Investigation into celiac disease in Indian patients with portal hypertension

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Received: 27 December 2013 / Accepted: 20 August 2014 © Indian Society of Gastroenterology 2014

Abstract

Background There is limited data on celiac disease in patients with cryptogenic cirrhosis or idiopathic noncirrhotic intrahepatic portal hypertension (NCIPH). Our objective was to evaluate for celiac disease in patients with portal hypertension in India.

Methods Consecutive patients with portal hypertension having cryptogenic chronic liver disease (cases) and hepatitis B- or C-related cirrhosis (controls) were prospectively enrolled. We studied tissue transglutaminase (tTG) antibody and duodenal histology in study patients.

Result Sixty-one cases (including 14 NCIPH patients) and 59 controls were enrolled. Celiac disease was noted in six cases (including two NCIPH patients) as compared to none in controls. In a significant proportion of the remaining study subjects, duodenal biopsy showed villous atrophy, crypt hyperplasia, and lamina propria inflammation, not accompanied by raised intraepithelial lymphocytes (IELs); this was seen more commonly in cases as compared to controls. An unexpectedly high rate of tTG antibody positivity was seen in study subjects (66 %) of cases as compared to 29 % in controls (p-value<0.001), which could indicate false-positive test result.

Conclusion In this study, 10 % of patients with unexplained portal hypertension (cryptogenic chronic liver disease) had associated celiac disease. In addition, an unexplained enteropathy was seen in a significant proportion of study patients, more so in patients with cryptogenic chronic liver disease. This finding warrants further investigation.

Keywords Chronic liver disease · Cirrhosis · Cryptogenic · Enteropathy · Intestinal permeability · Noncirrhotic

Introduction

Celiac disease is prevalent in 1 % of the general population in Western studies. A study from Sweden reported 15 times higher prevalence of celiac disease in patients with cryptogenic chronic liver disease, compared to general population [1]. “Cryptogenic” chronic liver disease is the most common cause of portal hypertension at our center currently [2]. At our center, in 2009–2010 [3] and 2005–2007 [4], 39 % to 48 % of patients with clinical diagnosis of cryptogenic chronic liver disease who underwent liver biopsy had idiopathic noncirrhotic intrahepatic portal hypertension (NCIPH). NCIPH is caused by a microangiopathy of portal vein branches. In a report from the UK, increased prevalence of celiac disease (16 %) was noted in NCIPH patients, and the presence of celiac disease was a determinant of liver transplant-free survival in these patients [5].

Recent studies have reported prevalence of celiac disease in India of 0.3 % to 1 % in the general population [6, 7]. This study aimed to investigate prevalence of celiac disease in portal hypertensive patients with cryptogenic chronic liver disease and NCIPH.
Methods

Ascertainment of cases and controls

Consecutive patients with portal hypertension managed in our department with cryptogenic chronic liver disease (cases) and those with hepatitis B- or C-related cirrhosis (controls) were prospectively recruited into the study between January 2009 and December 2010.

Patients having hepatocellular carcinoma, hepatic venous outflow tract obstruction, or portal vein thrombosis at time of initial presentation were excluded from the study. Patients who did not provide consent were also excluded from the study. Cirrhosis was diagnosed on the basis of suggestive clinical, biochemical, and imaging features. Portal hypertension was defined as presence of esophageal/gastric varices and/or high gradient ascites.

All study cases underwent the following etiological evaluation: history of alcohol intake, features of metabolic syndrome (body mass index, blood sugars, lipid profile), ultrasound abdomen with Doppler of portal vein and hepatic venous outflow tract, HBsAg, HCV antibody, autoantibodies (AMA, SLA, LKM, ANA), serum ceruloplasmin, and iron studies. Patients with cirrhosis with portal hypertension, with negative noninvasive evaluation for cause of liver disease, were labelled as cryptogenic chronic liver disease.

