ORIGINAL RESEARCH PAPER

General surgery

CHOLELITHIASIS IN PEOPLE WITH NORMAL SERUM CHOLESTEROL: ROLE OF SERUM IRON

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ABSTRACT

INTRODUCTION: The old axiom that a typical gallstone patient is a fat, fertile female of forty is only partially true, as the disease has been found in women soon after their first delivery and also in underweight and thin people. So while searching the literature for different factors, the Iron deficiency was found to be a new and interesting etiological factor in the formation of gall stones. Although the cause is still unclear, cholesterol Gallstones develop most commonly in multiparous women. This patient population is also prone to chronic iron deficiency anemia (IDA). Previous studies claimed a cause-effect relationship between iron deficiency and cholesterol gallstones.

AIM - To assess the association between Serum Iron and Bile Cholesterol levels in people with normal serum cholesterol levels, which reflects the effect of Serum Iron levels in the formation of Gallstones.

MATERIALS AND METHODS – The study was conducted in the Department of General Surgery, G.S.L Medical College, and General Hospital over 18 months. A total of 49 patients with cholelithiasis and normal Serum Cholesterol levels were included in the study. Serum iron and Bile cholesterol contents were analyzed.

RESULTS – It is observed that there is a significant association between increased incidence of Cholelithiasis and Low Serum Iron levels among people with normal levels of serum cholesterol.

CONCLUSION – Low Serum Iron was associated with increased Bile Cholesterol concentration, indicating a possible role of Serum iron in forming gallstones.

INTRODUCTION:

Biliary calculus disease is one of the most common disorders affecting the gastrointestinal tract and is an important cause of morbidity. There has been a marked rise in gallstone disease incidence in the west during the past century. The word cholelithiasis was derived from the Greek chol- (bile) + lith- (stone) + iasis- (process)

Autopsy reports show that gallstones are present between 10% and 15% of adults[1], with an incidence of 1.39 per 100 person-years. Approximately 1–2% of asymptomatic patients will develop symptoms requiring surgery per year[2]. Once a person develops biliary symptoms, the risk for recurrent symptoms is greater than 50%. Multiple stones increase the risk for complications such as acute cholecystitis or symptomatic gallstone disease[3]

The old axiom that a typical gallstone patient is a fat, fertile female of forty is only partially true. The disease has been found in women soon after their first delivery and underweight and thin people[4]. So while searching the literature for different factors, the Iron deficiency was found to be a new and interesting etiological factor in the formation of gall stones. Although the cause is still unclear, cholesterol Gallstones develop most commonly in multiparous women[5]. This patient population is also prone to chronic iron deficiency anemia (IDA). Previous studies claimed a cause-effect relationship between nutritional deficiency and cholesterol gallstones[6]

AIM OF THE STUDY :

To establish the role of iron levels in the concentration of bile cholesterol and formation of gallstones in people with normal levels of serum cholesterol

OBJECTIVES OF THE STUDY

1. To estimate Serum Iron levels in all patients included in the study
2. To study Bile Cholesterol levels in all patients included in the study
3. To assess the association between Bile Cholesterol and Serum Iron in all patients included in the study

MATERIALS AND METHODS:

Study setting: Patients attending the Department of General Surgery, G.S.L Medical College & General Hospital.
Study Design: Hospital-based cross-sectional study.
Study period: October 1ST 2018 TO March 31st, 2020
Study selection: Simple random sampling

STUDY SUBJECTS:
INCLUSION CRITERIA:

All patients with cholelithiasis and having serum cholesterol levels in the normal range (150-200 mg/dl) and had undergone cholecystectomy either by laparoscopy or open method were included in the study

EXCLUSION CRITERIA :

1. Patients with a history of hemolytic anemia
2. Patients on specific medications like Hormones like estrogen and Fibrates
3. Pediatric patients (below 12 years) with cholelithiasis
4. Patients on iron supplements.

METHODOLOGY

All patients who satisfy inclusion criteria are selected for the study. Written informed consent was obtained from all study subjects. Institutional ethical review committee approval was obtained before the study commencement, after obtaining a detailed history. All routine Pre-operative investigations and specific investigations required for the study were carried out in every study subject. Serum iron is estimated by the

Ferrozine kit method. Serum cholesterol is estimated by the cholesterol oxidase/peroxidase method. Bile is collected intraoperatively and sent for estimation of cholesterol by oxidase/peroxidase method

RESULTS -

A prospective study was performed to determine the role of serum iron levels in the pathogenesis of cholelithiasis. A total of 49 patients were studied who fulfilled the inclusion criteria. All these patients were admitted to the General Surgery department of GSL Medical College and General Hospital from 2018 October to 2020 August. The results obtained in the study are as follows

AGEWISE DISTRIBUTION
Table 1: Age distribution of the cases

Age group (years)	Number of cases
Age <25	6 (12%)
Age 26 -50	23 (47%)
Age 51-75	19 (39%)
Age >75	1 (2%)
Total	49

In the present study majority of the patients (47%) included in the study were in the age group of 26-50, followed by 39% of people in the 51-75 age group, With only 12% below 25 years of age. The mean age of the cases included in the study is 45.5 years.

