ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
September 2010; 9(3): 157-162.

The Prevalence of Celiac Autoantibodies in Hepatitis Patients

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Received: 16 February 2010; Received in revised form: 14 July 2010; Accepted: 21 August 2010

ABSTRACT

Celiac disease has been associated with other autoimmune disorders such as autoimmune hepatitis, moreover it is known that T cell mediated immune response to dietary gluten and released cytokines are important for the entheropathy seen in celiac disease. We investigated celiac autoantibodies in patients with autoimmune hepatitis (AIH), and chronic hepatitis B (CHB).

Sera from 84 patients with Autoimmune Hepatitis (AIH) type 1 and 88 patients with Chronic Hepatitis B (CHB) were tested for Immunoglobulin A and G antibodies to Gliadin, Immunoglobulin A antibodies to tissue transglutaminase using enzyme immunoassay, and Immunoglobulin A anti-endomysial antibodies by both indirect immunofluorescence, and enzyme immunoassay. The patients positive for anti-endomysial antibodies and/or anti tissue transglutaminase antibodies were considered for deuodenal biopsy. The study was approved by Research Center for Gastroenterology and Liver Disease Ethics Committee and all patients gave their written informed consent to participate.

Immunoglobulin A anti-endomysial and Immunoglobulin A anti-gliadin antibodies were positive in two out of 84 patients with AIH. Moreover, Immunoglobulin A anti-gliadin antibodies were positive in another patient who was also positive for anti tissue transglutaminase antibodies. Tissue transglutaminase antibodies were positive in eight (9.1%) of 88 patients with CHB, two of which were also positive for anti-endomysial antibodies. One of the patients with CHB was only positive for anti-endomysial antibodies.

Compared with the general population, the prevalence of celiac autoantibodies in CHB and AIH patients is relatively high, and it is noteworthy that most positive patients were asymptomatic for celiac disease. We suggest screening for celiac disease before and during treatment in patients with viral and autoimmune hepatitis.

Key words: Celiac disease; Hepatitis

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INTRODUCTION

Celiac disease (CD) is a gluten-sensitive enteropathy in which genetic, immunologic and environmental factors are implied.[1] The disease is

characterized by malabsorption, abnormal small bowel structure and function and intolerance to gluten. It has been proposed that gluten or its metabolites may initiate an immunological reaction in the intestinal mucosa.[2,3]

Recent screening studies in western countries suggest that the prevalence of adult CD is increasing.[4-6] In two studies in Iran, CD prevalence among healthy blood donors and general population were 1:166, and 1:104 respectively.[7,8] The symptoms range from significant malabsorption of multiple nutrients to a subclinical disorder with isolated nutrient deficiencies, or a completely asymptomatic process.[9] The asymptomatic form is more common, and is usually found in association with autoimmune disorders,[10-12] which are mostly associated with the same haplotypes as CD (especially haplotypes HLA B8, DR7, DR4 , DQ8, DR3, and DQW2), and their incidence is significantly higher in patients with long-standing, untreated CD.[11-13] Early recognition of the intestinal disorder is mandatory, as gluten restriction improves the symptomatic forms and can also reduce neoplastic (small intestinal lymphoma and carcinoma) and systemic (osteoporosis, infertility) complications as well as improvement in the associated autoimmune disease.[2,9] Non-specific hepatitis, with slight elevation of serum aminotransferase levels, is quite common in CD[11-17] (approximately in 40% of patients).[4] It usually represents a benign condition called gluten hepatitis in which aminotransferase levels are resolved in 2 to 8 weeks[12] with a gluten-free diet.[12,13,17] In addition, there are several case reports and retrospective studies describing an association of autoimmune liver diseases, such as primary biliary cirrhosis (PBC),[1,9,10,13-16] primary sclerosing cholangitis (PSC),[1,13-16] autoimmune hepatitis (AIH),[1,9-11,13,17] and chronic active hepatitis (CAH)[10,16] in patients with CD. For instance in Bardella et al study, the prevalence of hepatitis B surface antigen positivity in CD patients was 2.5%,[16] twice that reported in general population of Northern Italy.[16] The prevalence of coexisting CD in AIH ranges from 4% in type1 AIH to 8% in type2 AIH.[17] This is considerably higher than its prevalence (0.05-0.2%) in general population.[17] There is no data on prevalence of CD in patients undergone for the treatment of viral hepatitis.

To establish CD prevalence in patients with AIH and CHB, we performed serological screening for gluten sensitive enteropathy by means of IgA EmA (Endomysial Ab), IgA tTGA (tissue Trans Glutaminase Ab), and IgA and IgG gliadin antibodies (AGA) in a large series of patients with AIH and chronic hepatitis B (CHB). Then we examined wherever possible the small intestinal mucosa for histologic evidence of CD in serologically positive patients.

