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A HOSPITAL BASED OBSERVATIONAL STUDY OF ASSOCIATION OF SERUM IRON AND SERUM FERRITIN LEVEL WITH CHOLELITHIASIS

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Abstract

Background: Iron deficiency disrupts liver enzyme activity, leading to increased cholesterol saturation in bile and the formation of cholesterol crystals[1]. As a cofactor for nitric oxide synthase (NOS), iron is essential for maintaining normal gallbladder tone and motility; its deficiency causes biliary stasis, further contributing to gallstone development[2]. Given that ferritin reflects the body's iron status, this study aimed to explore the relationship between serum iron, ferritin levels, and the occurrence of cholelithiasis***. Methods of study***: A hospital-based case-control study was conducted in the Department of General Surgery, Assam Medical College and Hospital, Dibrugarh, over one year. Each group (cases and controls) included 91 participants. Low serum ferritin levels were found in 42.85% of cases and 23.3% of controls, with a 95% confidence interval and 80% study power. ***Results:***The study indicates a strong connection between lower blood iron and serum ferritin levels and the prevalence of cholelithiasis. The serum iron level was substantially lower in patients(49.2±20.65µg/dl) compared to controls(55.81±19.4 µg/dl) with p value of 0.0273. Serum ferritin levels were significantly lower in gallstone patients (28.81 ± 31.81 µg/dl) compared to controls (41.21 ± 23.11 µg/dl) with p-value of 0.003. This indicates that reduced iron and ferritin levels may be associated with the development of gallstones***.Discussion:*** Iron deficiency alters bile composition and reduces bile acid synthesis, promoting cholesterol buildup and gallstone formation. Low ferritin levels in patients suggest a link between iron deficiency and gallstones, though causation is

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unclear. Further studies are needed to confirm this relationship and explore iron's role in prevention and treatment.

***Key word**:* Cholelithiasis; Iron deficiency; Serum ferritin; Serum iron; Bile composition; Nitric oxide synthase (NOS); Gallbladder motility; Cholesterol saturation; Biliary stasis; Case-control study

Introduction

Cholelithiasis, a common abdominal disorder, develops primarily due to cholesterol supersaturation in bile. Recent evidence suggests that iron plays a crucial role in gallstone formation by regulating hepatic enzymes involved in bile acid and cholesterol metabolism[1], as well as maintaining gallbladder motility through nitric oxide synthase (NOS) activity[2]. Iron deficiency impairs these functions, leading to biliary stasis, altered bile composition, and cholesterol crystallization, which promote gallstone development. Studies indicate tindividuals with low iron or ferritin levels are more prone to gallstones, highlighting the importance of assessing iron status in at-risk patients. However, further research—particularly longitudinal and interventional studies—is needed to clarify the causal relationship and evaluate whether correcting iron deficiency can prevent cholelithiasis.

Aim and objectives

The primary aim of the study was to evaluate the relationship between serum iron and ferritin levels with the occurrence of cholelithiasis. Specifically, the objectives were to assess how variations in serum iron and ferritin concentrations are associated with the development of gallstone disease.

Review of Literature

The study by **M. I. Goldblatt et al. (2001)** found that iron deficiency affects liver enzyme activity, causing an increase in bile cholesterol saturation and promoting cholesterol crystalformation in the gallbladder. Iron serves as a cofactor for nitric oxide synthase (NOS)**,** which helps maintain normal gallbladder tone and relaxation. When iron is deficient, gallbladderand sphincter of Oddi motility are impaired, leading to biliary stasis (slowed bile flow) and, consequently, a higher likelihood of cholesterol crystal and gallstone formation[1]**.**

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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 ($p<0.001$)[2]

In the **2015 study by Prasad et al.**, it was found that 62% of female patients with gallstonedisease had low serum iron levels**,** compared to 38% of healthy females. Only 12% ofgallstone patients had normal serum iron levels, whereas 38% of healthy controls did. The study concluded that most gallstone patients with low serum iron levels were females, suggesting a strong link between iron deficiency and gallstone formation in women.[3]

In **2016** a study conducted by **Halgaonkar et al,** out of 100 patients withcholelithiasis about 93 patients had decreased serum iron, whereas only 7 had normal serum iron. None of their patients had increased serum iron levels .[4]

