COMPARATIVE STUDY OF CHEMICAL COMPOSITION OF STONE AND SERUM IN GALLSTONE PATIENTS


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ABSTRACT: Gallstones are classified into three types based on their composition as cholesterol, pigment and mixed stones, with each type having different epidemiology and risk factors. Additionally, each type of these stones is formed in a different site. Cholesterol stones and pigment stones are predominantly found in the gall bladder and bile canaliculi of liver respectively, while mixed stones occur across the entire biliary tract but finally grow and settle down in gall bladder.1 Gallstones comprise mainly of cholesterol. The other chief components are bilirubin and calcium salts (e.g. phosphates). These substances are also present in the serum of the gallstone patients.2 However, there is not much data available on the correlation between bile and serum levels of these constituents in this patient population. This study was conducted to study this correlation.

Keywords: Bilirubin, Calcium, Cholecystectomy, Cholelithiasis, Cholesterol, Gallstones, Mixed Stones, Pigment Stones.

MATERIALS AND METHODS
Gallstones and blood samples were collected from 40 cholelithiasis patients who underwent cholecystectomy at the Nalanda Medical College, Patna, India. Of the gallstone samples that were collected, 14 were cholesterol type, 13 of the pigment type and the remaining 13 were of the mixed type. With regard to blood samples, 5 ml of blood was collected from all patients. The stones were powdered in a pestle and mortar. For determining the total cholesterol and bilirubin levels, a solution was prepared by dissolving 30 mg of the stone powder in 3 ml chloroform in a test tube, which was then kept in a boiling water bath for 2 min. For determining phosphate and calcium levels, a solution was prepared by dissolving 30 mg of the stone powder in 3 ml 1N HCl in a graduated 10 ml tube and by adding distilled water to make the final volume up to 10 ml. This tube was kept in a boiling water bath for 1 h. Both the prepared solutions were stored at a temperature of 2–8°C when not in use. The blood samples were centrifuged at 2000 rpm for 20 min and the supernatant serum was collected and stored at 4°C when not in use. The various components were measured using the following techniques in both stone solution and sera and their contents were correlated using regression equation.

• Total cholesterol based on enzymic colorimetric estimation employing cholesterol esterase, cholesterol oxidase and peroxidase by commercial enzootic method of Bayer Diagnostic India Ltd.
• Total bilirubin based on bichromatic method by commercial kit method of Bayer Diagnostic India Ltd.
RESULTS
Table 1 depicts the correlation between total cholesterol, total bilirubin, calcium and inorganic phosphate in gall stone and sera of cholelithiasis patients (n=40).

Pigment stone patients: Although a good positive correlation was observed between bilirubin of sera and gall stones (r=0.843), no positive correlation between total cholesterol, calcium and inorganic phosphate of sera and gall stones was observed (r= -0.321, r=0.843 and r= -0.280, respectively).

Cholesterol stone patients: Although no correlation between inorganic phosphate content of sera and gall stones (r= -0.032), there was a moderately positive correlation between total cholesterol, bilirubin and calcium of sera and gall stone (r=0.354, r=0.099, r=0.158, respectively), with the correlation coefficient being highest for cholesterol.

Mixed stone patients: There was a moderately positive correlation between bilirubin and inorganic phosphate of sera and gall stone (r=0.246 and r=0.308, respectively), but not in total cholesterol and calcium of sera and gall stone (r=0.060 and r= -0.398) respectively.

DISCUSSION
The results of our study clearly indicate that there is a positive correlation between the type of stone and the serum level of its component. For example, in case of cholesterol gallstones, there was an elevation in the serum levels of total cholesterol and in pigment stones, serum bilirubin levels were elevated. With respect to calcium and phosphate, both these components showed a positive correlation in pigment stones, while only phosphate showed a positive correlation in cholesterol stones, and calcium did so in mixed stones. Additionally, the type and composition of the gallstone are related to symptoms, and they are a heterogenous clinical entity with different pathogenesis, clinical presentations and warranting different management strategies.7

CONCLUSION
Our study clearly indicates that the etiology for each type of stone is different. The changing epidemiology and the emerging non-surgical interventions for gallstone disease necessitate the definition of target populations for future therapies. By defining patterns of gallstone composition and identifying demographic predictors of gallstone composition in India, the treatment approach can be devised. However, since ours was a small study, further larger trials are warranted to confirm these findings.
Table 1: Correlation between composition of Stone and Serum in Indian Gallstone patients

<table>
<thead>
<tr>
<th>Stone Type</th>
<th>Composition of Stones</th>
<th>Composition of Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TC</td>
<td>Bil</td>
</tr>
<tr>
<td>Pigments Stones</td>
<td>Mean</td>
<td>498.92</td>
</tr>
<tr>
<td>STD</td>
<td>87.23</td>
<td>0.37</td>
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<tr>
<td>CC(r)</td>
<td>-0.321</td>
<td>0.84</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Mean</td>
<td>485.28</td>
</tr>
<tr>
<td>STD</td>
<td>95.47</td>
<td>0.21</td>
</tr>
<tr>
<td>CC(r)</td>
<td>0.354</td>
<td>0.099</td>
</tr>
<tr>
<td>Mixed Stones</td>
<td>Mean</td>
<td>450.61</td>
</tr>
<tr>
<td>STD</td>
<td>73.95</td>
<td>0.232</td>
</tr>
<tr>
<td>CC(r)</td>
<td>0.060</td>
<td>0.246</td>
</tr>
</tbody>
</table>

REFERENCES