PATIENTS AND METHODS

Among 112 patients with AIH, who were registered consecutively at the Research Center for Gastroenterology & Liver Diseases and Tehran Hepatitis Center, 84 patients satisfied international criteria for diagnosis of AIH, and had anti nuclear antibodies (ANA), anti smooth muscle antibodies (ASMA), or both which justified their diagnosis as AIH were included in our study. Also, among 876 patients with CHB, 88 subjects with HBsAg and HBc IgG Ab, who were matched in sex, age, and stage of disease with AIH patients were selected.

A questionnaire was filled out for every patient, which consisted of demographic, clinical, and Para clinical data such as age, sex, ethnicity, signs, symptoms, auto antibodies, protein electrophoresis, liver pathology, and whether they took medications and all of the patients had a liver histology compatible with their diagnosis.

The study was approved by Research Center for Gastroenterology and Liver Disease Ethics Committee and all patients gave their written informed consent to participate.

Determination of Antibodies

Serum samples were taken during treatment course, and stored frozen at –70 Cº before examination. All were tested for total IgA, AGA (IgA and IgG), EmA, and tTGA. Total IgA was sought using turbidimetry (The Binding Site, UK). IgA and IgG AGA were determined using a commercial ELISA (GENESIS, UK). Sera were diluted 1:100 for measurement of IgG and IgA. Normal range was considered < 10 u/ml for IgG, and < 4 u/ml for IgA. IgA EmA was analyzed using a commercial ELISA (Euro immune, Germany), the sera for analysis were diluted 1:200 with ready to use sample buffer, whereas calibration and control sera were prediluted when needed. Photometric measurement of the color intensity was done at wavelength of 450nm with a reference of 620nm, and more than 20 Relative(R) u/ml of antibody was considered positive. Also, IgA EmA was examined

using monkey's esophagus Immunofluorescence Assay (IFA) kit (The Binding Site, UK). IgA tTGA was measured using a commercial ELISA (GENESIS, UK). The test was quantitative and values were obtained in u/ml. Sera were diluted 1:100, and absorbance was determined at 450nm with a reference of 620nm. Values greater than 7u/ml were considered positive for IgA tTGA as recommended by the manufacturer.

Celiac Disease Diagnosis

According to high specificity of IgA EmA and IgA tTG for diagnosis of CD,[18-21] and considering the low accessibility of patients, endoscopy and duodenal biopsy were suggested for those with existence of IgA EmA and/or IgA tTGA. After the explanation of our findings to the patients, including the sensivity of the specific serological markers, the significance of celiac disease, and the effect of it on their liver disease, six biopsies were performed from the second part of duodenum. Mucosal biopsies were interpreted according to the Marsh's modified classification.[22]

Statistical Analysis

Student's t test for continuous variables and chi square analysis for frequency measures were used to determine statistically significant differences in Antibody levels between AIH and CHB groups.

RESULTS

The mean age of patients with AIH was 33.7±14.5 y, and 75 (89%) of them were female. All patients had chronic active hepatitis except 21 of them (25%), which had cirrhosis. Total IgA deficiency was not found in any patient. IgA and IgG AGA were present in one (1%) and nine (10%) patients respectively. Using ELISA and IFA tests, IgA EmA were present in three (3.5%) and two (2%) patients respectively. IgA tTGA was also present in two patients (2%). The mean age of patients with CHB was 35.5±14 y, and 77 (87%) of them were female. The hispathological findings were compatible with chronic hepatitis in 66 of them (75%), and the rest had cirrhosis. None of them was healthy carrier. There was no total IgA deficiency among them. IgA and IgG AGA were present in nine (10%) and twenty five (28%) patients respectively. IgA EmA was present in three (3%) and two (2%) patients when using ELISA and IFA tests respectively. IgA tTGA was present in eight patients (9%).

Five of these patients, including two patients with AIH, and three patients with CHB, who agreed, and were accessible, underwent upper tract endoscopy and duodenal biopsy. A subtotal villous atrophy and increasement of plasma cells and lymphocytes in lamina propria consistent with the diagnosis of celiac disease were found in all of them (Table 1). It is noteworthy that none of these patients had shown characteristic symptoms of celiac disease.

The frequency of IgA EmA and/or IgA tTGA in CHB patients were significantly higher than patients with AIH ($P<0.05$). Frequency of the history of IFN-α therapy in CHB patients with and without celiac autoantibodies has been shown in Table 2. The frequency of taking IFN-α was significantly higher in patients who had IgA EmA and/or IgA tTG Antibodies comparing to those without these antibodies ($p<0.05$).

DISCUSSION

Our results showed some interesting information about the high frequency of celiac autoantibodies in adults affected by AIH and CHB. There were some reports on CD prevalence in healthy populations,[4-8] but the most compatible reports with our study were two surveys conducted by Malekzadeh and colleagues[7,8] on Iranian population. They found the prevalence of CD to be 1/166 in Iranian blood donor population,[7] and 1/104 in Iranian general population,[8] thus in comparison with our results, it indicates that at least celiac autoantibodies are much more frequent in patients with AIH and CHB comparing with general population.

