INTRODUCTION
Gallstones are one of the most common problem associated with the gallbladder, affecting millions of people throughout the world. Bile is excreted from liver and gallbladder into Duodenum for digestion. After digestion, if the gallbladder is not emptied out completely, the Bile Juice that remains in the gallbladder can become too concentrated with cholesterol leading to gallstone formation. Cholesterol and calcium bilirubinate are the two main substances involved in gallstone formation. Gallstones derived from bile consists of mixture of cholesterol, bilirubin with or without calcium. Based on their chemical composition, gallstones found in the gallbladder are classified as cholesterol, pigmented or mixed stones. Iron deficiency has been shown to alter the activity of several hepatic enzymes, leading to increased gallbladder bile cholesterol saturation and promotion of cholesterol crystal formation.

AIMS & OBJECTIVE
Attempt to establish a correlation with gallstones and decreased serum iron levels.

MATERIAL & METHODS
This study was a prospective cohort study which included 100 consecutive patients with imaging studies suggestive of Cholelithiasis. The Gallstone surgically removed was crushed with mortar and pestle and then analysed for cholesterol, calcium, phosphate and bilirubin (pigment). Serum samples were analysed for Cholesterol, iron and iron binding capacity.

RESULTS
86% patients had increased cholesterol levels (p=0.04) and 93% had decreased serum Iron levels (p=0.96). The most common type of gallstone was found to be Cholesterol type of gallstone followed by Mixed and Pigment gallstones.

CONCLUSION
Serum cholesterol levels were found to be raised in majority of the patients and serum iron was found to be low in these majority of the patients indicating iron deficiency may play a role in gallstone formation.

KEYWORDS
Gallstones, Cholesterol, Iron, Pigment Stones and Mixed Stones.

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INTRODUCTION: Gallstones are one of the most common problem associated with the gallbladder, affecting millions of people throughout the world.1 Gallstones may occur as one large stone or hundreds of tiny stones. Cholesterol and calcium bilirubinate are the two main substances involved in gallstone formation.2 Gallstones derived from bile consist of mixture of cholesterol, bilirubin with or without calcium. Based on their chemical composition, gallstones found in the gallbladder are classified as cholesterol, pigmented or mixed stones. Gallstones can be mostly white, yellow, brown, black and green coloured. Approximately, 80% of the gallstones are cholesterol gallstones, which chiefly consist of cholesterol and bile salts.3

As per studies, gallstones represent a major problem in many countries. The prevalence of gallstones is 6% in India, 9.2% in Italy, 9.7% in Spain, 28.5% in Chile and 3.1% in Thailand. Mjaland et al 2012 have reported that the incidence of gallstones is correlated with socio-economic conditions and dietary factors.4 Other risk factors as identified by Marscall et al 2007 are marked obesity, family history of gallstone disease, high energy intake, ageing, multiple parity, cholecystitis, and sedentary lifestyle.5 Gallstones are more frequent among patients with certain conditions, such as Crohn’s disease and liver cirrhosis.

Role of Iron in Gallstone Pathogenesis: Iron deficiency has been shown to alter the activity of several hepatic enzymes, leading to increased gallbladder bile cholesterol saturation and promotion of cholesterol crystal formation. Iron acts as a coenzyme for nitric oxide synthase (NOS), and that is important for the maintenance of basal gallbladder tone and normal relaxation.6

Hence, an attempt is done to establish a correlation with gallstone formation and decrease serum iron levels.

**MATERIALS & METHODS:** This study was conducted on patients admitted in the ward of K.J. Somaiya Hospital and Research Centre, Mumbai. This study is a prospective cohort study which included 100 consecutive patients with pain in the upper quadrant of abdomen. Their basic investigation which included a complete Haemogram, Fasting Blood sugar, Urine routine in normal limits and imaging studies which included plain abdominal radiographs and ultrasound studies is in normal limits and the imaging studies suggestive of Cholelithiasis. Detailed history was recorded and thorough clinical examination was performed. The recorded data includes demographics and details like onset, duration, location and progression of abdominal pain, associated symptoms of patient and relevant clinical findings.

**Exclusion Criterion:** Patients who had undergone cholecystectomy, pregnant women, patients with coagulation defects, patients with critical illness or sepsis, medically unfit for surgery. Patients not subjected to imaging studies.

Fasting Blood sample was collected from the patient whose prior written consent was taken. Then basic investigations like CBC were done for the patients. Imaging studies included plain abdominal radiographs and ultrasound studies. The following investigations were done to estimate serum cholesterol and serum iron levels.