GENDER WISE DISTRIBUTION
Table 2 – Gender Distribution

Gender	Number of cases
Female	34 (69%)
Male	15 (31%)
Total	49

In this study, out of 100 cases, 34 are females, and 15 are males. Showing an increased incidence of cholelithiasis in the female population.

BMI DISTRIBUTION
Table 3: BMI Distribution

BMI Class	Number of cases
Normal BMI (18.5-22.9)	8 (16.3%)
Overweight (23-24.9)	32 (65.3%)
Obese (>25)	9 (18.4%)
Total	49

In this study, out of 49 cases, eight are of Normal BMI (18.5-22.9kg/m2), 32 are of Overweight BMI(23-24.9 kg/m2), and nine are of Obese(>25 kg/m2) BMI, with a Mean BMI of 24.1 (Overweight) Indicating an increased incidence of Cholelithiasis in Overweight.

DIET DISTRIBUTION
Table 4: Diet Distribution

Diet	Number of cases
mixed	41 (83.7%)
vegetarian	8 (16.3%)
Total	100

In this study, out of 49 cases, 41 are of the Mixed diet, and eight are of the Vegetarian diet. This shows an increased incidence of cholelithiasis in the Mixed Diet population.

SERUM IRON DISTRIBUTION
Normal value (Males – 65-175ug/dl, Females –50-170ug/dl)

Table 5: Serum Iron Distribution

Serum Iron level	No of cases
low	26 (53%)
normal	23 (47%)
Total	49

In this study, out of 49 cases, 26 cases have serum iron lower than normal (<65ug/ml in males, <50ug/dl in females), and 23 cases have serum iron levels in the Normal to high range. The Mean values of Serum Iron are 62 in males and 52 in Females, with Maximum and Minimum values at 170 ug/dl and 11 ug/dl, respectively.
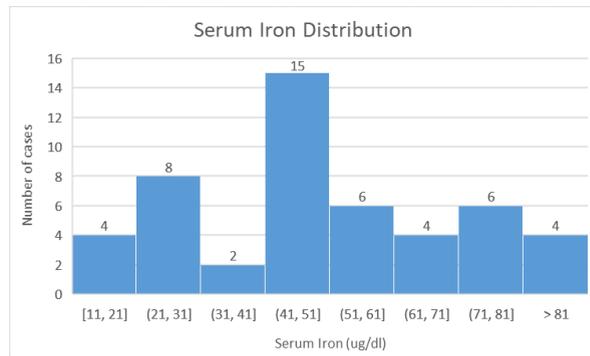

Fig 1: Serum Iron Distribution Histogram

Majority of 26 cases having serum iron values between 41ug/ml and 51ug/ml. With the Second peak between 21ug/ml and 31 ug/ml. This shows an increased cholelithiasis incidence in individuals with the Lower and lower limit of Normal serum iron values.

Bile cholesterol Distribution
(Normal Bile cholesterol 100-300mg/dl)

Table 6: Bile Cholesterol Distribution

Bile Cholesterol Groups	Number of cases
B Chol <300	16 (32.7%)
B Chol>300	33 (67.3%)
Total	49

In this study, out of 49 cases, most of the cases (67%) are in the higher range of bile cholesterol with values above 300mg/dl, with 32.7% cases having Bile cholesterol value lower than 300mg/dl. The mean Bile cholesterol value in the present study was observed to be 363.59.
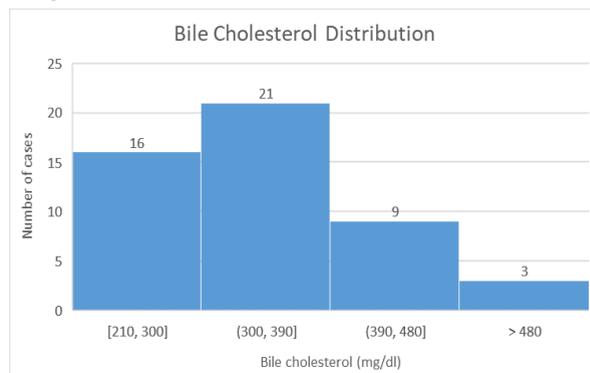

Fig 2: Bile Cholesterol Distribution Histogram

The maximum pooling of cases is observed around Bile Cholesterol of 300-390mg/dl, indicating an increased cholelithiasis incidence in people with high bile cholesterol.

To establish a relationship between the serum iron and bile cholesterol, Pearson correlation was performed. A non-significant (P-value – 0.306) Negative correlation (r-value = -0.149) was obtained between Serum Iron and Bile Cholesterol.

