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Exploring the Relationship Between Serum Micronutrients with Gallstone Disease in Adult Patients

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Abstract: ***Gallstone disease (GSD) is a common hepatobiliary disorder influenced by metabolic, dietary, and biochemical factors. This hospital-based cross-sectional study evaluated the relationship between serum micronutrients and GSD among 65 adult patients attending the Department of General Surgery, Baba Raghav Das Medical College, Gorakhpur, India. Serum calcium, iron, vitamin D, and vitamin B12 levels were measured using standard biochemical and immunoassay techniques. Descriptive statistics and correlation analyses were performed using SPSS version 24.0. The study population was predominantly female (93.85%), with a mean age of 39.80 ± 13.45 years. Hypercalcemia was observed in 44.62% of patients, while 55.38% had low serum iron levels. Vitamin D deficiency was present in 63.08% of participants, and vitamin B12 deficiency was detected in 96.92%. These findings indicate a high prevalence of micronutrient abnormalities among patients with GSD, suggesting that altered calcium metabolism, iron deficiency, and deficiencies of vitamin D and vitamin B12 may be associated with gallstone disease. Routine assessment of these biochemical parameters may facilitate early identification and appropriate management of nutritional deficiencies in patients with GSD. Further large-scale prospective studies are required to clarify the causal relationship between micronutrient status and gallstone disease.***

Keywords: Gallstone disease, Micronutrients, Vitamin D, Serum Iron

1. Introduction

Gallstone disease (GSD) is one of the most common disorders of hepatobiliary system often presenting as a significant cause of morbidity all over the world [1,2]. The most common feature associated with GSD is the formation of calculi in the gall bladder or the biliary system owing to complex interaction of hereditary, metabolic, and environmental factors [3]. The incidence of GSD varies among several groups affecting approximately 10.15% of population in India and approximately 20% of the overall western population [3]. Consequently, this high incidence poses significant medical and economic burden resulting from readmission to hospital, surgery, and complications associated with the disease [1,4,5].

The complications of GSD are primarily attributed to acute calculus cholecystitis and life-threatening conditions such as acute biliary pancreatitis and ascending cholangitis, posing a substantial burden of mortality and morbidity [6]. Gallstone is formed as a result of bile supersaturation with cholesterol or bilirubin crystal formation, aggregation, and impaired gall bladder activity [3]. Furthermore, nutritional and dietary factors are also recognized in gallstone formation, however, the role of micronutrients and trace minerals remains underexplored compared to established risk factors such as obesity, dyslipidemia, and metabolic syndrome [7,8].

Several trace elements including calcium, copper, and iron play an essential role in oxidative balance, enzymatic activity, and cellular metabolism. Alteration in the level of these trace elements may affect the cholesterol metabolism, bile composition, and gall bladder motility, thereby contributing to GSD [9]. Calcium is a vital component of pigment gallstones and is also present in cholesterol stones as calcium bilirubinate, carbonate, and phosphate. Elevation in calcium levels may promote biliary nucleation and stone formation while adequate level may inhibit cholesterol crystallization, highlighting their dual role in GSD [10,11]. Moreover, alteration in iron metabolism, including deficiency and overload has been associated with GSD. Iron overload such as hereditary hemochromatosis is associated with pigment gallstone owing to oxidative stress and increased bilirubin turnover. In contrast, iron deficiency increases the biliary cholesterol saturation impairing the cholesterol metabolism, thereby contributing to GSD [12,13].

Beyond trace elements, several vitamins such as vitamin D and B12 also plays a vital role in GSD. Low levels of vitamin D, a fat-soluble vitamin involved in calcium metabolism and immune regulation may increase GSD risk. This may occur because vitamin D may affect calcium regulation, bile acid synthesis, gall bladder motility, and chronic gallbladder inflammation, however, there is paucity of existing evidence [10,14]. In addition, deficiency of

vitamin B12 commonly observed in malabsorption disorders, may indirectly contribute to GSD through impaired lipid metabolism, altered hepatic function, and elevated serum cholesterol [15]. Therefore, the involvement of serum calcium, serum iron, vitamin D, and vitamin B12 in cholesterol mechanism, bile composition, and oxidative stress suggests their potential role in GSD. This study aims to assess the serum levels of calcium, iron, vitamin D, and vitamin B12 in patients with GSD and analyze the relationship between these nutrient levels and GSD.

2. Materials and Methods

This hospital-based cross-sectional study was conducted in the department of surgery, and patients transferred from other department Baba Raghav Das Medical College, Gorakhpur, Uttar Pradesh, India from 2024-2025, after obtaining approval from the Institutional Ethics Committee.

