Comparative Study of Serum Ferritin and Lipid Profiles (Serum Cholesterol, High-density Lipoprotein, and Low-density Lipoprotein) Between Normal Population and Patients Suffering from Cholelithiasis

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Abstract

Background: Calculous biliary disease by far, the most common pathology involving the gallbladder (GB) and biliary tree. The basic pathophysiology of gallstone (GS) formation is a complex interplay of supersaturation of secreted bile, concentration of bile in the gallbladder, crystal nucleation, and GB dysmotility. Thus, high concentrations of cholesterol and lipid in bile secretions from the liver predispose to cholesterol stone formation, whereas increased hemoglobin catabolism leads to pigment stone formation. The lipid profiles of patients with GS disease its implication have already been reported in the literature; however, the effect of patient iron profile and its association with GS disease has not been much evaluated so far; thus, we planned this prospective study to assess the iron status of patients of GS disease and compare it with normal population.

Aims and Objectives: The objective of the study was to compare the levels of serum ferritin and serum cholesterol, low-density lipoprotein, and high-density lipoprotein between normal population and patients suffering from GS disease.

Materials and Methods: The prospective study was conducted over a period of 12 months in the Department of General Surgery, Maharishi Markandeshwar Medical College and Hospital, Kumarhatti, Solan, Himachal Pradesh, India. A total of 100 subjects, 50 patients suffering from cholelithiasis admitted and confirmed by ultrasonography and 50 healthy volunteers as the control group, were included in this study.

Summary and Conclusion: Low serum iron level in one or the other way was leading to bile supersaturation with respect to cholesterol, which leads to GS formation. Serum ferritin levels were significantly lower in iron-deficient patient; however, as its value can vary due to other causes such as iron therapy, hepatocellular disease, and inflammations (since cholecystitis is an inflammatory condition, this could be the reason for the high level of serum ferritin) hence cannot be taken as sole diagnostic marker for iron deficiency anemia and indicator of GS disease.

Key words: Biliary tree, Cholelithiasis, Hemoglobin

INTRODUCTION

Calculous biliary disease by far, the most common pathology¹-³ involving the gallbladder (GB) and biliary tree. GB concentrates bile; however, the concentration of solutes in the GB differs from that in the rest of the biliary tree. This increase in solute concentration⁴ combined with stasis in the GB between meals predisposes to stone formation in the gallbladder. GS can be subclassified into two major subtypes, based on the major solute component of the stone. Majority of >70% of GSs in the United States of America⁵ are reported as combination of cholesterol and calcium, whereas pure cholesterol stones are only found in a small fraction of <10% patients. Pigment stones can be further subclassified as black or brownstones due to

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precipitation of concentrated bile pigments, the breakdown products of hemoglobin. The basic pathophysiology of GS formation is a complex interplay of supersaturation of secreted bile, concentration of bile in the gallbladder, crystal nucleation, and GB dysmotility. Thus, high concentrations of cholesterol and lipid in bile secretions from the liver predispose to cholesterol stone formation, whereas increased hemoglobin catabolism leads to pigment stone formation. The lipid profiles of patients with GS disease its implication have already been reported in the literature; however, the effect of patient iron profile and its association with GS disease has not been much evaluated so far; thus, we planned this prospective study to assess the ion status of patients of GS disease and compare it with normal population. Since serum ferritin is a good marker of body iron stores, hence, we included it along with lipid profile of the study subjects and controls for our study.

Aims and Objectives
The objective of the study was to compare the levels of serum ferritin and serum cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) between normal population and patients suffering from GS disease.

MATERIALS AND METHODS
The prospective study was conducted over a period of 1 year (January 2017–December 2017) in the Department of General Surgery, Maharishi Markandeshwar Medical College and Hospital, Kumarhatti, Solan, Himachal Pradesh, India. A total of 100 subjects, 50 patients suffering from cholelithiasis admitted and confirmed by ultrasonography and 50 healthy volunteers as the control group, were included in this study after obtaining a written and informed consent. The Normal reference values in our lab are shown in Table 2.

Inclusion Criteria
All patients suffering from cholelithiasis confirmed by ultrasonography were included in this study and willing to participate in the study.

