ANALYSIS OF BILIARY CHOLESTEROL LEVELS IN IRON-DEFICIENT PATIENTS OPERATED FOR GALLSTONE DISEASE

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ABSTRACT

BACKGROUND

Gallstone disease is a common gastrointestinal problem in day-to-day practice. The old concept that a typical gallstone sufferer is fat, fertile, flatulent female of 50. This is partially true as the disease has been found in women soon after their first delivery who are thin and underweight and in males also. Conditions that favour the formation of cholesterol gallstones are super saturation of bile with cholesterol, kinetically favourable nucleation and presence of cholesterol crystals in the gallbladder long enough to agglomerate into a stone. Recent studies have defined the role of trace elements (Fe, Ca, Zn and Cu) and defective pH in the formation of gallstones.

The aim of the study is to determine the association of iron deficiency in super saturation of bile. This cross-sectional study of 50 patients was conducted over a period of 12 months in the Department of General Surgery, Kilpauk Medical College, Chennai, India. Biliary cholesterol and serum cholesterol were compared in iron deficient and non-iron deficient patients having gallstones. A low serum iron level is a factor in bile super saturation with respect to cholesterol leading to gallstone formation.

MATERIALS AND METHODS

This study was conducted over a period of 12 months in the Department of General Surgery, Kilpauk Medical College, Chennai, India. 50 patients suffering from cholelithiasis confirmed by USG were divided into two groups based on serum iron values. Group A consists of patients with normal serum iron (non-anaemic) and Group B of patients with less than normal serum iron (anaemic).

RESULTS

Serum total cholesterol of the patients of cholelithiasis was not different among groups categorised based on serum iron levels. There were no significant variations in the serum cholesterol contents of both the groups. Also, there was no significant variation of the above parameter in the male and female patients.

CONCLUSION

Though, it is difficult to draw a causal relation between low iron levels and cholelithiasis with this study design alone in future prospective trials could aim at better defining if such causal relationship exists.

KEYWORDS

Gallstone, Serum Ferritin, Bile Cholesterol, Anaemia.

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BACKGROUND

Gallstone disease is a common clinical entity affecting the adult population of both sexes. The earliest known gallstones date back to the 21st Egyptian dynasty discovered in the mummy of a priestess of Amenen (1085-945 BC). Gallstones are classified into either pure cholesterol stones, black or brown pigmented stones or mixed stones. Conditions that favour the formation of cholesterol gallstones are super saturation of bile with cholesterol, kinetically favourable nucleation and presence of cholesterol crystals in the gallbladder long enough to agglomerate into stone. Recent studies have defined the role of trace elements (Fe, Ca, Zn and Cu) and defective pH in the formation of gallstones.[1,2]

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OBJECTIVES

The objective of the study determines the correlation between iron deficiency with biliary cholesterol levels and serum cholesterol levels.

MATERIALS AND METHODS

This study was conducted over a period of 12 months in the Department of General Surgery, Kilpauk Medical College, Chennai, India. 50 patients suffering from cholelithiasis confirmed by USG were divided into two groups based on serum iron values. Group A consists of patients with normal serum iron (non-anaemic) and Group B of patients with less than normal serum iron (anaemic). Gallbladder bile cholesterol with serum cholesterol of both the groups is compared by a Student's t-test.

The patients were selected based only on the USG confirmation of their gallbladder stones irrespective of their age, sex, physique, parity, etc. Only those patients were included whose serum as well as bile could be procured for analysis. Patients with empyema and mucocele of gallbladder were excluded.

