Bile composition in patients with noncirrhotic portal hypertension

H ullah wani, R Kochhar, B Nagi, C Nain, B Ahmed

Citation

Abstract
Background and Aims: Stasis is an important predisposing factor for gall bladder sludge and hence gall stones. The present study was done to find out whether bile composition is altered in patients with noncirrhotic portal hypertension with portal biliopathy. Methods: 35 patients with noncirrhotic portal hypertension with portal biliopathy documented on ultrasound, ERCP / MRCP were prospectively evaluated for gallbladder emptying response to fatty meal by ultrasonography using Ellipsoid method. In 10 patients with extra hepatic portal vein obstruction, bile was collected from common bile duct during ERCP for analysis. Ten healthy age and sex matched controls were taken for comparison. Results: The mean age of the patients in our study was 29.93 +11.7 years. Gallstones were seen in 16.1% and sludge was present in 9.6% of patients of extra hepatic portal vein obstruction . Gallbladder varices were seen in 29.03% patients on USG/ Doppler ultrasonography. Gallbladder ejection fraction was significantly less in extra hepatic portal vein obstruction patients than controls (40.6±16.73% vs 57.71±13.25%, p=0.007). Patients with extra hepatic portal vein obstruction had significantly higher cholesterol levels (10.32+2.96) than in controls (6.86+3.36) (p<.02) and the bile acid levels were also higher in patients than controls although statistically not significant (p=0.07)). The cholesterol saturation index was comparable in patients with extra hepatic portal vein obstruction and controls. Conclusions: Ejection fraction was significantly lower in extra hepatic portal vein obstruction compared to controls. Extra hepatic portal vein obstruction patients had significantly higher cholesterol levels than in controls and the bile acid levels were also higher in patients than controls although statistically not significant.

INTRODUCTION
Noncirrhotic portal hypertension is the portal hypertension caused by pre-hepatic or intra-hepatic lesions in the absence of cirrhosis or venous outflow tract obstruction. It includes extra hepatic portal venous obstruction (EHPVO) and noncirrhotic portal fibrosis (NCPF). Other causes are, Schistosomiasis, veno-occlusive disease and congenital hepatic fibrosis.

In the western countries, cirrhosis accounts for more than 90% cases of portal hypertension and EHPVO constitutes less than 5% of cases. In India, noncirrhotic portal hypertension constitutes up to 50% of all patients of portal hypertension. The most common site of block is at the portal vein formation [90%] and total block of splenoportal axis is seen in 10% of cases. The etiology and clinical features are different in children and adults. In children, the causes are umbilical sepsis, umbilical catheterization and developmental anomalies, other causes include dehydration, multiple exchange transfusions and sepsis. In adults, important causes are neoplastic diseases, infections, pancreatitis, myeloproliferative disorders and hypercoagulable state. The cause of portal vein block is obscure in 50% of cases. Such patients generally present with repeated well tolerated episodes of hematemesis and massive splenomegaly. EHPVO, on the other hand, is generally diagnosed in a young adult presenting with repeated hematemesis and evidence of occlusion in the main portal vein with portal cavernoma formation and normal histology of liver.

Portal biliopathy is defined as abnormalities of the extrahepatic, intrahepatic bile ducts and gall bladder wall in patients with portal hypertension. The abnormalities include strictures of intrahepatic and extrahepatic bile ducts,
segmental dilatation, ectasias and pruning and tortuous dilated blood vessels in or around the gall bladder wall or in the bed of the gall bladder fossa. Portal biliopathy has been reported to occur in 80-100% of patients of EHPVO, 40% patients of NCPF and 30% patients of cirrhosis. Presence of portal vein thrombosis is an important determinant for the development of gall bladder varices. Although, biliary abnormalities are common in portal hypertension, only a few patients present with jaundice, pain and cholangitis.

An association of defective gall bladder emptying with cholelithiasis is well known. Previous studies of gall bladder motor function in patients with portal hypertension have yielded conflicting results. Normal and impaired gall bladder emptying have been described previously. The present study was planned to know whether gall bladder varices and portal biliopathy is associated with defective emptying of gall bladder and whether it leads to increased stasis of bile and alters bile composition which may result in cholelithiasis or choledocholithiasis.