Sample size: Sample size was estimated using the formula for single population proportion, and the minimum required sample size was calculated to be 65. Consecutive sampling was followed during the study period.

Inclusion criteria: Adult patients aged between 18-65 years diagnosed with GSD confirmed ultrasound or imaging and who gave written informed consent were included in the study.

Exclusion criteria: Patients with chronic liver disease, pancreatitis, or any known metabolic disorders affecting nutrient absorption; pregnant women; and patients with significant renal, gastrointestinal, or endocrine disorders were excluded from the study.

Study Procedure:
After obtaining informed consent from, all patients underwent detailed clinical evaluation and their demographics and comorbidities were noted. Serum calcium was measured using an automated biochemistry analyzer by the Arsenazo III method. Serum iron was evaluated using colorimetric methods, and TIBC was determined to calculate transferrin saturation. Moreover, Ferrozine kit method was utilized to measure serum iron levels with normal reference values of 60–160 µg/dl for male and 35–145 µg/dl for females. Serum cholesterol was estimated by the Enzopa kit based on the cholesterol oxidase/peroxidase method. In addition, vitamin D and B12 was measured using a chemiluminescence immunoassay (CLIA) and enzyme-linked immunosorbent assay (ELISA, respectively. Notably, GSD was diagnosed based on clinical presentation (such as biliary colic, jaundice, or acute cholecystitis) and confirmed through abdominal ultrasound or other imaging techniques like CT or MRI, as appropriate

Statistical analysis: Data was entered in Microsoft Excel and and analysed using Statistical Package for the Social Sciences (SPSS) version 24.0. Descriptive statistics was used to summarize demographic and clinical characteristics of the study population. Pearson or Spearman correlation analyses were performed to assess the relationship between nutrient levels and gallstone presence or severity. Notably, multivariate logistic regression was conducted to control for confounding factors.

Ethical considerations: The study was conducted in accordance to institutional ethics committee and the principles of the Declaration of Helsinki, 2013 revision. Informed consent was obtained from all participants and confidentiality and data protection was maintained throughout the study.

3. Results

A total of 65 patients diagnosed with GSD were included in the study. Of these, 93.85% (n=61) were female and 6.15% (n=4) were males. The mean age of participants was 39.80 ± 13.45 years, with most patients belonging to the 41–50 years age group (30.77%), followed by 20–30 years (29.23%) and 31–40 years (24.62%), while only 7.69% each were aged 51–60 years and >60 years.

The mean serum calcium, serum iron, vitamin D, and vitamin B12 levels among these patients were found to be 10.44 ± 2.44 mg/dL, 86.18 ± 89.49 µg/dL, 20.43 ± 19.91 ng/mL, and 77.21 ± 147.90 pmol/L, respectively. Wide variations were observed across all biochemical parameters, with serum calcium ranging from 4.4–16 mg/dL, serum iron from 1.7–385 µg/dL, vitamin D from 1.9–100 ng/mL, and vitamin B12 from 14.4–1226 pmol/L, indicating considerable heterogeneity in micronutrient status among the study participants (Table/ Fig. 1).

Table 1: Serum Biochemical Parameters in GSD patients

Parameter	Mean ± SD	Minimum	Maximum
Serum Calcium (mg/dL)	10.44 ± 2.44	4.4	16
Serum Iron (µg/dL)	86.18 ± 89.49	1.7	385
Vitamin D (ng/mL)	20.43 ± 19.91	1.9	100
Vitamin B12 (pmol/L)	77.21 ± 147.90	14.4	1226

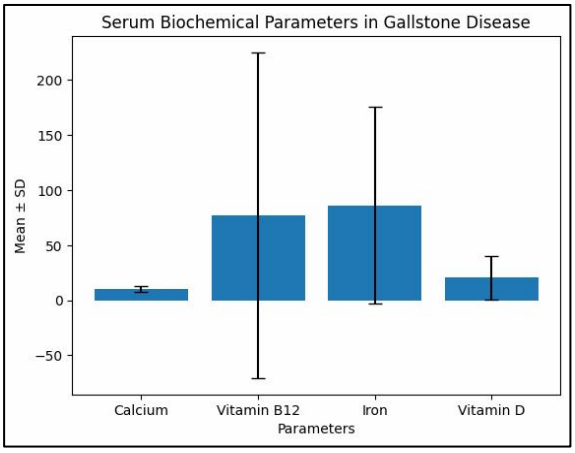

Figure 1: Serum biochemical parameters in patients with gallstone

Serum calcium levels were elevated ((>10.5 mg/dL) in in 44.62% of cases, followed by normal levels in 38.46% and low levels in 16.92%. In contrast, more than half of the patients (55.38%) had low serum iron levels (<60 µg/dL), while 27.69% had normal and 16.92% had elevated iron levels, suggesting a higher prevalence of hypercalcemia and iron deficiency in the study population (Table/ Fig. 2).

