Al Ameen J Med Sci 2025;18(4):307-313 ● US National Library of Medicine enlisted journal ● ISSN 0974-1143

ORIGINAL ARTICLE CODEN: AAJMBG

Assessing the Hidden Link: Iron Deficiency, Anemia, and Gallstone Formation

Ajinkya V. Kawalkar[1], Girish K. Kullolli[1*], Shailaja Bidri[2], Tejaswini Vallabha[1] and Shruti Sheelin[1]

[1]Department of General Surgery, Shri B.M. Patil Medical College, Hospital & Research Centre, Solapur Road, Vijayapura-586103, Karnataka, India, and [2]Department of Obstetrics and Gynecology, Shri B.M. Patil Medical College, Hospital & Research Centre, Solapur Road, Vijayapura-586103, Karnataka, India

Received: 11th August 2025; ***Accepted:*** 15th September 2025; ***Published:*** 01st October 2025

Abstract: *Background:* Gallstone disease (GSD) is more than just a painful inconvenience; it is a multifactorial hepato-biliary disorder with far-reaching implications. While obesity and dietary factors are well-documented risk factors, the role of iron deficiency and anemia remains shrouded in mystery. This study sheds light on the hidden correlation between these conditions and gallstone formation. *Objectives:* To assess the efficacy of serum hemoglobin, serum iron, and ferritin levels as risk factors for the development of gallstone disease. *Methods:* A cross-sectional study was performed at Shri B.M. Patil Medical College, Hospital & Research Center, B.L.D.E. (Deemed to be University), Vijayapura. A total of 122 patients with diagnosed GSD, as evidenced by radiological findings, were enrolled. Hemoglobin, serum iron, and serum ferritin were assessed and correlated with the prevalence of GSD, age, and gender. Statistical analysis was done by using descriptive and inferential statistics, which were achieved using Pearson's correlation and Chi-square tests. Software used in the analysis was SPSS 24.0 version, and $p<0.05$ was considered a level of significance. *Results:* The findings reveal a striking correlation between gallstone disease and reduced serum iron and hemoglobin levels. A staggering 72.1% of gallstone patients suffered from anemia, while 52.5% exhibited low serum iron levels. Aforementioned data was further solidified by the Statistical analysis, which established a positive association between gallstone formation and iron deficiency ($p < 0.05$). *Conclusion:* This study unearths a critical yet often unheeded factor in GSD pathogenesis, which is iron deficiency. A game-changing system for gallstone prevention can be provided with this study by the identification and management of iron deficiency in at-risk individuals.

Keywords: Gallstone disease, Iron deficiency, Anemia, Serum ferritin, Cholelithiasis, Hepato-biliary disorders, Nitric oxide synthase.

Introduction

GSD is one of the many prevalent and debilitating hepato-biliary conditions worldwide. While lifestyle and genetics are often blamed, an unexpected culprit, iron deficiency, may be lurking in the shadows. Iron plays a vital role in bile metabolism and gallbladder function, and its deficiency may trigger biochemical changes that foster gallstone formation. Iron is stored in the form of ferritin. It is an established concept that iron scarcity alters the action of several hepatic enzymes, raising bile cholesterol concentration and stimulating crystallization. Nitric oxide synthase (NOS), which creates Nitric Oxide (NO), necessitates iron as a cofactor [1].

Nitric oxide (NO) has been shown to be a crucial inhibitory nonadrenergic, noncholinergic (NANC) neurotransmitter in the gastrointestinal tract. Smooth muscle relaxation results from NO produced in response to myenteric plexus nerve stimulation. Neuronal NO synthase (nNOS) in the myenteric plexus is activated to produce NO. The nNOS enzyme in the myenteric plexus is activated to produce NO from arginine. NOS catalyses the oxidation of arginine to NO with stoichiometric production of citrulline using NADPH as an electron donor and five enzyme cofactors, one of which is iron [2].

