High Prevalence of Celiac Disease in Autoimmune Hepatitis Detected by Anti-Tissue Transglutaminase Autoantibodies

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Celiac disease (CD) may be found in association with other autoimmune diseases. We investigated the relation between autoimmune hepatitis (AIH) and CD by assessing the prevalence of IgA and IgG anti-tissue transglutaminase (tTG) antibodies in AIH, and by verifying whether the findings were associated with clinical and histological features of CD. Forty-seven consecutive patients with AIH (type I: $n = 39$; type II: $n = 8$) were studied. One hundred patients with chronic hepatitis C, and 120 healthy blood donors were also studied as controls. We analyzed sera for the presence of IgA and IgG anti-tTG antibodies using a specific human recombinant tTG immunoassay. Anti-tTG positive patients and controls were further tested for anti-endomysium antibodies (EMA) and HLA typing, and those found positive by either of these tests underwent duodenal biopsy to confirm a

possible diagnosis of CD. Three of the 47 AIH patients (6.4%) were positive for IgA anti-tTG and EMA antibodies, and were subsequently confirmed to be affected with CD by small-bowel biopsy findings. No IgG anti-tTG positivity was found in the AIH patients. None of the controls were positive for IgA anti-tTG, and only one with chronic hepatitis C had a low positive reaction for IgG anti-tTG, which resulted as a false positive. The crude prevalence rate of CD in AIH was 63.8 per 1,000 (95% CI, 13.2–186.1), and it was significantly higher than that found in the general population in Italy (4.9 per 1,000; 95% CI, 2.8–7.8). The results of this study showed a high prevalence of CD in patients with AIH. For this reason, early serological screening testing for CD is strongly recommended for all AIH patients. *J. Clin. Lab. Anal.* 19:6–10, 2005. © 2005 Wiley-Liss, Inc.

Key words: autoimmune hepatitis; celiac disease; anti-tissue transglutaminase antibodies; anti-endomysium antibodies

INTRODUCTION

Celiac disease (CD) is a gluten-sensitive enteropathy of autoimmune origin. It is characterized by inflammation and villous atrophy of the small-bowel mucosa, and affects mainly susceptible individuals bearing the human leukocyte antigens HLA DR3-DQ2 (DQA1*0501 and DQB1*02 alleles) or DR4-DQ8 (DQA1*0301 and DQB1*0302 alleles). Patients can achieve full recovery on a gluten-free diet (1,2). There is growing evidence that CD is a relatively common disorder. It may be

estimated from population-based screening studies that its frequency is 0.5% to 1% (3–5).

CD has been shown to be closely associated with certain autoimmune diseases, such as insulin-dependent

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diabetes mellitus (6,7), thyroid disorders (8,9), Addison's disease (10,11), alopecia (12), autoimmune myocarditis (13), and pernicious anemia (14). The association with other autoimmune diseases, including connective tissue diseases (CTDs) (15–20), autoimmune diseases of the digestive tract (20–23), and autoimmune cholestatic liver diseases (20,24–26), has also been extensively investigated; however, conflicting results were obtained, and an increased frequency of CD with these diseases cannot be firmly established.

Autoimmune hepatitis (AIH) is a rare chronic necroinflammatory liver disease of unknown origin. It is characterized by female predominance, histological features of periportal hepatitis in the absence of viral markers, hypergammaglobulinemia, autoantibody presence in serum, and association with the human leukocyte antigens HLA A1, B8, DR3 (DRB1*0301) and DR4 (DRB1*0401). CD is found occasionally in patients with AIH (27–29), but only one study has systematically evaluated a large number of patients with AIH for CD association (30). In that study, anti-gliadin and EMA were used as serological screening tests.

In this study we aimed to investigate the relation between AIH and CD by assessing the prevalence of IgA and IgG anti-tissue transglutaminase antibodies (tTG) in patients with AIH, using the more specific human recombinant tTG antigen, and verifying whether the findings were associated with clinical and histological features of CD.

