical association; its non-significant result indicates that across all categories there was no global association. However, the Kruskal–Wallis and post-hoc tests, which compare medians and pairwise differences respectively, revealed a specific association between low ferritin and low iron that Fisher's test did not detect. This distinction has been clarified in the text and abstract.

An AUC of  $\sim 0.80$  for serum iron suggests moderate discriminative ability for identifying low ferritin in this cohort. While promising, this finding should be interpreted cautiously — ROC-derived cut-offs require validation in larger, prospective case-control studies (including inflammatory markers) before clinical adoption.

Low serum iron was more frequent among females ( $\sim 69.5\%$ ) than males (50.0%). This likely reflects higher iron requirements and menstrual blood loss in women, as well as possible hormonal influences (e.g., pregnancy history, estrogen effects on biliary physiology) and dietary differences. These mechanisms are discussed and supported by prior literature.

Various studies indicate that low serum iron levels alter the activity of several hepatic enzymes and cause increased gallbladder cholesterol saturation, thus promoting the formation of cholesterol crystals (8).

There are various reasons proposed for the formation of gallstones in patients having deficiency of iron, although

the exact mechanism remains unclear. Mechanisms involved include alteration in hepatic enzymes, altered motility of the gallbladder, reduced biliary transferrin, metabolic dysfunction, alteration in the hepatic sterol metabolism and also abnormalities in the genes that control the serum iron and ferritin level (1) (9).

Various studies show an increased number of female patients with iron deficiency anemia having gallstones. Studies claim that one of the reasons for this could be the effect of estrogen and/or progesterone on the saturation of bile in the gallbladder (10) (11).

The gallbladder emptying and hepatic enzyme metabolism are impaired in patients with iron deficiency (12). Iron is an important component of co-factors, which are a part of the nitric oxide synthase complex (13). Nitric Oxide acts as a putative inhibitory neurotransmitter and is present all over the gastrointestinal tract (14). A decrease in the iron levels leads to reduced neuronal nitric oxide synthase levels. This results in abnormal gall bladder motility and dysfunction of the sphincter of Oddi. Compensatory mechanisms occur to return neuronal NOS levels to their baseline but over some time it leads to cholesterol crystal formation (15).

In this study, we noted that serum iron was significantly low in patients with gallstones. Serum ferritin levels, on the other hand, were mostly normal, with only 11.50% of patients having low ferritin levels.

This study has several important limitations. First, its cross-sectional design prevents any causal inference between serum iron levels and gallstone formation, limiting the ability to assess temporal or predictive relationships. Second, the absence of a healthy control group makes it difficult to determine whether the observed prevalence of low iron is specific to gallstone patients or reflective of the general population in this region. Third, this cross-sectional study cannot establish causation or temporality. The absence of a healthy control group limits assessment of whether observed iron abnormalities are specific to gallstone disease or reflect regional population prevalence. Importantly, inflammatory markers such as CRP, dietary iron intake, menstrual history in women, and possible chronic blood loss were not systematically collected; these factors can influence serum iron and ferritin and may confound our results. Some subgroup analyses had limited sample sizes. Lastly, the small sample size in certain subgroups reduced statistical power for subgroup analyses, further limiting the generalizability of the findings.

## CONCLUSION

Serum iron levels were frequently low in this cohort of patients with gallstone disease, whereas serum ferritin had limited utility as a standalone diagnostic marker for iron deficiency in this group. A low ferritin was associated with low iron in pairwise analysis; however,

normal or elevated ferritin did not reliably reflect iron status in all patients. Serum iron may be a potential indicator of altered iron metabolism in gallstone disease, but further prospective, controlled studies — including CRP and dietary/menstrual data — are needed before clinical recommendations can be made.

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