In patients with cryptogenic chronic liver disease, liver biopsy with measurement of hepatic venous pressure gradient (HVPG) was done when feasible. We defined idiopathic NCIPH when five criteria were met: presence of portal hypertension, patent hepatic and portal veins on Doppler, no etiology for liver disease identified, absence of cirrhosis or advanced fibrosis on liver biopsy, and lack of another pathology known to cause histological changes similar to NCIPH (e.g. sarcoidosis) [8].

Controls were patients with hepatitis B- or C-related chronic liver disease with portal hypertension.

Investigation into celiac disease

1. Serum IgA tissue transglutaminase (tTG) antibody determination was done in all study patients at baseline by a commercially available solid-phase enzyme immunoassay kit which employs human recombinant antigen (AESKULISA Celichek, Germany). Based on the manufacturer’s instructions, tTG antibody titers <15 U/mL were interpreted as negative, 15–20 U/mL as borderline positive, and >20 U/mL as positive. For our study, we took titers >20 U/mL as positive for celiac disease and ≤20 U/mL as negative.

2. In tTG antibody-positive patients, symptoms pertaining to celiac disease were assessed and, if present, graded in severity as per symptom score by Kurppa et al. [9] as none, mild (occasional abdominal pain, flatulence, diarrhea, belching, tiredness, or joint pains), moderate (symptoms more persistent, disturbing normal life), and severe (significant daily symptoms restricting normal life or excess weight loss).

3. Whenever feasible, multiple biopsies were obtained from the second part of duodenum. Duodenal mucosal histological changes were assessed by a pathologist who was blinded to clinical details. In particular, the following features (of celiac disease) were looked for on duodenal biopsy: raised intraepithelial lymphocytes, crypt hyperplasia, villous atrophy, lamina propria inflammation, and subepithelial fibrosis. Presence of any severity of villous atrophy was considered as duodenal mucosal architectural change.

4. In tTG antibody-positive patients, duodenal histological changes were interpreted according to Marsh-Oberhuber grading [10, 11]: accordingly, Marsh 0: normal; Marsh I: raised intraepithelial lymphocytes (IEL); Marsh II: crypt hyperplasia; and Marsh III: villous atrophy. Based on the degree of villous atrophy, Marsh III was further subclassified as follows: IIIA (mild villous atrophy), IIIB (moderate/subtotal villous atrophy), and IIIC (severe villous atrophy). As per Oslo definitions [12], celiac disease was defined as tTG antibody positive with duodenal biopsy showing raised IELs with or without architectural changes.

Gut permeability

This was assessed by lactulose-mannitol (L/M) assay, which assesses the relative percentage timed excretion of the ingested dose of lactulose and mannitol in the urine (L/M ratio=percent lactulose excretion/percent mannitol excretion). L/M ratio >0.07 was designated as positive and interpreted as an evidence of increased gut permeability.

Assay for cardiolipin antibody

Serum cardiolipin antibodies (IgM, IgG, and IgA) were tested using enzyme immunoassay (Varelisa kit). Titers <10 U/mL were considered as negative, 10–15 U/mL as equivocal, and >15 U/mL as positive.

Assessing severity of liver disease

Child’s score was used to grade liver disease severity. Patients in Child’s class A were termed to have early liver disease, while those in Child’s classes B or C were termed to have late liver disease.
Assessment of tTG antibody by different commercial kits

As we noted the unexpectedly high rate of tTG antibody positivity in cases and controls, we assayed serum samples (stored at −20 °C) by two other commercially available tTG antibody test kits—EUROIMMUN (EUROIMMUN AG, Germany; ≥20 relative units/mL as positive) and INOVA QUANTA Lite™ (INOVA Diagnostics, USA.; ≥20 units/mL as positive).

Follow up evaluation after gluten-free diet in patients with celiac disease

Patients with celiac disease were counselled by a dietician to take gluten-free diet and were followed up till the end of this study. In patients reviewed after being on gluten-free diet for >6 months, symptoms, tTG antibody titers, duodenal histology, intestinal permeability, and severity of liver disease were reassessed at follow up.