Smooth muscle relaxation results from NO produced in response to myenteric plexus nerve stimulation. Numerous areas of the GI tract depend on released NO for physiological functions. NO controls the sphincter's muscular tone in the pylorus, anus, sphincter of Oddi, and lower oesophagus [3]. In the past, the old maxim was that the archetypal gallstone patient is a fair, fertile, fat female in her fourth decade of life. Modern medicine considers it to be only partly true, as GSD was concurrently seen in postpartum females and also in thin, underweight people. Females, whilst in pregnancy, if diagnosed with cholelithiasis, should be tried to be managed conservatively with dietary modifications. If it doesn't subside, the symptoms, then laparoscopic cholecystectomy can be performed safely, preferably in the second trimester [4].

In India, about 53% of people have iron deficiency anaemia (IDA). The most frequent nutritional shortfall is iron deficiency, which is over 2.5 times more common whether anaemia is present or not. In developing nations, adolescents and women of reproductive age are more frequently impacted. Adolescent girls and women of reproductive age in underdeveloped countries are more frequently impacted due to heightened iron requirements resulting from monthly blood loss, inadequate nutrition, and limited access to healthcare [5].

Recurrence of gallstones is expected if gallstones are removed without cholecystectomy. Cholelithiasis can cause severe consequences like acute gallstone pancreatitis and gallbladder cancer if proper treatment is not received [6]. Gallbladder contractility is decreased by methyl scopolamine, atropine, and somatostatin. In addition to its cholecystokinetic effects, morphine causes the sphincter of Oddi to spasm.

From the intricate interplay of genetic variables, chronic carbohydrate overnutrition, dietary fibre depletion, and other inadequately characterised environmental influences, such as physical inactivity and infections, Gallstone disease phenotypes probably arise, all of which have been linked to 'westernised' nutrition.

Due to the growth in the incidence of cholelithiasis in both India and Western countries, there is a desperate need for a study that can offer in sight in to the disease's prevalence is desperately needed, the risk factors connected with the condition, so that the population at risk can be detected early and treatment could be initiated, thus reducing morbidity and mortality [7].

The genetic components of gallstone formation have been better understood by using mice species that are vulnerable to cholelithiasis (C57L/J) and mice resistant to cholelithiasis (AKR/J). These mice were used to discover the potential involvement of lithogenic genes ONE and TWO (Lith1 and Lith2) in gallstone development. A key factor in determining hepatic cholesterol hypersecretion is Lith1, which is found on the mouse chromosome. The ATP-binding cassette (ABC) transporters ABCG5 and ABCG8 are highly expressed in the intestine and hepatocyte cells. After combining to create heterodimers, these two proteins are extracted from the endoplasmic reticulum [8-9].

Because of the substantial decrease in bile cholesterol release caused by ABCG5/G8 inactivation, liver and plasma cholesterol levels are extremely sensitive to changes in dietary cholesterol intake. Consequently, this may raise the risk of early coronary heart disease, hypercholesterolaemia, and phytosterolemia. However, the gallbladder's cholesterol level rises due to the overexpression of the ABCG5/G8 protein, which raises the risk of cholesterol crystal precipitation [10].

This study aims to unravel this intriguing correlation, offering new perspectives on GSD prevention.

Material and Methods

This cross-sectional study was conducted at B.L.D.E. Shri B.M. Patil Medical College, Hospital & Research Center, Vijayapura. 122 patients diagnosed with GSD based on ultrasonographic evidence were included. Data was meticulously collected on hemoglobin, serum iron, and ferritin levels. Sample size was calculated in accordance with the study by Punnosse et. al With an anticipated Proportion of Iron deficiency

among gallstone patients of 81.8% [11], the study would require a sample size of 122 patients with a 95% level of confidence and 7% absolute precision.