MATERIALS AND METHODS

Forty-seven consecutive patients with AIH (type I: $n = 39$; type II: $n = 8$) who had been referred to seven different Italian gastroenterology or liver units were enrolled in the study. All of the patients satisfied international criteria for the diagnosis of AIH (31). The male/female ratio and the mean age were respectively 1/6 and 54 (range = 12–71) for type I AIH, and 1/1 and 50 (17–69) for type II AIH. As control groups, 100 patients with chronic hepatitis C, and 120 healthy blood donors were also studied. Oral informed consent was given by each patient and control. Serum samples were stored at -80°C and tested in batches for IgA anti-tTG and total IgA in one laboratory, by a single operator. IgG anti-tTG antibodies were also determined in all of the study subjects, since the existence of biopsy-proven CD patients characterized by an isolated IgG anti-tTG positivity (e.g., negative for IgA anti-tTG and with no selective IgA deficiency) was recently reported (32).

Total IgA was determined by nephelometry (Dade Berhing, Deerfield, IL). IgA and IgG anti-tTG antibodies were assayed with an enzyme-linked immunosorbent assay (ELISA) method with the use of human

recombinant tTG (Celikey for IgA and ReCombi tTransglutaminase for IgG; Pharmacia Diagnostics, Freiburg, Germany). Antibody levels were expressed as U/mL, and the cut-off values were set at 5 U/mL for both IgA and IgG tTG according to the manufacturer's indications. All samples with an IgA or IgG value above the cut-off level were tested for IgA and IgG anti-endomysium (EMA) antibodies by indirect immunofluorescence performed on frozen sections of monkey distal esophagus (Eurospital, Trieste, Italy) at the starting dilution of 1:5. Slides were evaluated at 400-fold magnification under an epifluorescence microscope by an observer who was unaware of the patients' diagnosis and anti-tTG results.

If the EMA was also positive, the patients were informed of their high likelihood of having CD and were invited to undergo fiber-optic upper-tract endoscopy and biopsy of the small intestine. Histological evaluations were graded according to the modified Marsh's classification (33). If the EMA was negative, HLA DQ2 (DQA1*0501-DQB1*02) and DQ8 (DQA1*0301-DQB1*0302) alleles were determined (Protrans HLA Celiac Disease, Kesch, Germany), and patients with a positive haplotype underwent an intestinal biopsy.

Statistical Analysis

We computed the crude prevalence of CD and its corresponding 95% confidence interval (CI) using the Poisson distribution (34).

RESULTS

Frequency of IgA and IgG anti-tTG Antibodies

Three AIH patients (6.4%) had IgA anti-tTG antibody levels (concentrations = 10.0, 14.5, and >100 U/mL, respectively) above the cut-off value. No IgG anti-tTG positivity was found in the AIH patients, and none had a selective IgA deficiency. None of the controls were positive for IgA anti-tTG, and only one control with chronic hepatitis C was IgG anti-tTG positive (7 U/mL).

Frequency of EMA Antibodies

The IgA EMAs were positive in all three IgA anti-tTG AIH patients at a titer ranging from 1:10 to 1:320. The IgG anti-tTG patient with chronic hepatitis C was negative for both IgA and IgG EMA. Since the HLA DQ2 and DQ8 alleles were also negative, this patient was not invited to undergo biopsy of the small intestine, and the IgG anti-tTG positivity was considered a false-positive result.

Intestinal Biopsy

All three AIH patients with positive IgA anti-tTG and IgA EMA findings underwent duodenal biopsy. A subtotal villous atrophy and an intraepithelial lymphocyte count of $>35/100$ enterocytes (consistent with the diagnosis of CD) were found in all the three patients. The clinical and laboratory findings for these patients are reported in Table 1.

Prevalence of CD

The crude prevalence rate of CD in AIH was 63.8 per 1,000 (95% CI, 13.2–186.1) and it was significantly higher than that found in the general population in Italy (4.9 per 1,000; 95% CI, 2.8–7.8) (5).

DISCUSSION

In this study we have shown that biopsy-proven gluten-sensitive enteropathy is highly prevalent in patients with AIH. Although we are aware that our case series is too small for us to draw any conclusions, our data confirm an association between AIH and CD (30). A possible explanation might be found in the sharing of common histocompatibility genes, as was shown for CD and other autoimmune conditions (e.g., autoimmune thyroid diseases and diabetes type I). The sharing of a common immunological basis may also be a factor. It is now widely accepted that immunological mechanisms are involved in the development of mucosal damage in patients with CD, and that tissue transglutaminase may play a crucial role. It is possible that, independently of the immune reaction caused by gliadin ingestion, tissue transglutaminase modifies other external or self antigens and generates different neoantigens (35). In turn, these new antigens may drive (auto)antibody production and induce various autoimmune phenomena outside the intestine, in genetically predisposed individuals.