**MATERIAL AND METHODS**

**PATIENT POPULATION:**

Thirty five patients of noncirrhotic portal hypertension attending the gastroenterology services of PGIMER, Chandigarh were enrolled for study.

**INCLUSION CRITERIA:**

Patients of non cirrhotic portal hypertension with portal biliopathy documented on ultrasonography endoscopic retrograde cholangiography or magnetic resonance cholangiography.

Ten healthy age and sex matched controls were taken for comparison.

**EXCLUSION CRITERIA:**

Previously diagnosed gall stone disease prior to the onset of EHPVO.

Hemolytic anemia.

Obesity.

Cirrhosis.

Systemic sclerosis, diabetes and other forms of autonomic neuropathy.

Drugs which interfere with gall bladder motility like erythromycin, pirenzpine, cisapride taken within 2 weeks of the study.

Patients with prior shunt surgery.

**PROCEDURE PROTOCOL:**

A written consent was obtained from each subject. A detailed history regarding duration of illness, number of episodes of GI bleed, variceal eradication therapy and history of biliary colic, jaundice and cholangitis was obtained. Routine liver function tests were done in all the patients. Upper gastrointestinal endoscopy for documentation of varices and ultrasonography / doppler for paracholedochal and pericholecystic collaterals were done in all patients. Endoscopic retrograde cholangiopancreatography / Magnetic resonance cholangiopancreatography (ERCP/MRCP) for documentation of portal biliopathy was done in all patients.

**GALL BLADDER FUNCTION:**

After an overnight fast, gall bladder volume was measured by real time ultrasonography (3.5 MHZ transducer RT 3600, Rancho, USA). All examinations were performed by the same investigator. Longitudinal and axial cross sectional images of gall bladder at its largest diameter was obtained. The volume of gall bladder was calculated using ellipsoid method as described by Dodd’s et al. Volume=0.52xLxWxH where L : is the length, W : is the width and H : is the height or depth of gall bladder.

Each measurement was made in triplicate in rapid sequence at each point and mean value was calculated. Gall bladder emptying was studied in response to fatty meal. The meal consisted of 50 grams of fat, 20 grams of protein and 50 grams of carbohydrate.

At time 0, patients were offered fatty meal and gall bladder images were obtained every 15 minutes for 60 minutes.

Parameters of gall balder contractility calculated for each subject and control were as follows.

Basal volume (ml) : the resting volume of gall bladder after an overnight fast.

Residual volume (ml) : the smallest volume observed at any time after fatty meal.
Ejected volume (ml) : the difference between basal volume and corresponding residual volume.

Ejection fraction(%) : \[1-(\text{residual volume}) / (\text{fasting volume}) \times 100\].

Abnormal gall bladder emptying was said to be present when the ejection fraction was lower than the lowest ejection fraction obtained in healthy controls.

**BILE COMPOSITION**

In 10 patients with EHPVO, bile was collected from CBD during ERCP. Intravenous infusion of 200 ml of essential amino acid solution (commercially available as Hermin) was given in each subject 30 minutes prior to ERCP. The bile collected was divided into 3 aliquots, one aliquot was examined within 30 minutes under a polarizing microscope. The second aliquot was stored at 37°C for repeat microscopic examination if required and third aliquot was subjected to chemical examination.

Bile Microscopy : A 2.5 ml of bile was centrifuged at 2000 rpm for 10 minutes, the supernatant was discarded and sediment was examined under polarizing microscope. The number of cholesterol crystals was counted under cover slip at low magnification (10x).

Chemical analysis of bile : The bile was subjected to chemical analysis for cholesterol, phospholipids and total bile acids.

Total lipid extraction was done by adding 1ml of bile to 20 volumes of chloroform methanol (2:1 volume / volume) mixture and keeping in a shaker for 2 hours. It was then filtered and washed with 0.2 volumes of 0.15 M aqueous sodium chloride solution. After thorough mixing two phases were formed. The upper phase was discarded and the lower phase was dried under nitrogen. The extract was saved at -20 degree centigrade till analysis. Cholesterol and phospholipids were estimated colorimetrically in the lipid extract.

