Prevalence of celiac disease in patients with hepatitis B and C virus related cirrhosis and cryptogenic liver cirrhosis.

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Abstract

Introduction: Celiac disease (CD) is a disease that involves many organs including the liver and is caused by autoimmune enteropathy. We aimed to compare the CD frequency between the cryptogenic cirrhosis and cirrhosis due to hepatitis B virus (HBV) and hepatitis C virus (HCV).

Materials and methods: Patients with cryptogenic cirrhosis (n=83), HCV related cirrhosis (n=42) and HBV related cirrhosis patients (n=36) were enrolled to the study.

Results: Abnormal celiac serology was present in 24 (14.9%) of 161 cirrhotic patients. In 6 of them (3.7%) CD was diagnosed. Remaining 18 patients had false positive celiac serology and biopsy results were not compatible with CD. The incidence of abnormal celiac serology in patients with cryptogenic cirrhosis was significantly higher than that of patients with HBV and HCV related cirrhosis (P<0.004). CD was not detected in patients with HBV related cirrhosis. Among HCV-related cirrhosis patients, 3 patients (7%) were diagnosed with CD. In cryptogenic cirrhosis patients, 3 patients (3.6%) were diagnosed with CD. Anti-tissue transglutaminase (anti-tTG) and anti-endomysium (EMA) IgA values were abnormal in all of the 6 CD patients diagnosed. Marsh type 3 changes were detected in duodenal biopsies of these patients

Conclusions: The prevalence of CD in cirrhosis patients was 3 times higher than in the normal population. However, contrary to expectations, the prevalence of CD in HCV-associated cirrhotic patients was 2-fold higher than in patients with cryptogenic cirrhosis. These results show that in cryptogenic cirrhosis cases, there is no significance of CD in etiology, but the ratio of abnormal celiac serology is increased.

Introduction

Celiac disease (CD) is a chronic immune mediated disease caused by damage to the small intestine (SB) mucosa due to gluten-containing food intake in genetically susceptible individuals [1,2]. Damage to the small bowel mucosa may be patchy, as well as up to total villus atrophy, and patients may present with malabsorption findings [3]. Extra intestinal involvement can be seen in about 30% of patients and, according to the current view, CD is considered as a systemic disease that can involve many organs, including the liver [4,5].

The most common liver diseases associated with CD have been reported as primary biliary cirrhosis, sclerosing cholangitis, autoimmune hepatitis, and non-alcoholic fatty liver disease [5-8]. Prevalence of elevated liver enzymes were determined in 40% of on the adult CD patients [9]. Rapidly developing research techniques help the clinicians in revealing the etiology of cirrhosis; however, cryptogenic cirrhosis still exists frequently in the clinical practice. Many studies report that the rate of cryptogenic cirrhosis averages between 3% and 10% [10-12].

Although there is a reported 0.7-1% CD frequency in the general population, a small number of studies about the CD frequency have been conducted in cirrhotic patients. In a recent study, the prevalence of CD in cirrhotic patients was found to be 2.5% and there was no study of CD prevalence in patients with cryptogenic liver cirrhosis. This study also showed improvement in liver function tests after gluten free diet (GFD) [13]. In studies conducted on chronic cryptogenic hepatitis cases, the prevalence of CD was between 1-4%. In this study, examination of anti-tissue transglutaminase (anti-tTG) IgA and IgG antibodies in patients with unknown etiology and chronic abnormal liver tests was suggested, and endoscopy and biopsy were recommended in both positive cases [14,15].

Also recent studies have suggested that diseases such as KHB and KHC trigger gluten intolerance and interferons used in treatment may cause CD activation [16]. The prevalence of CD

in autoimmune liver diseases was found to be 3.4% and significantly higher than the normal population (0.4%) [17].

In this study, we aimed to investigate (1) the frequency of celiac disease in cryptogenic cirrhosis etiology and (2) to compare the frequency of celiac disease between cryptogenic cirrhosis and cirrhosis due to hepatitis B virus (HBV) and hepatitis C virus.

Materials and Methods

Patients and data collection

Between January 2010 and December 2015, a total of 161 patients diagnosed with HBV and HCV-associated liver cirrhosis and cryptogenic liver cirrhosis in the hepatology clinic were included in the study. By using the medical records; the patients' demographic characteristics, laboratory tests, monitoring studies and endoscopy and biopsy results were examined retrospectively.