Formula used:

$$n=\frac{z^2\ p*q}{d^2}$$

Where Z= Z statistic at α level of significance
d^2 = Absolute error
P= Proportion rate
q= 100-p

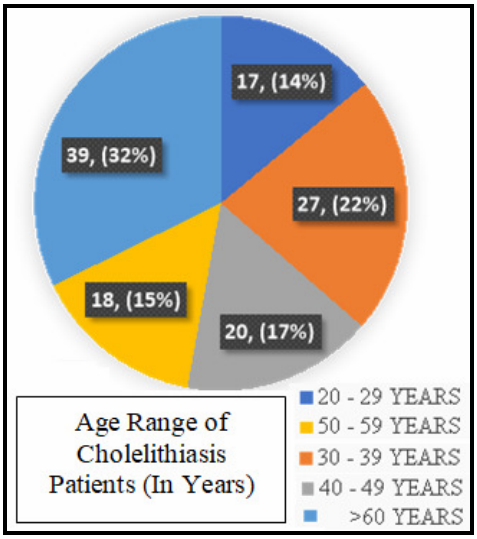
Analysis of statistics, including Pearson's correlation and Chi-square tests, was employed to determine the association between iron deficiency, low hemoglobin levels, and gallstone formation.

They underwent estimation of serum iron and ferritin levels, along with other biochemical parameters. The data obtained were assembled into MS Excel and evaluated statistically using SPSS software (IBM-SPSS Software, India). The principal biochemical parameters used were serum iron and hemoglobin as principal tools, along with serum ferritin and peripheral smear. A pro forma was filled out for personal information, history taking, and clinical examination. This study encompassed patients with symptomatic as well as incidental gallbladder stones. Those patients with a history of iron supplementation in any form and those with haemolytic anaemia, haemosiderosis, cystic fibrosis, or Crohn's disease were excluded. Quantitative parameters were expressed as mean ± Standard deviation. Analysis of statistics was done with the appropriate test. The significance level was set at $P \leq 0.05$, with a high level of significance indicated by $P \leq 0.01$.

Results

Among the 122 patients studied, 88 (72.1%) were diagnosed with anemia, and 64 (52.5%) had low iron levels. The average hemoglobin level was significantly lower in gallstone patients. Pearson's correlation analysis showed a substantial positive correlation between gallstone occurrence and serum iron levels ($p < 0.05$). The results highlight a strong link between iron metabolism and gallstone development, suggesting a previously underestimated risk factor.

Chart-1: Age-wise Distribution of Cases



As illustrated in Chart-1, A total of 122 patients with gallstone disease were analysed. The mean age of presentation was 44.8 ± 13.6 years (range: 21–78 years). The majority of cases were in the >60 years age group (39 cases, 32%), followed by the 30–39 years group (27 cases, 22%) and the 40–49 years group (20 cases, 17%). Patients aged 50–59 years contributed 18 cases (15%), whereas the 20–29 years group accounted for 17 cases (14%).

This distribution highlights that while gallstone disease is predominantly observed in the elderly, a significant proportion of younger adults are also affected, reflecting a shifting trend toward earlier age of onset.

Table-1: Serum iron and hemoglobin frequency

Parameter	Category	Frequency	%
Hemoglobin	Low hemoglobin	88	72.1%
Hemoglobin	Normal	34	27.9%
Serum Iron	Low iron	64	52.5%
Serum Iron	Normal	58	47.5%

Among 122 patients with gallstone disease, nearly three in four (72.1%, n = 88) presented with anemia (low hemoglobin), while only one in four (27.9%, n = 34) had normal hemoglobin levels. In contrast, just over half the cohort (52.5%, n = 64) showed low serum iron, with 47.5% (n = 58) maintaining normal iron status (Table-1).

Table-2. Hemoglobin (Hb) levels and statistical comparison between groups (where 'n'= frequency)					
Group	**n**	**Mean Hb (mg/dL)**	**Std. Deviation**	**Mann-Whitney U**	**p-value (2-tailed)**
Low Hb	64	10.75	±2.26		
Normal	58	11.37	±2.07	1441.000	0.033

The mean hemoglobin level was 10.75 ± 2.26 mg/dL in the low Hb group and 11.37 ± 2.07 mg/dL in the normal group. The Mann-Whitney test (U = 1441.000, p = 0.033) confirmed that this difference was statistically significant (Table-2).