Cholesterol estimation was done using standard (2mg / ml in isopropanol), 2.5% ferric chloride solution in orthophosphoric acid, digitoni (1% solution in absolute alcohol–water mixture), isopropanol, concentrated sulphuric acid and glacial acetic acid. 50 μl and 100 μl aliquots of each bile extract (diluted 1:5) were dried and 1 ml isopropanol was added to all samples. Free cholesterol was precipitated using digitonin. Standards containing 20 μg, 40 μg and 80 μg of cholesterol and a blank were set up in duplicates. 3 ml of concentrated sulphuric acid was added to all the tubes. After cooling for 10 minutes, absorbance was read at 560 nm.

Phospholipid estimation was done using standard potassium dihydrogen orthophosphate stock concentration 35mg/100ml (8mg/100ml of 70% perchloric acid), 2.5% ammonium molybdate in 5N sulphuric acid and Fiske-Subbarow reagent. The stock solution was diluted 8 times to get a working concentration of 1 mg /100 ml. Fiske-Subbarow reagent was prepared by dissolving 30 gms of sodium bisulphate in water to a final volume of 200 ml. To 195 ml of the above solution, 0.5 gm of 1-amino 2-naphthol 4-sulphonic acid was added and left for 3 hours to dissolve. 5 ml of 20% sodium sulphite was then added with mixing. The solution was filtered and stored in a dark bottle at 4 °C. 25 μl and 50 μl aliquots of lipid extract of each bile sample (diluted 1:5) were added to separate test and dried. Standards containing 1 μg, 2 μg and 4 μg of phosphorus and a blank were set up in duplicates. 0.5 ml of perchloric acid was added to all the test tubes. The bile samples were kept in a sand bath 2-3 hours to allow digestion of lipids. The final volume of each mixture was made to 3 ml with distilled water. The test tubes were vortexed to allow thorough mixing 0.5 ml of ammonium molybdate solution and finally 0.2 ml of Fiske-Subbarow reagent were added. The tubes were kept in boiling water bath for 10 minutes and then cooled for about 20 minutes. Absorbance was measured at 700 nm.

The total bile acids were estimated enzymatically using 3β-hydroxysteriod dehydrogenase assay as described by Turley and Dietschy. The enzyme was obtained in purified power form from Sigma chemical company, St Luis USA. The enzyme solution of strength 10 units per ml was prepared in cold Tris HCI-EDTA (0.03M-0.001M) buffer at pH 7.2. The reaction mixture consisted of 1) 0.5 ml of Tris HCI-EDTA (0.03M-0.001M) at pH 9.5

2) 0.33 ml of Hydrazine hydrate (1M) at pH 9.2

3) 0.1 ml of NAD (7mm) at pH 7.0 and

4)50 ml of sample bile diluted 1:100 in Tris HCI-EDTA (0.03M-0.001M) at pH 7.2.

Parallel reaction was conducted using 25 ml of diluted bile and 25 ml of diluant in place of 50 ml of diluted bile sample. 20 μl of the enzyme solution was then added to the above mixture and incubated at 37 C for 45 minutes. Spectrophotometric readings were taken at 340 nm.
The maximum cholesterol solubility in bile was estimated from molar phospholipids/(phospholipids + bile salt) ratio & total lipid concentration in gram/dl using Carey’s tables. Cholesterol saturation index was estimated by dividing the actual cholesterol concentration in moles by the maximum cholesterol solubility.

STATISTICAL ANALYSES:
Statistical analysis was done using Statistical Package for Social Sciences (SPSS), version 10.0, SPSS Inc. Illinois for PC Windows. The results were expressed as mean±SD (continuous variables) and percentages (Categorical variables). Comparison between categorical variables was done by Pearson Chi-square test (Fisher Exact Test wherever appropriate) and between continuous variables by student’s t test. Where the data was not normally distributed, comparison between continuous variables was done by Mann whitney U test. All t values were calculated as two-tailed and a P value of <0.05 was taken as statistically significant.