Variables were expressed as median and range (continuous) as or as numbers with percentage (discrete). Unpaired and paired nonparametric tests were used to compare various continuous variables in the two study groups and assess changes at follow up. Fisher’s exact test or chi-squared test was used for comparing discrete variables across groups. P-value of ≤0.05 was considered statistically significant. The study was approved by the institutional review board and ethics committee.

Results

Of 120 portal hypertensive patients enrolled into the study, 61 were cases with cryptogenic chronic liver disease and 59 were controls with hepatitis B- (39 patients)/C- (20 patients) related cirrhosis. Demographics and baseline investigations in the control patients with hepatitis B- (39 patients)/C- (20 patients) related cirrhosis. The liver biopsies were reassessed at follow up.

Variables were expressed as median and range (continuous) as or as numbers with percentage (discrete). Unpaired and paired nonparametric tests were used to compare various continuous variables in the two study groups and assess changes at follow up. Fisher’s exact test or chi-squared test was used for comparing discrete variables across groups. P-value of ≤0.05 was considered statistically significant. The study was approved by the institutional review board and ethics committee.

Celiac disease in study patients

Of the 40 cases and 17 controls who were tTG antibody positive, 37 and 11 underwent duodenal biopsies, respectively (Fig. 1). Duodenal biopsy was consistent with celiac disease in six cases (March I, 2; Marsh IIIA, 3; Marsh IIB, 1) as compared to none in controls. The tTG antibody titer in these six celiac disease patients was 88 U/mL; 21–300 U/mL; median, range.

Of these six patients with celiac disease (five males; age, 46, 34–57 years; median, range), three patients were from eastern India and one each from western, southern, and northern parts of India. Two of the six celiac disease patients had NCIPH, and the other four patients had cryptogenic chronic liver disease (they did not undergo liver biopsy). Five patients belonged to Child’s class A and one patient was Child’s class B. Only one patient had mild classical symptoms suggesting celiac disease, while five patients had iron deficiency anemia and one patient had low serum vitamin B₁₂ level at presentation. Three of six patients tested had increased gut permeability.

Three patients (two with NCIPH) were followed up (25, 12–58 months; median, range) for at least 1 year on gluten-free diet. In two of these patients, tTG antibody became negative with improvement in duodenal biopsy (IIIA → 0 and IIIA → 1). In one patient, although the antibody titer decreased (300 U/mL → 117 U/mL), there was no improvement in duodenal histology (IIIB → IIIB). In two patients who had repeat L/M ratio assessed, there was improvement in one. In one patient, there was worsening of liver disease (Child’s class A → Child’s class B), but in the other two, the liver disease remained stable (Child’s class A → Child’s class A). The platelet counts in these patients remained unchanged.

Enteropathy, other than celiac disease, in study patients

Duodenal histological findings in 86 (53 cases and 33 controls) study patients are shown in Table 2. While villous atrophy and lamina propria inflammation were significantly more common in cases as compared to controls, crypt
hyperplasia, raised intraepithelial lymphocytes, and fibrosis were similar in both the groups. Villous atrophy and lamina propria inflammation in cases compared to controls were even more pronounced in patients in Child’s A; in addition, crypt hyperplasia was also significantly more often noted in cases than controls in Child’s class A (Table 2). There was no difference in duodenal histology in cases and controls in late stage of liver disease (data not shown).

tTG antibody titers were higher in patients with villous atrophy (36, 0–300 U/mL; median, range; \( n = 26 \)) as compared to patients with no villous atrophy (16, 0–300 U/mL, \( n = 57, p\)-value=0.017). Duodenal biopsy done in the 6/7 patients (all cases) with tTG antibody >100 U/mL showed raised IELs (in three patients), mild villous atrophy (two patients), moderate villous atrophy (three patients), and normal histology (two patients).