The existence of a hidden CD was suspected in only one of the three anti-tTG and EMA-positive AIH patients because that patient had isolated iron deficiency

anemia and recurrent oral aftae—symptoms that may be related to CD. However, it is important to note that many of the AIH patients included in this study were treated with steroids, in some cases in association with azathioprine, which may have contributed to their asymptomatic CD state. Indeed, immunosuppressive treatment can sometimes heal a celiac small-bowel mucosal lesion (36) and lead to both normal villous architecture and false-negative antibody results in CD patients. As a consequence, the real prevalence of CD in treated patients with AIH may be underestimated.

For these reasons, CD should be investigated in all patients with AIH, possibly before immunosuppressive treatment is initiated. It has been shown that the early recognition of silent CD and the subsequent introduction of a gluten-free diet can reduce a patient's risk of developing certain important complications, such as osteoporosis and intestinal lymphoma. The impact of a gluten-free diet on the outcome of AIH patients with CD is less clear. Volta et al. (30) observed that autoimmune liver damage is usually unaffected by diet. However, Olsson et al. (37) showed that chronic active hepatitis may have a more serious outcome in patients with concomitant CD than in those with normal small-bowel villous architecture, and Kaukinen et al. (38) reported that the early detection and treatment of CD may prevent progression to end-stage liver failure in patients with AIH or hepatitis of unknown etiology. Therefore, further studies are needed to shed light on the response to gluten withdrawal in AIH patients.

Even though on the basis of the results of this and other studies (30) the early recognition of CD in AIH patients seems very important, some doubts remain regarding the use of anti-tTG as an optimal serological screening test in such patients. ELISA assays for detecting anti-tTG antibodies have been shown to be very sensitive for the diagnosis of CD; however, other studies reported a high rate of false positives in patients with liver disease, especially when guinea pig liver extracts were used as a source of tTG (39,40). In contrast, Carroccio et al. (40) showed that false-positive

TABLE 1. Clinical and laboratory findings of the three AIH patients with celiac disease.

Patient	Age (Year)	Sex	AIH autoantibodies	IgA-anti-tTG (U/mL)	IgG-anti-tTG (U/mL)	IgA EMA (titre)	CD-related signs or symptoms	Duodenal biopsy (Marsh's criteria)	Other autoimmune disorders
AIH type I	40	M	ASMA +	10	0.2	1:10	None	Type IIIa	None
AIH type I	26	F	ASMA +; ANA +	14.5	0.4	1:80	Sideropenic anemia Hb 10.4; Fe 30 µg/dl; Ferritin 6 ng/mL; oral aftae	Type IIIb	Raynaud
AIH type I	68	F	ASMA +; ANA +	>100	0.4	1:320	None	Type IIIb	Hashimoto

*The cutoff values for IgA anti-tTG and IgG anti-tTG antibodies were set at 5 U/mL; the starting dilution for IgA EMA was 1:5.

results were completely abolished when human recombinant tTG was used. These data were not confirmed by Vecchi et al. (41), who reported a high prevalence of positive anti-tTG results in cirrhotic patients even when human recombinant tTG was used. It is conceivable that such discrepancies are caused by the different methodologies used for the ELISA assay, and differences in the patients' clinical features, such as high levels of immunoglobulins and autoantibodies, may also play an important role.

In the current study, the human recombinant tTG assay was shown to be very specific, and positive results were confirmed by EMA and intestinal biopsy findings. No control patients showed positive IgA anti-tTG results, and only one patient with chronic hepatitis C showed an IgG anti-tTG aspecific positivity. Therefore, in our experience, the anti-tTG assay using human recombinant tTG antigen proved to be a very accurate screening test for detecting CD in AIH patients. However, because the EMA test has higher specificity, it should be used as a confirmatory test before an anti-tTG-positive patient is advised to undergo an intestinal biopsy.

In conclusion, the results of this study showed a high prevalence of CD in patients with AIH. For this reason, early serological screening testing for CD is strongly recommended for all AIH patients.

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