RESULTS
Thirty five patients with noncirrhotic portal hypertension on gastroenterology follow up were enrolled. Out of these 35 patients (EHPVO 31,NCPF 4) 30 (85.8%) had portal biliopathy on ERCP/MRCP. Gall bladder emptying was studied in 30 (EHPVO 26,NCPF 4) patients with portal biliopathy and bile analysis was performed in 10 (EHPVO 8,NCPF2) patients. Ten healthy controls were taken for comparison.

DEMOGRAPHIC FEATURES
AGE AND GENDER DISTRIBUTION
The mean age of patients was 29.9 ± 11.2 years with a range of 9 – 58 years and the age of controls was 26.9 ± 8.2 with a range of 17 – 47 years. Out of 35 patients 20 were males and 15 were females and in controls male and female were equal. Both age and gender were comparable in two groups (p=NS). (Table 1).

GALLBLADDER EMPTYING IN PATIENTS WITH NONCIRRHOTIC PORTAL HYPERTENSION AND CONTROLS
The fasting gallbladder volume was less in EHPVO patients than controls (17.6± 13.71 vs 19.2 ± 6.8, p= 0.72) and residual volume was more in patients than controls (10.32 ± 6.48 vs 7.7± 3.3, p= 0.31). Gallbladder ejection fraction was significantly less in patients with portal biliopathy than controls after a fatty meal (40.6± 16.73 vs 57.7± 13.2, p=0.005). (Table 2).

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Figure 1
Table 1: Age and Gender distribution

<table>
<thead>
<tr>
<th>Gender</th>
<th>Patients,N=35</th>
<th>Control,N=10</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>20(57.14%)</td>
<td>5(50%)</td>
<td>25(55.5%)</td>
</tr>
<tr>
<td>Female</td>
<td>15(42.85%)</td>
<td>5(50%)</td>
<td>20(44.5%)</td>
</tr>
<tr>
<td>Age(ys) Mean</td>
<td>29.93</td>
<td>26.98</td>
<td>5.02</td>
</tr>
<tr>
<td>Total</td>
<td>35(100%)</td>
<td>10(100%)</td>
<td>45(100%)</td>
</tr>
</tbody>
</table>

Figure 2
Table 2: Gallbladder emptying response to fatty meal in patients and controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total Patients</th>
<th>P value</th>
<th>Controls</th>
<th>P value</th>
<th>EHPVO</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting volume (ml)</td>
<td>17.71±12.7</td>
<td>0.72</td>
<td>19.21±6.89</td>
<td>0.72</td>
<td>17.81±13.71</td>
<td>0.005</td>
</tr>
<tr>
<td>Residual volume (ml)</td>
<td>9.82±6.21</td>
<td>0.31</td>
<td>7.7±3.31</td>
<td>0.005</td>
<td>10.32±6.48</td>
<td>0.005</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>40.72±14.49</td>
<td>0.005</td>
<td>57.13±13.25</td>
<td>0.007</td>
<td>40.6±16.73</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Gallbladder emptying in patients with noncirrhotic portal hypertension with and without gallbladder varices

The fasting gallbladder volume was less (15.08±9.48) in patients with gallbladder varices than without varices (18.83±13.93), p=0.46. There was no significant difference in the ejection fraction in both the groups. (Table 3).

Figure 3
Table 3: Gallbladder emptying response to fatty meal in patients with and without gallbladder varices

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GB Vancies (+)</th>
<th>GB Vancies (-)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting volume (ml)</td>
<td>15.08±9.48</td>
<td>18.83±13.91</td>
<td>0.46</td>
</tr>
<tr>
<td>Residual volume (ml)</td>
<td>7.78±2.22</td>
<td>10.7±7.16</td>
<td>0.24</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>38.46±20.58</td>
<td>41.69±14.88</td>
<td>0.63</td>
</tr>
</tbody>
</table>

BILE COMPOSITION IN PATIENTS WITH NONCIRRHOTIC PORTAL HYPERTENSION AND CONTROLS
Biliary cholesterol was significantly higher (10.8±3.09) in EHPVO patients with portal biliopathy than controls (6.87...
Bile composition in patients with noncirrhotic portal hypertension

Bile composition in patients with noncirrhotic portal hypertension

±3.36) (p=0.02). Bile acid levels were also higher in patients (86.64±42.8) than in controls (53.68±24.03) (p=0.06). However cholesterol saturation index was not significantly different than controls. (Table 4).