Complete blood cell count, hepatic function tests [ALT, aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total bilirubin, direct bilirubin, albumin, INR], total cholesterol, triglyceride, urea, creatinine, HBsAg, Anti-HBs, HBeAg, Anti-HBe, Anti-HBc IgM, Anti-HBc IgG, antibody to the hepatitis delta virus (Anti-HDV), HBV-DNA (Amplicor HBV MonitorTM test, Roche Diagnostic Systems, Inc., Branchburg, NJ), antibody to the hepatitis C virus (Anti-HCV), HCV-RNA (Amplicor HCV MonitorTM test, Roche Diagnostic Systems, Inc., Branchburg, NJ), antibody to the human immunodeficiency virus (Anti-HIV), and alpha-fetoprotein (AFP) were studied. The enzyme-linked immunosorbent assay (ELISA) method was used in the assessment of HBsAg, HBeAg, Anti-HBe, Anti-HDV, Anti-HCV, and Anti-HIV tests in all patients, while HBV-DNA and HCV-RNA were assayed using quantitative PCR.

Diagnostic criteria

Diagnostic criteria used for the diagnosis of liver cirrhosis were as follows:

1. Signs in accordance with cirrhosis in the laboratory analyses; low albumin level, AST/ALT ratio >1, inversion of albumin/globulin ratio, presence of thrombocytopenia, and prolonged prothrombin time.

2. Imaging findings [ultrasonography (USG) and/or computed tomography (CT)]; decrease in the liver size, parenchymal heterogeneity, superficial nodular changes, hypertrophy of the left lobe, and splenomegaly.

3. Clinical/endoscopic signs suggestive of cirrhosis; esophageal varices, ascites, hepatic encephalopathy.

In addition to the findings indicated above, having HBsAg (+) and HBV DNA positive patients were diagnosed as HBV related liver cirrhosis; having anti-HCV (+) ve HCV RNA (+) patients were diagnosed as HCV related cirrhosis.

Cryptogenic liver cirrhosis was diagnosed when tests performed on cirrhosis aetiology (preprandial blood glucose, cholesterol, triglyceride, HBsAg, Anti-HCV, ANA, ASMA, AMA, immunoglobulins, iron, iron binding capacity, ferritin, ceruloplasmin, 24 h urine copper test) and monitoring methods (portal doppler USG [in terms of vascular pathologies and steatosis]) were negative and chronic use of alcohol was absent.

Development of ascites, hepatic encephalopathy, jaundice or gastrointestinal bleeding in the cirrhotic patients were accepted as signs of decompensation. Model of end stage liver disease (MELD) scoring systems were used to determine the severity of the disease.

Celiac serology panel

In all patients with chronic hepatitis B, C and D related and cryptogenic cirrhosis, tissue transglutaminase IgA (IgA for hTTG, QUANTA lite™ ELISA, Inova diagnostics, San Diego CA), anti-endomysium IgA (IgA EMA, Immunofluorescence Inova diagnostics San Diego CA) and total IgA (Beckman Coulter Immage/image 800 Immunochemistry system and Calibrator 1, Fullerton CA) level were measured. IgG levels were studied in patients with selective IgA deficiency. Abnormal serology panel is any value for IgA EMA above 1:10 dilution or any value for IgG, anti-TTG, IgA anti-TTG or Gliadin antibodies above 20 U/mL. Upper endoscopy and duodenal biopsies were performed in patients with positive or elevated anti-TTG IgA and/or EMA IgA. In patients with Anti-tTG IgA, symptoms and accompanying diseases (alopecia, dermatitis herpetiformis, migraine, Fe deficiency anemia, selective IgA deficiency, diabetes mellitus, autoimmune thyroiditis, and autoimmune hepatitis) were investigated.

Histological diagnosis

Upper endoscopy and duodenal biopsies were performed in patients with positive or elevated anti-TTG IgA and/or EMA IgA. The biopsies were taken 2 from bulbus and 4 from the second part of duodenum. Biopsy specimens were placed in vials containing 10% of buffered formalin solution for fixation. Paraffin sections were prepared and stained by hematoxylin and eosin (HE) stains. Mucosal changes were assessed by the pathologist according to the Modified Marsh Staging system [18,19]. Normal mucosa and villous structure was evaluated as Marsh 0, increase of intraepithelial lymphocyte as Marsh I, intraepithelial lymphocyte increase together with the crypt hyperplasia as Marsh II, villous atrophy as Marsh III. Herein; partial atrophy was accepted as Marsh IIIa, subtotal atrophy as Marsh IIIb, total atrophy as March IIIc. The hypoplastic mucosa was accepted as Marsh IV. Patients with positive celiac serology and biopsy Marsh III or Marsh IV mucosal findings were diagnosed as CD.