Age Range	Total	Females	Males
<20 Yrs.	2	2	0
20-30 Yrs.	11	10	1
30-40 Yrs.	13	10	3
40-50 Yrs.	14	12	2
50-60 Yrs.	8	5	3
60-70 Yrs.	2	1	1
>70 Yrs.	0	0	0

Table 1. Distribution of Data According to Age and Gender

Serum iron was estimated by the Ferrozine kit method.[3] The normal reference values were supplied with the kit for males (60-160 µg/dL) and for females (35-145 µg/dL) were used to label the patients as anaemic and non-anaemic, i.e. males with serum iron <60 µg/dL and females with serum iron <35 µg/dL were labeled as anaemic. Serum cholesterol was estimated by the Enzopa kit based on the cholesterol oxidase/peroxidase method as devised by Allain et al.[4]

Bile was kept in a sterile container and sent for analysis. Serum cholesterol and gallbladder bile cholesterol of all patients were estimated. Bile was first subjected to the Folch method to extract lipids and then the cholesterol contents were estimated as for serum cholesterol. In the Folch method, lipids from bile were extracted using water, methanol and chloroform mixture in the ratio of 3:4:8 v/v and from the extracted lipids, cholesterol was estimated by Enzopak kit based on the cholesterol oxidase/peroxidase method. The enzymes used only the cholesterol as substrate and hence bilirubin is automatically eliminated from the procedure of cholesterol estimation.

RESULTS

Table 1 shows the Following-

- Out of 50 patients, 40 (80%) were females and 10 (20%) were males.
- The male-to-female ratio was 1:4.
- In the present study, the minimum age was 17 years and the maximum age was 68 years.
- The number of patients was highest in the age group of 40-50 years having 14 patients (28%) followed by 30-40 years having 13 patients (26%). The least was in the age group 60-70 years.
- 0-20 years having 2 patients each 94%.
- Mean age was 40.6 years. Standard deviation was 12.1 years.
- Median age was 39.5 years.

Patients	No.	%	Range of Serum Iron (µg/dL)	Mean±SD
Males				
Non-Iron Deficient	9	90	95.5-163	119.8±25.3
Iron Deficient	1	10	40	-
Total	**10**	**100**	**95.5-163**	**111.8±34.7**
Females				
Non-Iron Deficient	18	45	36.3-140.1	79.8±31.1
Iron Deficient	22	55	7.4-34.6	26.2±8.3
Total	**40**	**100**	**7.4-140.1**	**50.4+34.5**

Table 2. Distribution of Serum Iron Among the Groups

Group	Number of Patients	Serum Iron Range µg/dL	Serum Cholesterol Mean±SD
A (Non-Iron Deficient)	27	36-163	93.2±34.6
B (Iron Deficient)	23	7-40	26.8±8.6
p-Value			<0.0001

Table 3. Distribution of Patients into Groups A and B

Table 2 and 3 show the Following-

- The 50 patients who were gallstones sufferers were divided into two groups based on the serum iron levels.

Group A- Patients with normal serum iron. (Male- 60-160 µg/dL, female 35-145 µg/dL).

Group B- Patients with less than normal serum iron.

- 27 (54%) of them were in normal serum iron group A and 23 (46%) were in serum iron deficient group B.

Group	Number of Patients	Biliary Cholesterol Range mg/dL	Biliary Cholesterol Mean±SD
A (Non-Iron Deficient)	27	3-147	754.5±398.3
B (Iron Deficient)	23	26-181	1184.7±405.2
p-Value			<0.0004

Table 4. Distribution of Biliary Cholesterol According to Anaemia

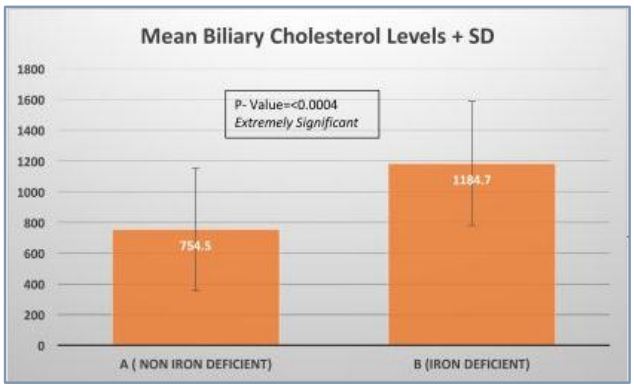

Figure 1. Biliary Cholesterol Levels in Groups A and B

Table 4 and Figure 1 Show the Following-

- A statically significant difference between the biliary cholesterol values of iron deficient and non-iron deficient groups.
- P value and statistical significance-
- The two-tailed P value equals 0.0004.
- Confidence interval-

The mean of group one minus group two equals- 430.200, 95% confidence interval of this difference- from -659.252 to -201.148.