**Serum Cholesterol Test:** Serum cholesterol was determined using the Siemens Auto-Pak in vitro Diagnostic Kit.

**Serum Iron:** Serum Iron was measured with the help of the in vitro kit of Clniqa Corporation. Patients were subjected to Cholecystectomy after complete workup and anaesthetic fitness. Gallbladder was sent for histopathological examination and physiochemical analysis of gallstones.

1) The Gallstone surgically removed was crushed with mortar and pestle and then analysed for cholesterol, calcium, phosphate and bilirubin (pigment).

   a) **Test for Cholesterol:** Powdered stone + small portion of ether in a test tube. Typical cholesterol crystals with notched corners are obtained.

      1. Liebermann-Burchard reaction: Chloroform + mixture of acetic anhydride and sulphuric acid. A dark green colour develops rapidly.

   b) **Test for Phosphates:** Powdered stone with molybdate. A canny yellow colour develops rapidly.

   c) **Test for Calcium:** Powdered stone. Add acetic acid and ammonium oxalate solution. If calcium is present, a precipitate of calcium oxalate is formed.

   d) **Test for Bilirubin (Bile pigments):** Powdered stone + Diazro reagent used in Van den Bergh test.

Data was analysed using SPSS (Statistical Package in Social Sciences) version 16 to present percentage distribution.

**RESULTS:**

**Gallstones:** The most common type of gallstone in our study was found to be Cholesterol stone followed by Mixed stone and Pigment stone as seen in Fig. 1 which shows Cholesterol stone-76%, Mixed stone-19% and Pigment stone 5%.

![Fig. 1: Graph showing Frequency of Gallstones](image1)

As seen in Fig. 2, 85% females and 15% males had stones.

![Fig. 2: Graph showing Distribution of Stones Gender Wise](image2)

In Fig. 3, it has been indicated that increased level of Serum Cholesterol (Above 200 mg%) was found in 86% Stone formers and 14% of the stone formers had normal Serum Cholesterol levels (150-200 mg%). This difference between the two levels of Serum Cholesterol (p=0.04) is significant.

![Fig. 3: Graph of Serum Cholesterol Status](image3)
In fig 4, it was seen that in 93% patients, Serum Iron level was decreased (normal range 70-130 µg/dL).

The mean serum iron was found to be highest in mixed gallstones followed by cholesterol stones followed by pigment stone. However, this difference was found to be insignificant (p=0.96).

It was seen that patients having increased serum cholesterol had decreased serum iron though this finding was not statistically significant.

DISCUSSION: Gallstones formation can be induced by many factors other than cholesterol and iron. In our study, it was found that of the 100 patients we studied; 76%, 19% and 5% had cholesterol stones, mixed stones and pigment stones respectively. In similar studies carried out by Saqib et al (cholesterol stones 55%),7 Taher M.A. (cholesterol stones 49%),8 Shareef MK et al(cholesterol stones 54%, mixed stones 40%, pigment stones 6%);9 Jarrar BM et al (cholesterol stones 54 %),10 Channa NA et al (cholesterol stones 68%).11 But in a study carried out by Jaraari et al12 in 2008, in a study on 41 gallstone patients, 39% had pigment stones, 34% mixed stones and 27% cholesterol stones. They concluded that although pigment gallstone was the most common type of gallstones, cholesterol seemed to be the major component in all types of stones. High cholesterol content in cholesterol stones especially suggests super saturation of cholesterol in bile consequent to dyslipidaemia which is an aetiological factor. Even across India, the distribution of type of gallstone is uneven which was confirmed by the study of Tendon RK et al13 and Ananthankrishnan K et al.14

For the formation of cholesterol gallbladder stones, three mechanisms are of major importance: (i) cholesterol super saturation of bile (ii) gallbladder hypomotility and (iii) kinetic, pronucleating protein factors. Wang DQ et al15 found out that cholesterol is only slightly soluble in aqueous media, but is made soluble in bile through mixed micelles with bile salts and phospholipids, precipitation of cholesterol occurs when cholesterol solubility is exceeded (cholesterol saturation index >1) and cholesterol crystals occur at low phospholipid: cholesterol ratios and at relative low phospholipid and high bile salt concentrations. Multilamellar vesicles then fuse and may aggregate as solid crystals. Thus, super saturation of cholesterol in bile may be caused by hypersecretion of cholesterol, or from hyposecretion of bile salts or phospholipids. Serum Cholesterol was found to be increased in 86 patients.