Exclusion Criteria
Patients not willing to participate and having following disorders/disease were not included: Hematological disorders, cirrhosis of liver, and pregnant females.

Based on the hemoglobin of the patients, all cases were divided into two groups: Non-anemic (i.e., hemoglobin >13 g% in males and >12 g% in females) and anemic (i.e., hemoglobin ≤13 in males and <12 in females). Serum cholesterol, LDL, HDL, and ferritin contents of both groups were compared with each other and the control group.

Observations
Out of fifty patients of GS disease during the Study period from April 2017 to March 2018, 40 (80%) were female and 10 (20%) were male patients. The mean age in the study group (GS s present) was 37.5 years (range 22–57 years) for males and 33.5 (range 25–65 years) for females. It was 33.5 years (18–65) for the control group of healthy volunteers. Both groups were comparable as far as age distribution was considered. 12% males and 48% females in the study group were anemic, whereas 32% of the control group subjects were anemic, of which two-third was female. The overall serum ferritin was low in female patients, both in anemic (n = 24.17 ± 5 [9–55]) and non-anemic (n = 16, 27 ± 5 [19–135]) subjects as compared to their male counterparts as shown in Table 1. Serum ferritin in control group anemic subjects was comparable (15 ± 4 [10–60]) to the study group; however, it was higher in non-anemic males (57 ± 18 [25–260]) in study group and non-anemic (52 ± 16 [26–272]) control group subjects. The serum ferritin levels were lowest in anemic female patients and significantly lower than (P < 0.05; Table 1) the other two groups (non-anemic patients and controls). The lipid profile of anemic female patients (serum cholesterol - 219 ± 28 [163–369]; serum LDL - 128 ± 23 [102–267]; and serum HDL - 28 ± 9 [27–63]) was significantly (P < 0.05) higher than the non-anemic female patients and the male patients in Table 1.

Table 1: Lipid and serum ferritin profile of study and control groups

<table>
<thead>
<tr>
<th>Patient and Lab parameters</th>
<th>Males n=10</th>
<th>Non-anemic n=4</th>
<th>Females n=40</th>
<th>Non-anemic n=16</th>
<th>Controls n=50</th>
<th>Non-anemic n=34</th>
<th>Anemic n=16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Hb&lt;11 2g/dl)</td>
<td></td>
<td>(Hb&lt;11 2g/dl)</td>
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<td>(Hb&lt;11 2g/dl)</td>
<td></td>
<td>(Hb&lt;11 2g/dl)</td>
</tr>
<tr>
<td>Serum cholesterol (mg/dl)</td>
<td>98±25 (72–167)</td>
<td>108±18 (92–197)</td>
<td>128±23 (102–267)</td>
<td>115±21 (84–193)</td>
<td>103±17 (77–172)</td>
<td>124±24 (101–256)</td>
<td></td>
</tr>
<tr>
<td>Serum LDL (mg/dl)</td>
<td>38±5 (28–76)</td>
<td>26±8 (22–67)</td>
<td>28±9 (27–63)</td>
<td>35±12 (27–98)</td>
<td>52±15 (35–65)</td>
<td>38±9 (24–69)</td>
<td></td>
</tr>
</tbody>
</table>

P<0.05; *P>0.05; HDL: High-density lipoprotein, LDL: Low-density lipoprotein
Table 2: Normal laboratory values for the assessed parameters are as below

<table>
<thead>
<tr>
<th>Lab parameter</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>22–322</td>
<td>10–29</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>0–200</td>
<td>0–200</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>60–130</td>
<td>60–130</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>30–80</td>
<td>30–80</td>
</tr>
</tbody>
</table>