On further analysis using Chi-square to establish an association between Serum Iron and Bile cholesterol. A Statistically Significant association was observed with a Chi-square value of 4.538 (P-value– 0.033)

DISCUSSION :

Iron functions as a cofactor in many intracellular enzyme systems, including hepatic enzymes involved in bile salt regulation[7]. Iron deficiency is further associated with raised serum transferrin. It is shown that transferrin is elevated in the bile of patients with cholesterol gallstones and that transferrin is a potent cholesterol pronucleator[8]. In addition, iron-containing cofactors are fundamental components of the nitric oxide synthase complex[9]. Nitric oxide has been identified as a putative inhibitory neurotransmitter present throughout the gastrointestinal tract[10,11]. Nitric oxide has been shown to be important for the maintenance of basal gallbladder tone in prairie dogs and is an inhibitor of the contractile response of the gallbladder to physiological stimulators[12], resulting in Gallstone formation in Iron deficient individuals[13].

In our study majority of the cases are within the 26 to 50 years of age group, and the same trend was observed among Halgaonkar et al. [14], Prasad et al. [15], Arora et al. [16], Sharma et al. [17], Dube et al. [18] and Pamuk et al. [19]. While the mean age is 45.53 in the present study, the remaining studies in the comparison showed an almost similar Mean age of their cases.

There was an increased frequency of cholelithiasis in the female Gender in our study, with 69% of the cases being females, which is also reflected in the other studies Halgaonkar et al. [14], Prasad et al. [15], Arora et al. [16], Sharma et al. [17], Kumar et al. [20], Misra et al. [6], Dube et al. [18] and Pamuk et al. [19]. This can be explained by the preponderance of gallstones in fertile women, suggesting that female sex hormones play a role in cholelithiasis pathogenesis [21].

The majority of the cases in our study were in the Overweight BMI with a Mean BMI of 24.1 kg/m^2, while Kumar et al. [20] and Sarhan et al. [22] show more number of their cases in above the Normal BMI (i.e. Overweight and Obese combined), the mean BMI in the study by Pamuk et al. [19] is in the Obese range with 25 BMI, which are in accordance with the study done by Erlinger. S[23], on the increased prevalence of gallstones in people with high Body Mass Index.

Regarding Dietary habits, an increased prevalence of cholelithiasis in the population was observed in cases consuming a mixed diet accounting for 83.7% of our study population. Similar results were observed by Halgaonkar et al. [14], with 65% of cases having mixed dietary habits. This increased prevalence of gallstones in people with Mixed Dietary habits was also reported by Bansal et al. [24], who stated the probable cause for this association between Mixed diet and gallstones to be increased consumption of fats and proteins.

Halgaonkar et al. [14] and Prasad et al. [15] show that most of the cholelithiasis cases are associated with low Serum Iron levels, which was also observed in the present study. Studies by Arora et al. [16] and Dube et al. [18] showed more cases with cholelithiasis being present in the Normal serum iron range, with Mean Serum iron levels of both Males and Females being in the Normal range. Misra et al. [6] showed the Mean Serum Iron in their study was in the Low Serum Iron level. In the present study, the Mean serum Iron vales of Males are in the Low Serum Iron range (62ug/dl), while for the Females, the Mean Serum Iron levels (52ug/dl) are at the lower limit of the Normal Serum Iron range.

The Mean Bile Cholesterol is 363, similar to the values obtained in the study by Sahu et al. [25] and Misra et al. [6] While the values of Mean Bile cholesterol reported by Dube et al. [18], Kumar et al. [20] and Sarhan et al. [22] were higher than what was obtained in the present study.

Our results indicated a higher frequency of cholelithiasis in patients with Low Serum Iron levels, which was complementary to what was stated in the study by Johnston et al. [14], that in certain animal studies, iron-deficient diets altered hepatic enzyme metabolism, which resulted in a higher level of GB bile cholesterol, thereby promoting the formation of cholesterol crystals.

As indicated in the age-old axiom, the characteristics of 'Fat, Female, Fertile, Forty' for Cholelithiasis, our study showed an increased frequency of cholelithiasis in the Overweight BMI group, Female population and Increased prevalence in the age group of around 40 years, which was also reflected in the results of other studies.

It was observed that the relation between Serum Iron levels and Bile Cholesterol levels is inversely correlated, and the association between them is significant (P-value – 0.033)

This significant association between Serum Iron and Bile Cholesterol obtained in People with Normal Cholesterol indicates a possible role of Low serum Iron levels associated with cholelithiasis in these populations even with Normal levels of Serum Cholesterol.

LIMITATION :

The present study was a single-center study. This could have an impact on the reproducibility of the reported results. The sample size was 49, which further might have an impact on the reproducibility of the results. Only a few works of literature are available on the correlation between serum iron, and bile cholesterol in patients with cholelithiasis, so further multi-centric studies with a larger sample size need to be done to validate our hypothesis.

CONCLUSION :

In conclusion, our study showed that low Serum Iron levels are associated with increased cholesterol levels of bile and cholelithiasis in people with Normal serum cholesterol levels.

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