Among patients with low hemoglobin, 58% (51/88) also had low serum iron, while 42% (37/88) had normal iron. In patients with normal hemoglobin, 38% (13/34) had low iron and 62% (21/34) had normal iron (Chart-2)

Chart-2: Correlation of serum iron with hemoglobin

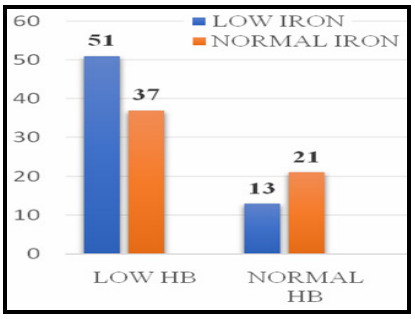


Table-3: Distribution of serum iron as per hemoglobin levels					
Iron * Hb (mg/dL)					
			Hb (mg/dL)		**Total**
			< 12.0	**12.0+**	
Iron	Low	Frequency	46	18	64
		Percentage	60.5%	39.1%	52.5%
	N	Frequency	30	28	58
		Percentage	39.5%	60.9%	47.5%
Total			76	46	122
			100.0%	100.0%	100.0%
Chi-Square Tests					
			Value	**Asymptotic Significance (2-sided)**	
Pearson Chi-Square			5.260	.022	

From the data in Table 3, we can observe that among individuals with low iron status, 60.5% (46/76) had Hb levels below 12.0 mg/dL, whereas only 39.1% (18/46) had Hb levels equal to or above 12.0 mg/dL. Conversely, in the normal iron group, 60.9% (28/46) of participants had Hb ≥12.0 mg/dL compared to 39.5% (30/76) with Hb <12.0 mg/dL.

The observed differences in the distribution of Hb levels between the iron status groups were statistically significant (Pearson Chi-square = 5.260, p = 0.022), indicating a substantial association between iron deficiency and lower hemoglobin levels.

Chart-3: Distribution of serum iron as per hemoglobin levels.

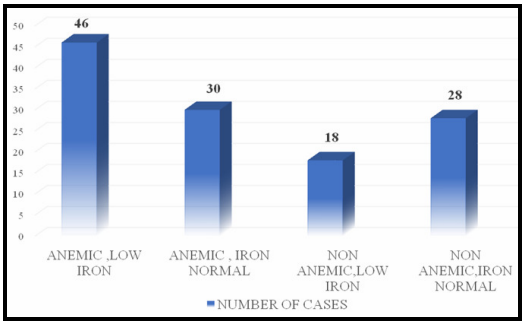


Chart 3 illustrates the distribution of serum iron status stratified by hemoglobin levels.

Most patients (46/122; 37.7%) had both anemia and low iron, confirming iron deficiency as the main cause. Thirty patients (24.6%) were anemic with normal iron, suggesting other causes. Eighteen patients (14.8%) had normal hemoglobin but low iron, likely early deficiency, while 28 (23.0%) had normal values for both. Assessing hemoglobin and iron together thus provides a clearer understanding than either alone. These results underscore the importance of assessing both hemoglobin and serum iron for accurate diagnosis.

Discussion

This study involved a total of 122 patients diagnosed with cholelithiasis by any radiological modality who had visited B.L.D.E. Shri B.M. Patil Medical College, Hospital & Research Center, Vijayapura from the period of 2023 to 2025. At the end of the study. The majority of cases, which were 39(32%), were in the age group above 60 years. The study also found a significant number of cases in younger age groups, like in the 30–39 years group (27 cases, 22%). A mean age of 44.8 ± 13.6 years was observed among the 122 patients with gallstones. This suggests a potential shift toward an earlier age of onset for the disease.

In a study by Pamuk GE, Umit H, Harmandar F, et al. Gallstones become more common as people age, with most cases seen between 40 and 60 years. This is likely because aging affects bile composition and slows gallbladder movement, making stones more likely to form. Iron deficiency anemia seems to worsen this risk by further reducing gallbladder function and altering bile quality, which may even cause stones to appear earlier than expected. Therefore, doctors should be especially attentive to middle-aged and older patients with iron deficiency, as they may be silently developing gallstones [12].