HDL: High-density lipoprotein, LDL: Low-density lipoprotein

**DISCUSSION**

Our results revealed a higher frequency of GSs in female patients with iron deficiency anemia (IDA) than in the control subjects. In animal studies, it has been observed that iron-deficient diets alter hepatic enzyme metabolism which results in a higher level of GB bile cholesterol levels, thereby promoting the formation of cholesterol crystals.\(^{[4,5]}\) We were unable to analyze the biochemical constituents of GS in our patients. Nevertheless, studies from our country reported that the most frequent type of GS was cholesterol GS which is similar to the situation in western countries. The three main factors which play roles in the formation of cholesterol GS is supersaturation of bile with cholesterol, hypersecretion of mucin from GB mucosa and crystal nucleation, and GB hypomotility.\(^{[6,7]}\) In many studies, GSs were found to be more prevalent in females.\(^{[10,11]}\) Our study, similarly, found a higher frequency of GS in female IDA patients, although this was not significant (11.4%). Various studies claimed that this condition was mediated through the effects of estrogens and/or progesterone on bile saturation.\(^{[1,5]}\) Pregnancy is one of the factors which is held responsible for GS formation in females.\(^{[2]}\) Nevertheless, the number of pregnancies in our IDA and control groups was similar. There was a trend toward a higher frequency of GS in male IDA subjects (12%). However, the number of male IDA patients was not high enough. Studies about the prevalence of GS in the normal population reported that age was an important risk factor for GS development.\(^{[1,2]}\) The median age of our IDA patients with GS was higher. It was also claimed that diabetes and chronic liver disease were associated with GS. The results of our study did not reveal a significant role for diabetes in GS formation in IDA patients. Elevated serum triglyceride and decreased HDL levels are also risk factors for GS formation.\(^{[12,14]}\) Obesity is another well-known risk factor for the formation of GS.\(^{[3]}\)

In our study, we found a statistically significant association between lipids, obesity, and GS formation as the levels were significantly higher in females as compared to the males and control population. The innervation of the GB is mainly supplied by the autonomic nervous system; and parasympathetic innervation from the vagus nerve and sympathetic innervation from the splanchnic nerves are also provided to the GB.\(^{[5]}\) GB stasis and difficulty in emptying are factors gaining increasing importance because they might predispose to GS formation.\(^{[16]}\) This mechanism is probably responsible in conditions such as diabetes mellitus, hyperglycemia, pregnancy, progesterone usage, and total parenteral nutrition.\(^{[17,18]}\) Iron is known to have an important role in hepatic enzyme metabolism.\(^{[19]}\) Iron-containing cofactors are fundamental components of the nitric oxide (NO) synthase complex.\(^{[20]}\) NO acts as a putative inhibitory neurotransmitter and it is present throughout the gastrointestinal system.\(^{[21]}\) In addition, it has been demonstrated in prairie dogs that NO is an important for maintenance of basal GB tone and that it acted as an inhibitor of the contractile response of the GB to physiologic stimulators.\(^{[15]}\) In iron deficiency, there is diminished GB neuronal NO synthase which results in altered biliary motility without affecting the hepatic metabolism of cholesterol\(^{[8]}\) and contributes to GB stasis.\(^{[5]}\) In addition, the motilities of GB and sphincter of Oddi are suppressed acutely in iron deficiency because of decreased neuronal NO synthase levels, and compensatory mechanisms return neuronal NO synthase to baseline levels while cholesterol crystal formation increases over time.\(^{[6]}\) NO synthase was demonstrated to be present in neurons of the GB in humans.\(^{[29]}\) Nevertheless, the effect of iron deficiency on NO synthase in humans has not been studied yet. In our study, we found that the GB dysmotility observed in iron-deficient animals was present also in humans. Although it is difficult to explain the mechanism exactly, the higher frequency of GS in our IDA patients might be a result of the tendency to impaired GB motility. One of the limitations of our study was that the number of patients, especially that of males, was not much. Another limitation of our study was that we were unable to perform the biochemical analysis of the constituents of GS. The design of our study was cross-sectional; therefore, cholecystectomy and analysis of GS were not undertaken in our IDA patients with GS. As a result, we found a significantly increased prevalence of GS in our IDA patients. Impaired GB emptying in these patients might have contributed to the higher frequency of GS in IDA.

**CONCLUSION**

In the present prospective study of 100 cases, the following conclusions were drawn. Serum lipid profile of female patients with cholelithiasis was significantly higher and lower ferritin levels than the males and general population. The low serum iron level in one or the other way could have contributed to bile supersaturation with respect to cholesterol and GS formation. However, serum ferritin cannot be taken as a sole diagnostic tool in the diagnosis of...
IDA as its value can vary due to other causes such as iron therapy, hepato cellular disease, and inflammations (since cholecystitis is an inflammatory condition, this could be the reason for the high level of serum ferritin).

REFERENCES