The **2018 study by Kumar, Badyal, and Gupta** found that low serum iron and ferritin levels were present in 18% of gallstone cases, with the highest prevalence in females aged 30–39years ($p<0.05$). This age group showed significantly low body iron stores**,** indicating an increased risk of cholelithiasis**.** The authors recommended screening gallstone patientsover 30 years **for** serum iron and ferritin to detect and manage iron deficiency early.[5]

The **2018 study by Arora and Yadav** found that iron deficiency, reflected by low serumferritin**,** is linked to a higher risk of gallstone disease, especially in females. Among males, 64.5% of cases showed low ferritin, while in females, 35.5% of cases had low ferritin compared to 15.38% of controls. The study concluded that low body iron stores contribute to cholelithiasis, and serum iron and ferritin can serve as useful markers for early detection of iron deficiency.[6]

The **2020 study by WaghMadhukarRajaram and Joshi Sunil**found that 65.7% of gallstone patients had low serum iron levels (<40 μg/dl), with most affected patients being females. Although serum ferritin levels were often normal and not significantly associated due to its rise in inflammatory conditions, the study concluded that low serum iron is significantly linked to a higher risk of cholelithiasis, likely due to bile supersaturation, and that gallstones are more common in females.[7]

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The **2020 study by Patil et al.** found that 30% of cholelithiasis patients had low serumferritin levels**,** which were associated with elevated bile cholesterol. The study concluded that low ferritin, indicating iron deficiency**,** may cause biliary stasis**,** thereby increasing the risk of gallstone formation.[8]

The **2020 study by BandamSriniva Sulu and Raju T. Narayana** found that low serumiron levels were more common in female gallstone patients, showing a significant association ($p<0.05$). The study concluded that low body iron stores increase the risk of cholelithiasisinfemales, and serum iron and ferritin can serve as early markers of iron deficiency.[9]

The **2021 study by Mahana and Singal** found that low serum iron and ferritin levels were significantly more common in cholelithiasis patients than in controls ($p=0.001$), affecting over half of males and most females. The study concluded that iron deficiency is strongly associated with an increased risk of gallstone disease.[10]

The **2022 study by Punnose and Kailasanadhan** showed that 36.9% of gallstonepatients had low serum iron, compared to 7.7% of controls, with a significant association ($p<0.05$). Among those with low iron, 90.5% had gallstones, indicating that iron deficiencyis strongly linked to gallstone disease.[11]

The **2023 study by Vaidehi et al.** found that patients with cholelithiasis had significantlylower serum iron levels (60.74 ± 10.21 μg/dL) than controls (78.29 ± 9.16 μg/dL, $p<0.001$) and showed elevated liver enzymes and ferritin, indicating that iron deficiency and liverdysfunctionare associated with gallstone disease.[12]

Material and methods

*STUDY AREA***:** Department of General Surgery Assam Medical

College and Hospital, Dibrugarh.

STUDY POPULATION: All cases admitted in the General surgery ward with cholelithiasis in Assam Medical College and Hospital, Dibrugarh.

STUDY PERIOD : One Year

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STUDY DESIGN : Hospital based case control study.

SAMPLE SIZE : Taking serum ferritin level as low in 42.85% cases and 23.3% control , confidence interval level 95% and power 80%; Sample size is calculated to be 91 in each group.

Inclusion criteria for case:

- All patients with Cholelithiasis confirmed by ultrasonography

within the age group of 20 - 70 years.

- Who are willing to give consent for the study .
- Patients admitted in General surgery ward .

Exclusion criteria for case:

- Patient aged below 20 years.
- Patients taking iron supplements in any form.
- Previous case of biliary tract surgery.
- Patient of empyema, hematological disorders, cirrhosis of liver, cystic fibrosis, blood dyscrasias, Crohn's disease, familial hyperlipoproteinemia, hyperlipoproteinemia type-3, pyruvate kinase deficiency and pregnant females will also be excluded.

Inclusion criteria forcontrol:

- Patients aged 20 - 70 years.
- Who are willing to give consent for the study
- Patients admitted in General surgery ward who are not suffering from cholelithiasis.

Exclusion criteria for control:

- Patient aged below 20 years.
- Patients taking iron supplements in any form.

Methodology:

1. Ethical clearance was obtained from the Institutional Ethics Committee, and written informed consent was taken from all participants.