The trend across age groups, a gradual increase from young adults to older adults, is fully consistent with published literature, supporting your discussion points about age as a major risk factor. This study highlights a significant association between iron deficiency, anemia, and gallstone disease (GSD). Among the 122 patients evaluated, 72.1% were anemic and 52.5% had low serum iron levels. A statistically significant difference in mean hemoglobin levels was observed between groups ($p = 0.033$), and Pearson's chi-square analysis confirmed a strong correlation between low hemoglobin and iron deficiency ($p = 0.022$) in cholelithiasis patients. Furthermore, combined analysis revealed that 37.7% of patients had both anemia and iron deficiency, underscoring iron deficiency as a predominant contributor to anemia in GSD.

This study demonstrates that 72.1% of gallstone patients were anemic and 52.5% had iron deficiency. The strong association between low hemoglobin, low iron, and gallstone formation suggests that iron deficiency may play a significant role in gallstone pathogenesis.

Investigational research by Johnston SM, Murray KP, Martin SA, et al. has established that iron deficiency can modify hepatic cholesterol metabolism, thus increasing the cholesterol inundation in bile and promoting gallstone development. In a study involving prairie dogs, those on a diet lacking iron exhibited a higher incidence of cholesterol conglomerations and gallstones compared to those on an iron-supplemented diet. This suggests that iron deficiency may play a previously obscure role in the pathogenesis of cholesterol gallstones [1].

These observations are strongly supported by previous research. Pamuk GE, Umit H, Harmandar F, et al. studied 163 patients with iron deficiency anemia and 200 healthy controls, reporting that gallstones were present in 13.5% of anemic patients compared to 6.2% of controls, a statistically significant difference ($p < 0.05$). They also noted that gallbladder dysmotility was more common among the anemic group, suggesting that impaired emptying of the gallbladder could be one mechanism linking anemia to stone formation [12].

Furthermore, a 2015 study by Prasad PC, Gupta S, Kaushik N, et al compared serum iron and serum ferritin levels amongst healthy individuals and cholelithiasis patients, observing that cholelithiasis patients had lower levels of these markers. The study came to the conclusion that iron deficiency could

lead to supersaturation of bile with cholesterol, thereby enhancing cholelithiasis [14].

In our study, most patients (46/122; 37.7%) had both anemia and low iron, confirming iron deficiency as the main cause. 30(24.6%) patients were anemic with normal iron, suggesting other causes. 18 (14.8%) patients had normal hemoglobin but low iron, likely early deficiency, while 28 (23.0%) had normal values for both. Assessing hemoglobin and iron together thus provides a clearer understanding than either alone.

In a manner comparable, Kshirsagar et al. prospectively examined 120 patients in the general surgery department at Krishna Institute of Medical Sciences, Karad, over a two-year period. Twenty cholelithiasis patients' serum iron and cholesterol levels were compared to those of healthy people. The majority of gallstone patients, the researchers found, had low serum iron levels. Patients with cholelithiasis had serum cholesterol levels that were not appreciably different from those of healthy, normal people. They came to the conclusion that low serum iron causes bile to become supersaturated with cholesterol, which in turn causes gallstones to develop, and that low serum iron correlated strongly with gallstone presence, supporting the notion that correction of iron deficiency may modify disease risk [15].

Evidence from Naik et al.also demonstrated a significantly higher prevalence of iron deficiency anemia among patients undergoing laparoscopic cholecystectomy, suggesting that perioperative screening could influence both surgical outcomes and long-term prognosis [5].

Conclusion

This study offers compelling evidence that iron deficiency and anemia aren't just coincidental findings in cholelithiasis patients but may play a crucial role in disease progression. Iron deficiency leads to increased oxidative stress, bile cholesterol supersaturation, and impaired gallbladder motility, all of which set the stage for GSD. However, the relationship is complex, as evidenced by findings on heme iron intake. The implications of these findings extend beyond GSD, hinting at a broader connection between iron metabolism and hepato-biliary disorders. Supplementary research is warranted in delineating the underlying mechanisms and in exploring probable preventive strategies aiming at iron metabolism in cholelithiasis patients.