In our study, Serum Cholesterol was found to be increased in 86 patients, this difference between those with increased level and normal was found to be significant (p=0.04). The mean serum cholesterol was found to be highest in patients with Mixed gallstones (255.1) followed by cholesterol gallstones (254.9) followed by pigment gallstone patients (192). Further, Jonkers IJ et al16 and Wang HH et al17 stated that hypertriglyceridaemia is identified as a cause for gallbladder hypomotility as it reduces the gallbladder sensitivity to cholecystokinin, a paracrine hormone that regulates the gallbladder contraction. Gallbladder hypomotility is one of the main causes for cholesterol crystallisation. Ruhl CE et al18 concluded that the lipid profile pattern seen among these patients should be evaluated with caution as impaired lipid homeostasis is a main risk factor for the cardiovascular disease as well. Thus, the development of gallstone disease can be considered as a warning sign to have high risk of cardiovascular disease for the patients with cholesterol gallstone. Aulakh R et al19 suggested -that mean total serum cholesterol and serum triglyceride was found to be higher among gallstone sufferers. Also, Smelt AH20 reported that high serum triglyceride in patients with cholesterol gallstone is a supportive evidence to indicate hypertriglyceridaemia as a risk factor for cholesterol gallstone.

In our study, we found out that 93 patients had decreased serum iron. The mean serum iron was highest among mixed stone patients followed by cholesterol stone followed by pigment stone; however, the difference was found to be insignificant. Sahu S et al21 in a study on 100

### Table 1: Serum Iron Status and gallstone

<table>
<thead>
<tr>
<th>Serum Iron</th>
<th>No. of Patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased</td>
<td>93</td>
<td>93%</td>
</tr>
<tr>
<td>Normal</td>
<td>7</td>
<td>7%</td>
</tr>
</tbody>
</table>

### Table 2: Serum Iron Status and Gallstone

<table>
<thead>
<tr>
<th>Gallstone</th>
<th>No. of patients</th>
<th>Serum Iron Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed stone</td>
<td>19</td>
<td>54.42</td>
<td>18.15</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>76</td>
<td>53.42</td>
<td>16.56</td>
</tr>
<tr>
<td>Pigment stone</td>
<td>5</td>
<td>52.40</td>
<td>12.56</td>
</tr>
</tbody>
</table>

### Table 3: Correlation of Serum Iron with Serum Cholesterol levels

<table>
<thead>
<tr>
<th>Correlation of Serum iron with cholesterol</th>
<th>Pearson Coefficient (R)</th>
<th>R²</th>
<th>Significance (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Cholesterol</td>
<td>-0.001</td>
<td>1.876</td>
<td>0.989</td>
</tr>
</tbody>
</table>
gallstone patients concluded that low serum iron level is a factor in the bile super saturation with respect to cholesterol leading to gallstone formation. Johnston SM et al.22 in a study suggested, Iron deficiency alters the activity of several hepatic enzymes leading to increased gallbladder cholesterol saturation and promotion of cholesterol crystal formation, it also acts as a coenzyme for nitric oxide synthase which synthesises nitric oxide important for the maintenance of gallbladder tone and normal relaxation. Goldblatt MJ et al.23 concluded that iron deficiency leads to alteration of motility of the gallbladder and sphincter of Oddi leading to biliary stasis resulting in cholesterol crystal formation. Roslyn JJ et al.24 found that consumption of diets rich in carbohydrate but deficient in iron alters hepatic metabolism of cholesterol and may be an important aetiologic factor in gallstone formation. Pamuk EG25 in a study on 111 gallstone patients suffering from iron deficiency studied the fasting volume, residual volume and ejection fraction and concluded that increased prevalence of gallstones in patients suffering from iron deficiency might be explained with impaired gallbladder motility. As we have seen, iron is required as a coenzyme for most hepatic enzymes.

Also cholesterol is converted to bile acids, requires NADPH and cytochrome P450 which requires iron containing compounds. Therefore, iron deficiency could be associated with decreased catabolism of cholesterol and thus increase in its level in bile. This in turn leads to precipitation of cholesterol leading to gallstone formation.

Our study showed a negative correlation between serum iron levels and serum cholesterol levels indicating that if serum iron decreases, serum cholesterol increases. However, this correlation was found to be insignificant (p=0.989). Prasad PC26 in a study on 50 gallstone patients also concluded low serum iron level is a factor in bile super saturation with respect to cholesterol leading to gallstone formation which was also supported by studies conducted by Muneesh K.27

CONCLUSIONS: Serum cholesterol levels were found to be raised in majority of the patients and serum iron was found to be low in these majority of the patients indicating iron deficiency may play a role in gallstone formation.

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REFERENCES