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2. Data were collected using a predesigned proforma.
3. Both patients with and without gallstone disease were included in the study.
4. Each participant underwent a detailed history taking, general, local, and systemic examination.
5. Diagnosis was based on clinical findings, laboratory investigations, and confirmed through ultrasonography.
6. A 4 ml venous blood sample was collected in a red vacutainer for serum iron and ferritin estimation.
7. Operation theatre (OT) records and postoperative monitoring notes were reviewed.
8. Serum iron and ferritin levels were analyzed using the **VITROS 5600 Automated Analyzer** in the Biochemistry Laboratory of AMCH.

Results and observations

1. **Age Distribution:** The study included patients aged 20–70 years, excluding those outside this range. The mean age was 42.29 ± 13.14 years in the case group and 42.34 ± 12.79 years in the control group. Gallstones were least common in the youngest and oldest age groups, with the highest prevalence observed in individuals aged 31–40 years.

2. **Gender Distribution :**In this current study, incidence of cholelithiasis was found to be more in females.

3. **Serum iron in male:**In male participants, with a normal serum iron reference range of 37–170 µg/dl, 42.86% of cases and 33.33% of controls had low serum iron, while 57.14% of cases and 66.67% of controls had normal levels. The mean serum iron was 79.05 ± 20.61 µg/dl in cases and 68.67 ± 16.32 µg/dl in controls. The difference was not statistically significant ($p > 0.05$).

4. **Serum iron in female:**In female participants, with a normal serum iron range of 37–170 µg/dl, low serum iron was observed in 25.71% of cases and 20% of controls,

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while normal levels were seen in 74.29% of cases and 80% of controls. The mean serum iron was 40.24 ± 8.96 µg/dl in cases and 56.16 ± 20.33 µg/dl in controls, with the difference being statistically significant (< 0.05).

5. **Serum ferritin in male:** In male participants, with a normal ferritin range of 6.24–137 µg/dl, low serum ferritin was observed in 61.9% of cases and 28.57% of controls, while normal levels were seen in 38.1% of cases and 71.43% of controls. The mean serum ferritin was 8.04 ± 6.57 µg/dl in cases and 47.53 ± 24.54 µg/dl in controls, with the difference being statistically significant (p < 0.05).

6. **Serum ferritin in female:** In female participants, with a normal ferritin range of 6.24–137 µg/dl, low serum ferritin was observed in 35.71% of cases and 14.29% of controls, while normal levels were seen in 64.29% of cases and 85.71% of controls. The mean serum ferritin was 35.04 ± 33.72 µg/dl in cases and 46.32 ± 31.51 µg/dl in controls, with the difference being statistically significant (p < 0.05).

7. **Distribution of Serum Iron and Serum Ferritin among Study Group:** Among the 91 cases, 4 (22.2%) had both serum iron and ferritin below the normal range, 14 (77.8%) had low serum iron but normal ferritin, 35 (48%) had low ferritin with normal serum iron, and 38 (52%) had both values within the normal range. These results indicate that abnormalities in iron or ferritin often occur independently, with only a minority of cases showing deficiencies in both parameters.

Table–1: Distribution of Serum Iron and Serum Ferritin among Study Group

Serum Ferritin *(µg/dl)*		Serum Iron*(µg/dl)*				
		<37		37–170		*p value**
		N	*%*	*N*	*%*	
Low	<6.24	4	22.22	35	47.95	0.048

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Normal	6.24–137	14	77.78	38	52.05	
TOTAL		18	100.00	73	100.00	

n : Number of Cases; % : Percentage

**Fisher Exact Test; The p-value issignificant at 5% level of significance*

8. Distribution of Serum Iron and Serum Ferritin among control Group:

Among the 91 controls, 3 (16.7%) had both serum iron and ferritin below normal, 15 (83.3%) had low serum iron but normal ferritin, 13 (17.8%) had low ferritin with normal serum iron, and 60 (82.2%) had both values within the normal range. Overall, most controls had normal iron and ferritin levels, while deficiencies in either iron or ferritin occurred less frequently and usually in isolation.

Table–2: Distribution of Serum Iron and Serum Ferritin among Control Group

Serum Ferritin *(µg/dl)*		Serum Iron*(µg/dl)*				
		<37		37–170		*p value**
		N	*%*	*N*	*%*	
Low	<6.24	3	16.67	13	17.81	0.909
Normal	6.24–137	15	83.33	60	82.19	
	TOTAL	18	100.00	73	100.00	

n : Number of Cases; % : Percentage

**Fisher Exact Test; The p-value is not significant at 5% level of significance*

9. correlation between serum iron and serum ferritin in Study group:

Below table shows the correlation between serum iron and serum ferritin in Study group . Here *r value* (Pearson correlation coefficient) is – 0.244 . This indicates a weak negative correlation between serum iron and serum ferritin . P value 0.019 indicates that the correlation is statistically significant at 5% level of significance .