Our findings shine a spotlight on iron deficiency and low hemoglobin as hidden players in gallstone disease. Routine screening for iron deficiency and anemia in at-risk populations could revolutionize gallstone prevention strategies. Forthcoming research should probe deeper into the molecular mechanisms at play and explore whether correcting iron levels could serve as a novel therapeutic approach to mitigate gallstone risk.

Acknowledgments

The authors express gratitude to the participants and staff of B.L.D.E.D.U. Shri B.M. Patil Medical College for their invaluable contributions to this research.

Financial Support and sponsorship: Nil

Conflicts of interest: There are no conflicts of interest.

References

1. Johnston SM, Murray KP, Martin SA, et al. Iron deficiency enhances cholesterol gallstone formation. *Surgery*. 1997; 122(2):354-362.
2. Takahashi T. Pathophysiological significance of neuronal nitric oxide synthase in the gastrointestinal tract. *Journal of Gastroenterology.* 2003; 38:421-430.
3. Verma GR, Pandey AK, Bose SM, Prasad R. Study of serum calcium and trace elements in chronic cholelithiasis. *ANZ Journal of Surgery.* 2002; 72(8):596-599.
4. Shaffer EA. Review article: control of gall-bladder motor function. *Aliment Pharmacol Ther.* 2000; 14 Suppl 2:2-8.
5. Naik S, Abuji K, Dahiya D, Sharma P, Das R, Behera A, Kaman L. Prevalence of iron deficiency anaemia in patients of cholelithiasis undergoing laparoscopic cholecystectomy. *International Surgery Journal.* 2022; 9(7):1335-1339.

6. Reshetnyak VI. Concept of the pathogenesis and treatment of cholelithiasis. *World J Hepatol.* 2012; 4(2):18-34.
7. Courtney M, Townsend Jr, Daniel Beauchamp R, Mark Evers B et al. editors. Sabiston Textbook of Surgery: The Biological Basis of Modern Surgical Practice. 19th ed. New York: *Elsevier Health Sciences.* 2012.
8. Wang J, Mitsche MA, Lütjohann D, Cohen JC, Xie XS, Hobbs HH. Relative roles of ABCG5/ABCG8 in liver and intestine [S]. *Journal of lipid research.* 2015; 56(2):319-330.
9. Van Der Bruggen P, Zhang Y, Chaux P, Stroobant V, Panichelli C, Schultz ES et al. Tumor-specific shared antigenic peptides recognized by human T cells. *Immunological Reviews*. 2002; 188(1):51-64.
10. Wang HH, Liu M, Portincasa P, Wang DQ. Recent Advances in the Critical Role of the Sterol Efflux Transporters ABCG5/G8 in Health and Disease. *Adv Exp Med Biol.* 2020; 1276:105-136.
11. Punnoose SK, Kailasanadhan SN. A Study on Association of Iron Deficiency and Gall Stones in Cholelithiasis Patients. *Kerala Surgical Journal.* 2022; 28(1):17-19.
12. Pamuk GE, Umit H, Harmandar F, Yeşil N. Patients with iron deficiency anemia have an increased prevalence of gallstones. *Ann Hematol*. 2009; 88(1):17-20.
13. Prasad PC, Gupta S, Kaushik N. To study serum iron levels in patients of gall bladder stone disease and to compare with healthy individuals. *Indian J Surg*. 2015; 77(1):19-22.
14. Kshirsagar AY, Kabra MV, Reddy M, Panicker S, Phadke A. A study to correlate low serum iron levels with gallstones. *Journal of Evolution of Medical and Dental Sciences.* 2015; 4(104):16912-16915.

Cite this article as: Kawalkar AV, Kullolli GK, Bidri S, Tejaswini V and Sheelin S. Assessing the Hidden Link: Iron Deficiency, Anemia, and Gallstone Formation. *Al Ameen J Med Sci* 2025; 18(4): 307-313.

*All correspondence to: Dr. Girish K. Kullolli, Professor, Department of General Surgery, Shri B.M. Patil Medical College, Hospital & Research Centre, Solapur Road, Vijayapura-586103, Karnataka, India. Email: drgirishkullolli@gmail.com