Table 2: Distribution of Serum Calcium and Iron Levels in GSD patients

Parameter	Category	Frequency (%)
Serum Calcium	Low (<8.5 mg/dL)	11 (16.92)
	Normal (8.5–10.5 mg/dL)	25 (38.46)
	High (>10.5 mg/dL)	29 (44.62)
Serum Iron	Low (<60 µg/dL)	36 (55.38)
	Normal (60–170 µg/dL)	18 (27.69)
	High (>170 µg/dL)	11 (16.92)

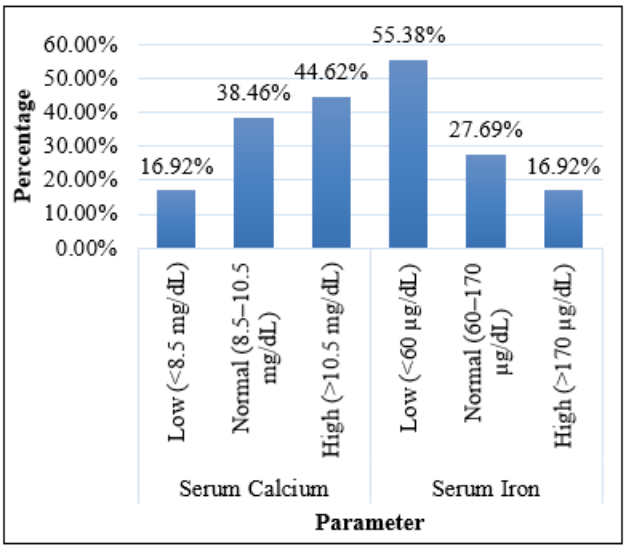

Figure 2: Distribution of Serum Calcium and Iron Levels in GSD patients

Vitamin D deficiency (<20 ng/mL) was observed in 63.08% of patients, while 15.38% had insufficient and 21.54% had sufficient vitamin D levels. Similarly, vitamin B12 deficiency (<148 pmol/L) was highly prevalent, affecting 96.92% of patients, with only 3.08% showing borderline levels and none having normal vitamin B12 levels, indicating a substantial burden of micronutrient deficiencies in the study population (Table/ Fig. 3).

Table 3: Vitamin D and Vitamin B12 level in GSD patients

Parameter	Category	Frequency (%)
Vitamin D	Deficient (<20 ng/mL)	41 (63.08)
	Insufficient (20–30 ng/mL)	10 (15.38)
	Sufficient (>30 ng/mL)	14 (21.54)
Vitamin B12	Deficient (<148 pmol/L)	63 (96.92)
	Borderline (148–221 pmol/L)	2 (3.08)
	Normal (>221 pmol/L)	0 (0.00)

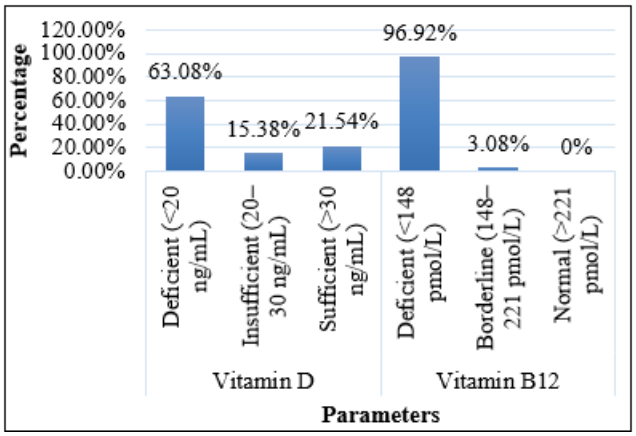

Figure 3: Distribution of vitamin D and vitamin B12 in GSD patients

4. Discussion

In this current study, 65 patients were enrolled who were diagnosed with GSD. In our study, the largest percentage of cases was reported in the 41 years to 50 years age followed by 20 years to 30 years and 31 years to 40 years. The results are in line with **Bhatti et al.** [16] that revealed higher occurrence in people between the ages of 31 and 45 years. The younger age group and the older age group had lower frequencies.

The age distribution in our study was similar with results reported by **Kamal VS et al**. [17] where 25% of patients were aged 20-30 years, 30% were aged 31-40 years, 19% were aged 41-50 years, and 26% aged above 50 years. This demonstrates that the presence of GSD may be attributes to people falling within the age ranges of the third to fifth decades of life.