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Table–3: Correlation between Serum Iron (µg/dl) and Serum Ferritin (µg/dl) in Study Group

	Serum Iron *(µg/dl)*	
	*r value**	*p value*
Serum Ferritin *(µg/dl)*	–0.244	0.019*
**Pearson correlation; The p-value is significant at 5% level of significance*		

10.correlation between serum iron and serum ferritin in control group:

Belowtable shows the correlation between serum iron and serum ferritin in control group . Here *r value* (Pearson correlation coefficient) is 0. 054 . This indicates a very weak positive correlation between serum iron and serum ferritin . P value 0.611 indicates that the correlation is not statistically significant at 5% level of significance .

Table– 4: Correlation between Serum Iron (µg/dl) and Serum Ferritin (µg/dl) in control Group

	Serum Iron *(µg/dl)*	
	*r value**	*p value*
Serum Ferritin *(µg/dl)*	0.054	0.611
**Pearson correlation; The p-value is not significant at 5% level of significance*		

Discussion

A hospital-based case-control study was conducted to evaluate the association between serum iron and ferritin levels with cholelithiasis. The study included 91 patients with gallstones

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confirmed by ultrasonography and 91 healthy controls. Serum iron and ferritin were analyzed using the VITROS 5600 Automated Analyzer. The findings indicated that iron deficiency alters hepatic enzyme activity, leading to cholesterol supersaturation in bile and increased cholesterol crystal formation, thereby contributing to gallstone formation[1]. Iron also acts as a coenzyme for nitric oxide synthase (NOS), which is essential for maintaining gallbladder tone and motility[2]. Deficiency of iron causes biliary stasis, further promoting gallstone development. Ferritin, as a specific marker of body iron stores, was emphasized for its potential role in understanding the relationship between iron status and gallstone formation.

Participants aged 20–70 years were included in the study, with a mean age of around 42 years in both case and control groups. The highest prevalence of cholelithiasis was observed in the 31–40-year age group, which is consistent with previous studies.Cholelithiasis was found to be more common in females, aligning with earlier findings. In terms of serum iron levels, low serum iron was observed in 42.86% of male cases compared to 33.33% of male controls; however, the difference was not statistically significant ($p > 0.05$). Similar findings were reported in earlier studies. Among females, low serum iron was found in 25.71% of cases compared to 20.00% of controls, and this difference was statistically significant ($p < 0.05$), consistent with the earlier findings. Regarding serum ferritin, 61.90% of male cases had low ferritin compared to 28.57% of male controls, a statistically significant difference ($p < 0.05$), consistent with previous results. Among females, low ferritin was found in 35.71% of cases compared to 14.29% of controls, also a significant difference ($p < 0.05$), similar to findings reported byearlier studies.

The study acknowledges several limitations, including its single-center design, which may limit the generalizability of findings due to regional and genetic variations in iron metabolism and gallstone risk. Potential confounders such as dietary iron intake, subclinical inflammation, liver or kidney disorders, and genetic factors affecting iron regulation could have influenced serum iron and ferritin levels. Additionally, demographic and lifestyle factors like age, sex, smoking, alcohol use, and obesity may also modulate iron status and gallstone formation. Despite these constraints, the study provides valuable insights into the possible link between iron deficiency and cholelithiasis, underscoring the need for larger, multi-center, longitudinal studies to validate and expand upon these findings.

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Conclusion

This study reveals a strong link between low serum iron and ferritin levels and an increased risk of cholelithiasis, suggesting that iron deficiency may contribute to gallstone formation. Iron plays a crucial role in bile metabolism and gallbladder motility; its deficiency can lead to biliary stasis and cholesterol supersaturation—key steps in gallstone development.

Clinically, the findings highlight the value of assessing iron status, especially in high-risk individuals such as premenopausal women, those with anemia, or chronic inflammatory and metabolic conditions. Early detection and correction of iron deficiency through diet or supplementation may help lower gallstone risk, though further research is needed to confirm causality.

Until then, a holistic strategy—encompassing iron monitoring, balanced nutrition, and healthy lifestyle practices—remains the most effective approach to gallstone prevention.

Conflict of interest

There is no conflict to disclose .

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