**Figure 4**
Table 4: Bile composition in patients and controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P value</th>
<th>Control (n=10)</th>
<th>EHPVO (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mM/L)</td>
<td>0.02</td>
<td>2.96</td>
<td>3.36</td>
</tr>
<tr>
<td>Phospholipid (mM/L)</td>
<td>0.1</td>
<td>10.53</td>
<td>24.03</td>
</tr>
<tr>
<td>Bile acid (mM/L)</td>
<td>0.07</td>
<td>53.68</td>
<td>66.44</td>
</tr>
<tr>
<td>Total Lipid (g/dL)</td>
<td>0.17</td>
<td>4.26</td>
<td>2.25</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.03</td>
<td>2.50</td>
<td>1.07</td>
</tr>
</tbody>
</table>

**CORRELATION OF BILE COMPOSITION IN PATIENTS WITH NONCIRRHOTIC PORTAL HYPERTENSION WITH DISEASE DURATION**

The cholesterol levels were not effected by duration of disease. The values were comparable in patients with duration of more or less than one year (10.48±3.85 vs 10.15±2.21, p=1). (Table 5).

**Figure 5**
Table 5: Bile composition in patients with different symptom duration

**BILE COMPOSITION IN PATIENTS WITH NONCIRRHOTIC PORTAL HYPERTENSION WITH AND WITHOUT GALLBLADDER VARICES**

Biliary phospholipids and bile acids were higher in patients without varices and cholesterol saturation index was significantly higher (3.53±1.7 vs 1.56±0.7) in patients with gallbladder varices. p =0.03. (Table 6).

**DISCUSSION**

Cholangiographic findings described in EHPVO are similar to those of primary sclerosing cholangitis and include strictures of intrahepatic and extrahepatic bile ducts, segmental dilatation, ectasias and pruning. The pathogenesis of these changes revolves around compression of intrahepatic and extrahepatic bile ducts, by paracoledochal varices and ischemic insult. Similar varices are also seen around the gallbladder. Gallbladder varices are tortuous dilated blood vessels in or around the gall bladder wall or in the bed of the gall bladder fossa. Whether these collaterals impede the contractility of the gall bladder wall or impair motor function of the gall bladder is exactly not known. Gall bladder varices seen in patients with portal biliopathy may be responsible for gallbladder stasis. Increased incidence of cholelithiasis and choledocholithiasis has been reported in patients with portal biliopathy. There are conflicting reports about the significance of these collaterals.

In the present study, gall bladder varices were present in 9 (29.03%) of 31 patients of EHPVO. In the study by Chawla et al, 12 (34%) of 35 patients with EHPVO, 5 (13%) of the 38 cirrhotics and 7 (24%) of 29 NCPF patients were reported to have gall bladder varices. In another study by Malkan et al gall bladder varices were detected in 11 (55%) and choledochal varices in 9 (45%) of 20 patients. Endoscopic ultrasound (EUS) has higher sensitivity for detection of gall bladder varices and choledochal varices than transabdominal USG and doppler. In the only such study by Palazzo et al 9 (43%) of the 21 EHPVO patients were having gall bladder varices and 11 (52%) were having pericholedochal varices. The varices were seen in the wall of gall bladder and surrounding the common bile duct on EUS.

The gall bladder motility in EHPVO can be affected in two
ways. Firstly in the presence of strictures or indentations in the CBD (as demonstrated by ERCP or MRCP) can lead to the dilatation of biliary radicals and increased gall bladder volume with or without decrease in ejection fraction. On the other hand presence of gall bladder varices can decrease the compliance of gall bladder and thereby decreasing gall bladder distensibility. 20

The fasting gall bladder volume of the patients of EHPVO (17.7±2.7 mm$^3$) was comparable with controls (19.21±6.89 mm$^3$), p=0.72. The ejection fraction of these patients of EHPVO was significantly low (p<0.007) as compared to controls. The explanation for this low ejection fraction could be because of the presence of gall bladder varices in the wall and in the gall bladder fossa which makes it less compliant.