Of patients with negative tTG antibody who underwent duodenal biopsy, 4/16 (25 %) of cases and 4/22 (18 %) of controls had villous atrophy.

Enteropathy in NCIPH patients

Celiac disease was noted in 2 of the 14 (14 %) patients with NCIPH. Details of enteropathy in the 14 NCIPH patients are given in Table 3.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cryptogenic chronic liver disease (( n = 61 ))</th>
<th>Hepatitis B/C-related cirrhosis (( n = 59 ))</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, median (range)</td>
<td>42 (7–67)</td>
<td>46 (21–67)</td>
<td>0.3</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>46:15</td>
<td>53:6</td>
<td>0.05</td>
</tr>
<tr>
<td>Presentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jaundice</td>
<td>13</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Ascites</td>
<td>28</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Variceal bleed</td>
<td>22</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Serum total bilirubin (mg/dL), median (range)</td>
<td>1 (0.4–30.8)</td>
<td>1.2 (0.4–17.6)</td>
<td>0.85</td>
</tr>
<tr>
<td>Serum total protein (g/dL), median (range)</td>
<td>7.5 (5.4–9.1)</td>
<td>7.6 (5.2–10)</td>
<td>0.78</td>
</tr>
<tr>
<td>Serum albumin (g/dL), median (range)</td>
<td>3.6 (1.8–5.2)</td>
<td>3.1 (1.6–5.1)</td>
<td>0.04</td>
</tr>
<tr>
<td>International normalized ratio for prothrombin time, median (range)</td>
<td>1.2 (0.9–2.9)</td>
<td>1.3 (0.9–3.4)</td>
<td>0.01</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL), median (range)</td>
<td>0.9 (0.5–1.6)</td>
<td>1 (0.4–6.1)</td>
<td>0.002</td>
</tr>
<tr>
<td>Hemoglobin (g/dL), median (range)</td>
<td>10.6 (5.2–15.1)</td>
<td>11.2 (6.1–17.3)</td>
<td>0.26</td>
</tr>
<tr>
<td>Platelet count (( \times 10^9/\text{mm}^3 )), median (range)</td>
<td>0.68 (0.2–2.6)</td>
<td>0.67 (0.06–5.5)</td>
<td>0.5</td>
</tr>
<tr>
<td>Child’s class (A/B/C)</td>
<td>39/15/7</td>
<td>29/19/11</td>
<td>0.25</td>
</tr>
<tr>
<td>Child’s score, median (range)</td>
<td>5 (5–12)</td>
<td>7 (5–14)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Table 1** Baseline characteristics in 120 portal hypertensive study patients

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**Fig. 1** Flow chart depicting results of celiac serology (tissue transglutaminase antibody) and duodenal biopsy (D2 Bx) in study patients. **tTG** tissue transglutaminase, **D2 Bx** biopsy from 2nd part of duodenum, **CD** celiac disease.
Increased gut permeability (L/M ratio >0.07) was documented in 9 of 30 patients with cryptogenic chronic liver disease tested. Of these nine patients, eight were tTG antibody positive and duodenal biopsy showed mild villous atrophy (six patients), moderate villous atrophy (two), and normal histology (one).

Cardiolipin antibodies in study patients

IgM cardiolipin antibody was negative in 28 cases and 32 controls tested while IgA cardiolipin antibody was borderline positive in four cases and four controls, and both IgG and IgA cardiolipin antibodies were positive only in one patient with hepatitis B-related cirrhosis.

tTG antibody test by other commercial kits

Stored samples were available for retesting tTG antibody by other kits for 92 (52 cases and 40 controls) study patients. tTG antibody titers by EUROIMMUN and INOVA QUANTA Lite™ in cases were 7.5 (0–547) and 12 (4–147)U/mL, respectively, whereas in controls, the titers were 15.5 (0–170) and 12 (4–65)U/mL, respectively.