Ethics statement

The study was carried out in accordance with principles of the Helsinki Declaration of 1975 as revised in 2000, and with the

approval number 04.05.2015/151 of the University of Gaziantep Faculty of Medicine Clinical Research Ethics Committee. All patients provided written consent prior to study entry with all clinical investigations conducted according to the principles expressed in the Declaration of Helsinki.

Statistical analyses

Statistical analyses were performed using licenced Statistical Package for the Social Sciences (SPSS) software (version 15.0, SPSS Inc. Chicago, IL, USA). Values are presented as mean ± standard deviation, median (25th, 75th percentiles) or N (%). Prevalence of celiac disease (CD) was estimated by calculating the percentage of patients with Marsh III and IV. In addition, univariable analysis was performed to assess differences between patients without CD and abnormal serology and those without CD and normal serology. Analysis of Variance (ANOVA) for continuous variables and Pearson's chi-square test for categorical factors were used to compare the groups. A P value ≤ 0.05 was considered statistically significant.

Results

Patient characteristics

47.8% of the patients were female and the mean age was 57.7 ± 13. Of the 161 patients studied, 83 (52.6%) had cryptogenic cirrhosis, 42 (26.1%) had HCV-associated cirrhosis, and 36 (22.4%) had cirrhosis associated with HBV (Table 1).

Table 1. Demographic and clinical characteristics of patients with cirrhosis.

Factor	Total (n=161)
Female	77 (47.8)
Age	57.7 ± 13
Cryptogenic	83 (52.6)
HCV	42 (26.1)
HBV	36 (22.4)

HBV: Hepatitis b virus; HCV: Hepatitis c virus;

Values presented as Mean ± SD or N (%).

Small bowel biopsy and serology

CD was diagnosed in 6 of all cirrhosis patients (3.7%). CD was not detected in patients with HBV related cirrhosis. CD patients were diagnosed in 3 patients (7%) among HCV-related cirrhosis patients. In cryptogenic cirrhosis patients, CD was diagnosed in 3 patients (3.6%).

Abnormal celiac serology was present in 24 (14.9%) of the cirrhotic patients. Six of them were diagnosed with CD. The remaining 18 patients had false-positive celiac serology and the biopsy results were not compatible with the CD. Marsh type 2 changes were detected in 1 of 18 patients, but this was not considered as CD. There was normal serology in 137 patients (85%) (Table 2).

Table 2. Celiac serology and distribution of marsh scores in patients with cirrhosis.

Factor	Total (n=161)
IgA deficiency	1(0.6)
EMA IgA (+)	4(3.6)
Anti-tTG (+)	21 (13)
Abnormal celiac serology	24 (14.9)
Marsh type	
Type1	0
Type2	1 (0.6)
Type3	6 (3.7)

Anti-tTG: anti-tissue transglutaminase; EMA IgA: anti-endomisyum IgA; EMA IgA not available for 49 subjects.

Values presented as Mean ± SD or N (%).

Three of the 6 CD patients diagnosed were female and all patients had abnormal hTTG and EMA IgA levels. Marsh type 3 changes were detected in duodenal biopsies of these patients. Patient laboratory data and MELD scores are shown in Table 3.

Table 3. Demographic, clinical and laboratory characteristics of patients with Celiac disease.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Sex	Female	Female	Male	Male	Male	Female
Age, years	61	47	37	52	56	29
Etiology	HCV	HCV	HCV	Cryptogenic	Cryptogenic	Cryptogenic
EMA Ig A	Positive	Positive	Positive	Positive	Positive	Positive
Anti-TTG, U/mL	200	200	200	200	16.8	328
Marsh, stage	3	3	3	3	3	3
Total bilirubin, mg/dL	1.1	1.4	0.4	1.1	1.8	1.1

AST, U/L	48	38	19	72	35	67
ALT, U/L	31	27	1	71	26	39
Albumin, g/dL	3.8	3.5	4.2	4.1	3	2.5
ALP, U/L	123	153	321	332	174	110
INR	1.08	1.96	1.04	1.13	1.42	1.5
Creatinine, mg/dL	0.59	0.6	5.28*	0.62	0.82	0.51
MELD	8	15	21	8	13	11

anti-tTG : anti-tissue transglutaminase; EMA IgA: anti-endomisyum IgA; MELD: model of end stage liver disease.