Gall stones were seen in 5 (16.1%) and sludge was present in 3 (9.6%) of 31 patients. The prevalence of gallstones in local population where the study was done is 3.3% . 21 This is much less as compared to the patient population in this present study thereby suggesting increased prevalence of gallstones in noncirrhotic portal hypertension. The low ejection fraction seen in patients with EHPVO suggests gallbladder stasis and thus increased propensity to gallstone formation. However some workers have reported the occurrence of hepatolithiasis in patients with EHPVO. Tanaka et al reported five (45.5%) cases of hepatolithiasis in 11 patients of EHPVO. The stones were usually made of calcium bilirubinate. 22

Initially cholesterol super saturation was thought to be sufficient for gall stone formation. But later it was discovered that super saturated bile is commonly secreted in many persons without gall stones. Cholesterol is essentially insoluble in water and therefore relies on the detergent activity of the bile salts and polar phospholipids to stay in solution. Cholesterol, phospholipids and bile salts are major lipid components in the bile . A change of either of these would result in alteration of the bile composition and therefore lithogenicity of the bile . Carry and Small investigated the solubility limits of the cholesterol in relation to varying amounts of lecithin and bile salts and calculated cholesterol saturation index (CSI). If CSI is greater than one, saturated bile is present and cholesterol can precipitate out of solution. 15 The composition of bile acid pool is also important determinant of bile lithogenicity. The more hydrophobic bile acids are the greater is its ability to induce cholesterol secretion and suppress bile acid synthesis. The combination of increased cholesterol secretion and decreased bile acid synthesis leads to more lithogenic bile.

In the present study the patients with EHPVO had significantly higher cholesterol levels(10.32±2.96) than in controls (6.86±3.36) ( p<0.02) and the bile acid levels were also higher in patients than controls although statistically not significant (p=0.07) . The cholesterol saturation index were comparable in patients with EHPVO and controls.

Bile acid concentration was increased in patients with microlithiasis than the control group. Sharma et al found that patients with microlithiasis had biliary lipid pattern similar to the patients with cholesterol gall stone disease. 23 Super saturation of bile with cholesterol is a necessary for gall stone formation. The primary defect is currently believed to the hyper secretion of cholesterol. However other factors which effect the formation of cholesterol crystals as well as abnormal motility of gall bladder or biliary stasis are important. Changes in the composition of bile can alter the bile lithogenicity.

In this study higher biliary cholesterol seen in EHPVO patients may be due to impaired gallbladder motility in response to a fatty meal. The presence of varices within gallbladder wall may also impair cholesterol absorption thus, increasing bile cholesterol in a subgroup of patients.

SUMMARY

Thirty five patients with noncirrhotic portal hypertension (EHPVO 31, NCPF 4) with portal biliopathy were prospectively evaluated for gallbladder function. Ten patients of portal biliopathy and 10 controls were studied for bile composition.

Gallstones were seen in 16.1% and sludge was present in 9.6% of EHPVO patients. Gallbladder varices were seen in 29.03% patients on USG/ Doppler ultrasonography.

Gallbladder emptying response to fatty meal was studied in 30 patients ( EHPVO 26,NCPF 4), with portal biliopathy by ultrasonography using Ellipsoid method. Gallbladder ejection fraction was significantly less in EHPVO patients than controls(40.6±16.73% vs 57.71±13.25%, p=0.007) . Bile analysis in EHPVO patients revealed that mean cholesterol level is significantly higher than in controls (10.30±2.96mM/L vs 6.86±3.36 mM/L,p=0.02) however cholesterol saturation index was not significantly different in the two groups (p=NS).

CONCLUSION

Gallbladder contractility is poorer in patients with EHPVO.
having biliopathy, their ejection fraction is significantly lower as compared to controls. Analysis of bile of patients with EHPVO showed that they had a higher mean cholesterol level than controls. These two observations may explain the high prevalence of gallstones in EHPVO patients.

References

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