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.

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.

<table>
<thead>
<tr>
<th>Age Range</th>
<th>Total</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20 Yrs.</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>20-30 Yrs.</td>
<td>11</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>30-40 Yrs.</td>
<td>13</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>40-50 Yrs.</td>
<td>14</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>50-60 Yrs.</td>
<td>8</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>60-70 Yrs.</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>&gt;70 Yrs.</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1. Distribution of Data According to Age and Gender

Serum iron was estimated by the Ferrozine kit method. 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 Enzopak kit based on the cholesterol oxidase/peroxidase method as devised by Allain et al.

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.

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 iron deficient group B.

<table>
<thead>
<tr>
<th>Patients</th>
<th>No.</th>
<th>%</th>
<th>Range of Serum Iron (µg/dL)</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td>95.5-163</td>
<td>119.8±25.3</td>
</tr>
<tr>
<td>Non-Iron Deficient</td>
<td>9</td>
<td>90</td>
<td>95.5-163</td>
<td>119.8±25.3</td>
</tr>
<tr>
<td>Iron Deficient</td>
<td>1</td>
<td>10</td>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>100</td>
<td>95.5-163</td>
<td>111.8±34.7</td>
</tr>
</tbody>
</table>

Table 2. Distribution of Serum Iron Among the Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Patients</th>
<th>Serum Iron Range µg/dL</th>
<th>Serum Cholesterol Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Non-Iron Deficient)</td>
<td>27</td>
<td>36-163</td>
<td>93.2±34.6</td>
</tr>
<tr>
<td>B (Iron Deficient)</td>
<td>23</td>
<td>7-40</td>
<td>26.8±8.6</td>
</tr>
<tr>
<td>p-Value</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Distribution of Patients into Groups A and B

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Patients</th>
<th>Biliary Cholesterol Range mg/dL</th>
<th>Biliary Cholesterol Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Non-Iron Deficient)</td>
<td>27</td>
<td>3-147</td>
<td>754.5±398.3</td>
</tr>
<tr>
<td>B (Iron Deficient)</td>
<td>23</td>
<td>26-181</td>
<td>1184.7±405.2</td>
</tr>
<tr>
<td>p-Value</td>
<td>&lt;0.0004</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Distribution of Biliary Cholesterol According to Anaemia
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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**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.

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

**Table 5. Distribution of Serum Cholesterol According to Anaemia**
REFERENCES