Intermediate values used in calculations- t=37763, df=113920.

Group	Number of Patients	Serum Cholesterol Range mg/dL	Serum Cholesterol Mean±SD
A (Non-Iron Deficient)	27	93-254	184.8±35
B (Iron Deficient)	23	79-270	171±49.3
p-Value			0.2544

Table 5. Distribution of Serum Cholesterol According to Anaemia

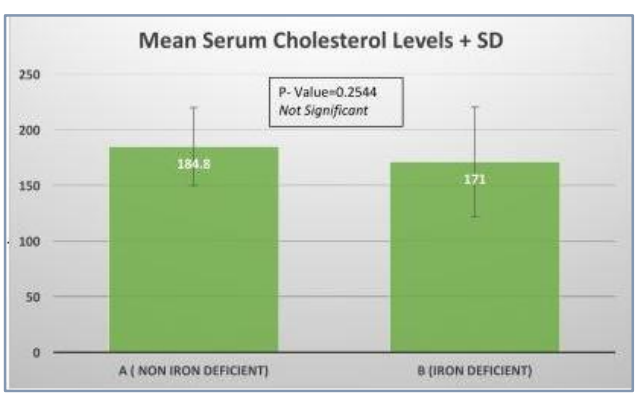

Figure 2. Serum Cholesterol Levels in Group A and Group B

Table 5 and Figure 2 Shows-

- No statistically significant difference in serum cholesterol values of iron deficient and non-iron deficient groups.
- P value and statistical significance-

The two tailed P value equals 0.2544.

- Confidence interval-
  - The mean of A (non-iron deficient) minus B (iron deficient) equals 13.800.
  - 95% confidence interval of this difference- from -10.254 to 37.854.

DISCUSSION

Iron deficiency has been shown to alter the activity of several hepatic enzymes leading to increased gallbladder cholesterol saturation and promotion of cholesterol crystal formation.[5,6] Iron acts as a coenzyme for Nitric Oxide Synthase (NOS), which synthesises Nitric Oxide (NO) important for the maintenance of gallbladder tone and normal relaxation.[7,8] Alteration of motility of the gallbladder and sphincter of Oddi leading to biliary stasis results in cholesterol crystals formation, which has been reported with iron deficiency.[9]

The present study shows that the gallbladder bile cholesterol level was significantly higher in anaemic patients as compared to that of non-anaemic patients. Further, no significant variations in the serum cholesterol values were detected between the anaemic and the non-anaemic group.

Animal studies had suggested that iron may have a role to play in the pathophysiology of gallstone disease. More importantly the finding that iron deficient mammals were more prone to develop gallstones has sparked the interest in its probable role in humans.

This study suggests that iron deficiency leading to anaemia plays a significant role in the super saturation of bile. The serum cholesterol of the anaemic group was found to be similar to the non-anaemic group. Iron deficiency probably alters the hepatic enzymes metabolism, leading to super saturation of gallbladder bile with respect to cholesterol irrespective of serum cholesterol levels, hence promoting the cholesterol crystal formation.[10]

CONCLUSION

In the present prospective study of 50 cases based on serum iron, biliary cholesterol and serum cholesterol, the following conclusions were drawn-

1. Serum total cholesterol of the patients of cholelithiasis was not different among groups categorised based on serum iron levels. There were no significant variations in the serum cholesterol contents of both the groups. Also, there was no significant variation of the above parameter in the male and female patients.
2. The low serum iron levels were associated with bile super saturation with respect to cholesterol. This was shown by a statistically significant difference in biliary cholesterol values between groups divided based on serum iron levels.
3. Our study had a few limitations. It is difficult to draw a causal relation between low iron levels and cholelithiasis with this study design. Future, prospective trials could aim at better defining if such causal relationship exists.

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