Using manufacturer specified cutoffs, tTG antibody was positive by EUROIMMUN in 16 (31 %) cases and in 15 (38 %) controls. Similarly, tTG antibody was positive by INOVA in 12 (23 %) cases and 8 (20 %) controls.

Of patients tested, 15 (25 %) cases and 12 (20 %) controls had positive tTG antibody (as per manufacturer specified cutoffs) by at least two of the three assay kits used. Seven cases and two controls had tTG antibody titer >100 U/mL by any one of the three assays (Table 4).

Discussion

In this study, we document that celiac disease is more prevalent in patients with cryptogenic chronic liver disease (10 %) as compared to hepatitis B/C-related cirrhosis (0/59). In patients with NCIPH, 2/14 (14 %) had celiac disease. The association of celiac disease with NCIPH and cryptogenic chronic liver disease has been previously reported from India [13–15].

Of the three celiac disease patients who took gluten-free diet for at least 12 months, there was a decrease in titer of tTG antibody in all (two became negative) and improvement in duodenal histology in two patients. There was no increase in titer of anti-tTG antibodies in the third case.

Table 2  Duodenal mucosal biopsy in portal hypertensive subjects who had cryptogenic chronic liver disease (cases) or hepatitis B- or C-related cirrhosis (controls)

<table>
<thead>
<tr>
<th>Variables</th>
<th>In all study patients who had duodenal biopsy</th>
<th>In Child’s class A patients who had duodenal biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (n=53)</td>
<td>Controls (n=33)</td>
</tr>
<tr>
<td>Crypt hyperplasia</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>Villous atrophy (mild/moderate)</td>
<td>18/3</td>
<td>6/0</td>
</tr>
<tr>
<td>Raised intraepithelial lymphocytes</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Lamina propria inflammation (mild/moderate)</td>
<td>34/13</td>
<td>22/1</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 3  Celiac serology and duodenal mucosal biopsy in portal hypertensive subjects who had idiopathic noncirrhotic intrahepatic portal hypertension or hepatitis B- or C-related cirrhosis (controls)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NCIPH (n=14)</th>
<th>Controls (n=59)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celiac serology (anti-tissue transglutaminase antibody)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-tTG positive; n (%)</td>
<td>6 (43)</td>
<td>17 (29)</td>
<td>0.35</td>
</tr>
<tr>
<td>Anti-tTG titers (U/mL), median (range)</td>
<td>11 (0–300)</td>
<td>7.5 (0–300)</td>
<td>0.38</td>
</tr>
<tr>
<td>Anti-tTG titer &gt;100 U/mL (n)</td>
<td>1</td>
<td>1</td>
<td>0.36</td>
</tr>
<tr>
<td>Duodenal histology (NCIPH, 12; controls, 33)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crypt hyperplasia</td>
<td>4</td>
<td>6</td>
<td>0.4</td>
</tr>
<tr>
<td>Villous atrophy</td>
<td>5</td>
<td>6</td>
<td>0.13</td>
</tr>
<tr>
<td>Raised IELs</td>
<td>2</td>
<td>1</td>
<td>0.17</td>
</tr>
<tr>
<td>Lamina propria inflammation</td>
<td>11</td>
<td>23</td>
<td>0.2</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

NCIPH idiopathic noncirrhotic intrahepatic portal hypertension, IELs intraepithelial lymphocytes
improvement in liver disease severity. It is possible that longer duration of gluten exclusion is needed for improvement of liver disease/portal hypertension in these patients.

In addition to celiac disease, we also demonstrate a much higher prevalence of duodenal mucosal inflammation and architectural changes in patients with cryptogenic chronic liver disease as compared to hepatitis B/C cirrhosis controls (Table 2). The exact cause of these changes remains unclear, but the presence of these changes in early liver disease suggests a role of gut inflammation in pathogenesis of these patients.