Our study reveals that the gender distribution was predominant in female population compared to male. These findings are consistent with the previous research that has indicated that incidence of GSD is high among women. For instance, **Chen et al.** [18] noted a preponderance of 71% in females while males with GSD were only 29%. Moreover, according to **Mishra et al.** [19], it was demonstrated that female occurrence was more than that of males. This predominance was observed due to several factors, hormonal factors being the most important one. The role of estrogen is notable as it increases the saturation of the cholesterol in the bile and reduces the motility of gallbladder, ultimately leading to the formation of gallstones [17].

Our study observed a substantial variability in serum biochemical parameters with serum calcium levels ranging from normal to high and no wide deviation. However, serum iron, vitamin D, and vitamin B12 reported wide variations indicating a significant difference in the status of micronutrients among the GSD patients. These findings are in concordance with previous research studies. **Sharma et al.** [20] reported inverse relationship between calcium intake and GSD occurrence in a sample of middle-aged men. Moreover, **Song et al.** [21] found calcium and potassium levels to affect the occurrence of dyslipidaemia as triglycerates were correlated with the low level of calcium and potassium. However, all biochemical parameters were not evaluated in the study.

In our study, a large percentage of population were hypercalcaemic. However, in study by **Mishra et al**. [19], it was found that hypocalcaemia was more predominant than hypercalcemia. In another study by **Shareef et al.,** [11] it was observed that serum calcium and chemical composition of gallstone had moderate correlations with positive and negative correlations existing between calcium and cholesterol, pigment and mixed stones, this confirms that presence of calcium in the pathogenesis of gallstones. Although variations exist across studies, the present findings underscores the potential involvement of altered calcium homeostasis in GSD.

In the present study, majority of the patients were vitamin D deficient with 15.38% of patients having insufficient vitamin

D levels. This finding is in agreement with the study of **Singh et al.**, [30] who reported a markedly low level of serum vitamin D3 (13.16 ± 6.76 ng/mL) among GSD patients. However, **Bin et al.**, [22] reported a positive correlation between consumption of vitamin D and gallstone risk analyzing vitamin D intake as continuous variable. These finding underscore the important role of vitamin D in GSD, though the exact mechanism remains unclear.

Moreover, serum iron levels in the present study was low in more than half of the GSD patients suggesting an elevated occurrence of iron deficiency. In addition, **Prasad et al.**, [23] also reported a higher percentage of gallstone patients with low serum iron levels and more than half of the patients amongst them were anaemic. This finding further strengthens the association between GSD and iron deficiency. The study by **Pamuk et al.**, [13] support this evidence as they demonstrated that gallbladder motility was reduced in patients with iron-deficiency anaemia further increasing the GSD risk when compared to non-anaemic patients. Similarly, **Halgaonkar et al.**, [24] reported low serum iron levels in majority of gallstone patients, underscoring iron deficiency as a vital contributing factor. Notably, **Sahu et al.**, [25] in his study concluded that low levels of serum iron results in cholesterol supersaturation of bile, thereby promoting GSD development.

In our study, none of patients exhibited normal vitamin B12 levels. Vitamin B12 deficiency was seen in a vast majority (96.92%) of our study population, indicating a significant association between GSD and vitamin B12 deficiency. However, **Chen et al.**, [18] reported contrast findings where vitamin B12 levels were marginally lower among patients with gallstones. Notably, there is a paucity of literature or experimental studies examining the relationship between vitamin B12 status and GSD, underscoring the need for further research in this area. In our study, no notable variations was observed between age and serum levels of calcium, vitamin B12, iron, or vitamin D among patients with GSD. These findings could not be adequately validated due to the limited availability of comparable studies in the existing literature. Therefore, further well-designed studies are warranted to better understand the interaction between age and serum nutrient profiles in patients with GSD and to clarify their potential medical significance.

5. Conclusion

Serum biochemical parameters evaluation in GSD patients indicated substantial abnormalities in the nutrient status with the study participants. A significant proportion of patients were found to be hypercalcaemic with vitamin D deficiency observed frequently. Moreover, more than half of the population were found with low serum iron levels indicating a prevalence of iron deficiency among GSD patients. It is important to note that approximately all of the study population was found to be vitamin B12 deficient. The study establish the association of micronutrient deficiency and biochemical disorders among GSD patients. There is a need to regularly determine the levels of serum nutrients in patients with gallstones because early detection and treatment of deficiency can be of medical significance. More extensive studies on a larger scale are needed to have a better understanding of the causal relation and the mechanism behind correlation between micronutrient status and GSD.

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