Abnormal serology panel is any value for IgA EMA above 1:10 dilution, or any value for IgG anti-TTG, IgA anti-TTG or gliadin antibodies above 20 U/mL.

*The patient has chronic renal failure.

Patients with normal celiac serology and abnormal celiac serology with normal small bowel biopsy were compared in all patient groups. When the patient groups were examined, the incidence of abnormal celiac serology in patients with cryptogenic cirrhosis was significantly higher than the other groups (p<0.004). In patients with HBV and HCV related cirrhosis, the incidence of abnormal celiac serology is lower than in normal serologic group. There were no significant age and gender differences in the patient groups (Table 4).

Table 4. Characterization of 18 patients with abnormal serology and no Celiac disease.

Factor	Normal serology (n=137)	Abnormal serology (Normal biopsy) (n=18)	P-value
Female	65 (47.4)	9 (50)	1
Age, years	58.3 ± 13	56.5 ± 11.8	0.574
Cryptogenic	65 (47.4)	15 (83.3)	0.004
HBV	35 (25.5)	1 (5.6)	0.059
HCV	37 (27)	2 (11.1)	0.144

HBV: Hepatitis B virus; HCV: Hepatitis C virus.

Values presented as Mean ± SD with ANOVA, or N (column %) with Pearson's chi-square test.

Discussion

In recent years, association of CD with various liver diseases has been reported increasingly. In the normal population, the CD frequency is reported as 0.7-1% [2,20]. In our study, the CD frequency in cirrhotic patients was 3.7% and the prevalence was more than 3-fold compared to the normal population. The prevalence of CD in cirrhotic patients has not been clarified sufficiently in studies conducted. Although there is insufficient information on how CD affects the liver, some pathophysiological mechanisms have been proposed. Because CD has genetic features, it is thought to be associated with autoimmune liver diseases [21]. The kupffer cells, cleaning the harmful pathogens and antigens inside the blood, exist intensely in both liver and intestine lamina propria. In consequence of any immunological stimulation, antimicrobial agents together with IL-4 ve IL-10 are released to the circulation and these cytokines contribute to liver damage [22]. In addition, disruption of mucosal integrity in CD accelerates entry of antigen, antibody and cytokine to portal circulation, which can lead to hepatic damage [23,24]. It has also been shown that malnutrition and bacterial overgrowth accelerate hepatic damage in CD patients [25,26].

In our study, CD prevalence in cirrhotic patients was three times higher than in the normal population. However, contrary to expectations, the prevalence of CD in HCV-associated cirrhotic patients was 2-fold higher than in patients with cryptogenic cirrhosis. CD was not detected in patients with HBV related cirrhosis. The great majority of patients with abnormal celiac serology and normal small bowel biopsy were patients with cryptogenic cirrhosis. These results show that in cryptogenic cirrhosis cases, there is no significance of CD in etiology, but the ratio of abnormal celiac serology is increased. It may be useful to follow these cases in terms of progression to the CD, perhaps with a prospective study.

Also interestingly, in our study, the prevalence of celiac disease in HCV-associated cirrhosis patients was found to be 6 times higher than in the normal population. Previous studies suggest that CD activation may be present in HCV patients treated with interferon (IFN-alpha), whereas no significant HCV and CD association was observed in those without treatment [16,27,28]. Our HCV related cirrhosis patients did not receive IFN-alpha treatment and unlike previous studies CD prevalence was extremely high. This can lead to think that HCV could cause mucosa damage in the CD by immune system activation and cytokine mediated cell damage.

In our study CD wasn't detected in patients with HBV related cirrhosis. This result supports previous studies [29]. Our study has some limitations. First, small bowel biopsy was not performed on all cirrhotic patients included in the study. However, we only received small bowel biopsy in patients with abnormal celiac serology. Second, in patients with abnormal celiac serology, there was no long term and prospective follow-up in terms of progression to CD. Thirdly, there was no follow-up in CD-diagnosed cirrhosis patients whether there was any improvement in post-dietary liver function.

In conclusion, although it has been shown in many studies that there is a positive correlation between liver disease and CD, it is not known exactly how the liver damage occurs in CD.

There is also a debate on whether CD has a role in cryptogenic cirrhosis etiology. Therefore, prospective studies with longer follow-up in terms of liver damage (including liver biopsy) and serological and small bowel biopsies demonstrating the development of CD in cirrhotic patients are needed.

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Competing Interests

We declare that we have no conflict of interests

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