Duodenal mucosal histological changes can be caused by tropical sprue, tropical enteropathy, small intestinal bacterial overgrowth, and gluten sensitivity enteropathy [16]. In India, incidence of tropical sprue is decreasing, while celiac disease may be on the rise [16, 17]. Absence of raised IELs in duodenal mucosa in our study patients may suggest that these changes are secondary to portal hypertensive duodenopathy [18–22]. We did not systematically document use of antibiotics in our study patients; hence, whether antibiotic use affected the IEL population in duodenal biopsy is not known.

An unexpectedly high prevalence (48 %) of positive tTG antibody was found in the 120 portal hypertensive patients studied. Human IgA anti-tTG antibody testing, being highly sensitive and specific to diagnose celiac disease and also being easy to standardize, is generally recommended as the initial serological test in diagnosis of celiac disease [23–26]. Significantly different results on retesting stored sera samples with two other ELISA kits raise the question of false positivity. False-positive tTG antibody results increase with advancing liver disease [27, 28]. Anti-endomysial antibody may help clarify this but was not performed in this study.

NCIPH is more common in India [3, 29] and enteropathies driving pathogenesis of NCIPH maybe one explanation for this. In our study, 5 of the 14 NCIPH patients had enteropathy (two celiac disease and three with unclear cause). Enteropathy may provide a pro-thrombotic milieu in the liver by imbalance of primary hemostatic mechanisms (ADAMTS13 deficiency and increased vWF levels), which in turn may drive the portal venular occlusion in NCIPH [30, 31]. IgA cardiolipin antibody is another putative pro-thrombotic agent in NCIPH [32, 33]; however, we did not find increased prevalence of this antibody among our study subjects.

NCIPH closely mimics cirrhosis and is often mis-labelled as cryptogenic cirrhosis [3, 4, 34]. Hence, we decided to include patients with cryptogenic chronic liver disease as cases for our study. In this study, 14 of the 20 cryptogenic chronic liver disease patients (70 %) who had liver biopsy were diagnosed as NCIPH, which is consistent with earlier reports from our center.

Celiac disease associated with portal hypertension raises the possibility of cryptogenic [13], autoimmune or cholestatic cirrhosis, NCIPH [15], or Budd-Chiari syndrome [14, 35, 36]. As in other reports from India [13, 14], in our study, the commonest cause of chronic liver disease associated with celiac disease was cryptogenic chronic liver disease.

In conclusion, our data suggests the need to search for celiac disease in all patients with unexplained portal hypertension. Secondly, higher prevalence of enteropathy in cryptogenic chronic liver disease patients suggests a need to investigate etiology of this enteropathy and to assess if these mucosal changes in gut have a causative role in development/progression of portal hypertension in these patients.

Acknowledgments This study was funded by Fluid Research at Christian Medical College, Vellore, India.

Conflict of interest RM, AG, ABP, SB, JS, CP, KAB, BR, SK, GJF, PA, GK, BSR, EE, and CEE all confirm that they have no conflict of interest to declare.

Ethics statement The study was performed in a manner to conform with the Helsinki Declaration of 1975, as revised in 2000 and 2008 concerning Human and Animal Rights, and the authors followed the policy concerning informed consent as shown on Springer.com.

References


Table 4 Results of tTG antibody testing in 92 study patients in whom sera were tested by all three ELISA kits

<table>
<thead>
<tr>
<th></th>
<th>AESKULISA</th>
<th>EUROIMMUN (positive/negative)</th>
<th>INOVA (positive/negative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases (n=52)</td>
<td>Positive</td>
<td>13/20</td>
<td>11/22</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>3/16</td>
<td>1/18</td>
</tr>
<tr>
<td>Controls (n=40)</td>
<td>Positive</td>
<td>9/3</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>6/22</td>
<td>2/26</td>
</tr>
</tbody>
</table>

AESKULISA AESKULISA Celichek, Germany, ≥20 U/mL as positive, EUROIMMUN EUROIMMUN AG, Germany, ≥20 relative units/mL as positive, INOVA INOVA diagnostics, USA, ≥20 units/mL as positive.