In patients with celiac disease, impairment of liver function is frequently found at diagnoses, which is usually normalized on a gluten-free diet.[2,13,16] Reactive hepatitis is a common finding in liver biopsies of patients with celiac disease, especially in those with elevated transaminase levels.[9-16] On the other hand, celiac disease is underdiagnosed in primary and secondary cares,[4-6] which delays in making the diagnosis. This may lead to an associated morbidity, and potentially the development of celiac-related complications related to the duration of gluten exposure; so that the prevalence of coexisted autoimmune disorders increases seven fold compared with normal population if CD is not diagnosed in adolescence.[23] Also, it has been observed that CD may be silent and only recognized after the diagnosis of other autoimmune diseases.[2,9,13-16]

Table 1. Demographic and clinical data of the patients who were positive for EmA or anti-tTG antibodies.

Diagnosis	Age (years)	Sex	Duration (years)	Liver biopsy	IgA EmA u/ml	IgA AGA u/ml	IgG AGA u/ml	IgA tTGA u/ml	Intestinal biopsy	Medication
AIH	23	F	3	Mild non specific reactive changes	<1	>200	70.5	24.5	Subtotal villous atrophy	Prednisone and AZA
AIH	28	F	6	Chronic active hepatitis	36.6	<1	22	16.5	Not done	Prednisone and AZA
AIH	21	F	7	Chronic active hepatitis	40.4	<1	16.5	>100	Subtotal villous atrophy	Prednisone and AZA
CHB	55	M	12	moderate chronic hepatitis	29.7	98.5	82.5	25.3	Not done	Interferon
CHB	60	M	7	cirrhosis	<1	4.4	20	8.4	Expired	Interferon
CHB	44	M	5	Sever chronic hepatitis	<1	<1	<1	59.8	Subtotal villous atrophy	Interferon
CHB	46	M	8	Sever chronic hepatitis	<1	<1	<1	18.5	Subtotal villous atrophy	Interferon
CHB	35	M	5	moderate chronic hepatitis	<1	<1	11.9	8.4	Not done	Interferon
CHB	60	M	10	cirrhosis	<1	5.06	<1	12.5	Not done	Unknown
CHB	11	M	2	moderate chronic hepatitis	<1	<1	<1	10.1	Subtotal villous atrophy	Lamivudine
CHB	43	M	9	Sever chronic hepatitis	>200	22.9	>200	>100	Not done	Interferon

AGA= Anti Gliadin Antibody; AIH= Autoimmune Hepatitis; CHB= Chronic Hepatitis B; EmA= Endomysial Antibody; tTG-Ab= tissue Transglutaminase Antibody.

Table 2. Frequency of IFN-α or Lamivudin therapy in CHB patients with and without IgA EmA and/or IgA tTGA

Patients	Total	IFN_α	Lamivudin	Unknown
CHB patients without antibodies	80	26	36	18
CHB patients with antibodies	8	6	1	1

Therefore, an early diagnosis might protect against the development of these disorders.

The coexistence of CD and other autoimmune disorders such as AIH had been reported previously,[9-16] and may propose a common autoimmune pathogenetic factor responsible for the injury of the intestine and other tissues such as liver. The most likely and frequently evoked hypothesis in the field of liver disorders is that a dysregulated immune process which would induce liver damage by autoantibody. The other hypothesis implicates that liver damage is a consequence of increased intestinal permeability resulting in the arrival of toxins or auto antigens into the liver through portal vein.

Interestingly, this study also showed that the frequency of celiac autoantibodies in CHB patients are much more than even AIH patients, which is an incidental and also novel finding in our study. Of course, there are some reports indicating autoimmune disorders such as insulin dependent diabetes mellitus and celiac disease which can develop during treatment with Interferon for viral hepatitis, because this drug has immunomodulatory properties that can induce a silent autoimmune disorder like CD.[24-28] Therefore, we can conclude that the IFN-α therapy can trigger CD in susceptible patients, and the most probable pathogenesis for this process can be due to dysregulation of the balance between the need to recognize antigens of pathogenic microorganisms and the need to prevent inappropriate immune responses to foods and normal flora. This balance is managed in the organized lymphoid tissue of peyer's patches[29] in which IFN-α prevents apoptosis of T cells and increases local expression of accessory molecules. Gluten reactive CD4+ T cells arriving from peyer's patches could therefore recognize low amounts of dietary gluten presented by lamina propria antigen presenting cells and be triggered because of the high expression of co-stimulatory molecules.[29-31]

Thus, this high frequency can be due to the history of IFN-α therapy in our CHB patients. However, we need more studies evaluating celiac disease before and after IFN- α therapy to be able to prove that this high frequency is related to treatment with IFN- α and nothing else.

Another interesting finding in our study is that none of the patients with proved celiac disease had shown characteristic symptoms for it, which can be probably due to medications such as Prednisone in AIH patients, however we did not find any justification for this asymptomatic feature in CHB patients. These findings suggest that CD should be sought prior to interferon and corticosteroid therapy for early diagnosis and prevention of CD complications.

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