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Celiac Disease in Autoimmune Cholestatic Liver Disorders

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OBJECTIVES: In this study, serological screening for celiac disease (CD) was performed in patients with autoimmune cholestasis to define the prevalence of such an association and to evaluate the impact of gluten withdrawal on liver disease associated with gluten sensitive enteropathy.

METHODS: Immunoglobulin A endomysial, human and guinea pig tissue transglutaminase antibodies, and immunoglobulin A and G gliadin antibodies were sought in 255 patients with primary biliary cirrhosis, autoimmune cholangitis, and primary sclerosing cholangitis.

RESULTS: Immunoglobulin A endomysial and human tissue transglutaminase antibodies were positive in nine patients (seven primary biliary cirrhosis, one autoimmune cholangitis, and one primary sclerosing cholangitis), whose duodenal biopsy results showed villous atrophy consistent with CD. Two of these patients had a malabsorption syndrome, and one had iron-deficiency anemia. Clinical and biochemical signs of cholestasis did not improve after gluten withdrawal in the three patients with severe liver disease. A longer follow-up of the six celiac patients with mild liver damage is needed to clarify whether gluten restriction can contribute to slow down the progression of liver disease.

CONCLUSIONS: The high prevalence of CD (3.5%) in autoimmune cholestasis suggests that serological screening for CD should be routinely performed in such patients by immunoglobulin A endomysial or human tissue transglutaminase antibodies. (Am J Gastroenterol 2002;97:2609–2613. © 2002 by Am. Coll. of Gastroenterology)

INTRODUCTION

An increased finding of celiac disease (CD) has been reported in patients with autoimmune cholestatic liver disease (1, 2). The association between CD and primary biliary cirrhosis (PBC) has been extensively investigated; however, the conflicting results obtained have not allowed one to establish whether these pathological conditions are strictly related or whether their combined occurrence is due to chance alone (3–5). On the one hand, a 12-yr study of a stable population in South Wales found a 3% prevalence of PBC in 143 patients with CD and a 6% prevalence of CD in 67 patients with PBC (3). This trend was confirmed by the finding of an increased PBC risk in two national cohorts of CD patients from Denmark and Sweden (4). On the other hand, opposite results were obtained by an Italian study, which reported only one case of PBC among 336 adults with CD and no case of CD among 65 PBC patients (5).

The association between CD and primary sclerosing cholangitis (PSC) is substantiated from several case reports (6–10). However, only one study has so far been performed in a large series of PSC patients, which displayed a 3% prevalence of CD (11).

The relationship between CD and autoimmune cholangitis (AIC), a rare, chronic cholestatic disease, also defined as mitochondrial antibody negative PBC, has been recently hypothesized, after the description of the concomitant occurrence of the two disorders in a 65-yr-old woman (12).

At present, immunoglobulin A (IgA) endomysial antibodies (EmA) are universally regarded as the standard marker with the highest predictive value for CD (13). Recently, IgA tissue transglutaminase antibodies (tTGA) proved to be a valid alternative to IgA EmA for CD screening (14–16). The sensitivity of IgA tTGA for CD has been found to be identical to that of IgA EmA, whereas the former showed a slightly lower specificity than the latter.

To determine CD prevalence in patients with autoimmune cholestatic liver diseases, we performed serological screening for gluten sensitive enteropathy by means of IgA EmA, human recombinant tissue transglutaminase (h-tTGA), and guinea pig tissue transglutaminase (gp-tTGA), and IgA and IgG gliadin antibodies (AGA) in a large series of patients with PBC, PSC, and AIC. Moreover, we aimed to evaluate the impact of gluten withdrawal on cholestatic liver disease associated with CD.

MATERIALS AND METHODS

Study Population

The study population comprised 255 consecutive patients with autoimmune cholestatic liver diseases (173 with PBC, 61 with PSC, and 21 with AIC) diagnosed in the Gastroen-

terology Department of Central Hospital of Asturias, Oviedo, Spain; and in the Department of Internal Medicine and Hepatology of the University of Bologna, Italy, over the last 10 years. All patients were evaluated for CD antibody tests. A serum sample had been obtained from each patient, stored frozen at −40°C, and tested in batch in one laboratory (Volta *et al.*). Of the 173 PBC patients, 86 came from Oviedo and 87 from Bologna. All patients satisfied international criteria for the diagnosis of PBC (17). All of them were positive for mitochondrial antibodies, and all showed cholestatic biochemistry and liver biopsy consistent with PBC.

The patients ranged in age from 25 to 80 yr (mean age 56 yr), and 155 of them (90%) were female. Six of the 21 AIC patients were diagnosed in Oviedo and the remaining 15 in Bologna. Diagnostic criteria for AIC were as follows: non-PBC, non-PSC chronic cholestatic liver disease, inflammatory bile duct damage on liver biopsy, and serum antinuclear antibodies on HEp2 cell lines (multiple nuclear dot pattern) with negativity for mitochondrial antibodies (18). All of them were female, ranging in age from 39 to 76 yr (mean age 55 yr). Of the 61 PSC cases studied, 13 were Spanish and 48 Italian. These patients satisfied criteria for PSC (19). The diagnosis was based on the cholangiographic finding of strictures or irregularities in the intrahepatic and extrahepatic bile ducts. Liver biopsy was performed in all patients at the time that PSC was diagnosed. Evidence of inflammatory bowel disease was investigated with colonoscopy and multiple biopsy in all patients. Ages ranged from 17 to 74 yr (mean age 42 yr), and 41 (67%) were male.

IgA EmA

IgA EmA were detected by indirect immunofluorescence using human umbilical cord cryostat sections (4 μm) as substrate. Sera were tested at the initial dilution of 1:5 and, when positive, were titrated up to the endpoint (13).

IgA h-tTGA and gp-tTGA

IgA h-tTGA were detected by ELISA using a commercial kit (Eurospital, Trieste, Italy) (16), whereas IgA gp-tTGA were tested for using a home-made ELISA, as previously described (14), using tTG from guinea pig liver (lot T-5398; Sigma, St. Louis, MO) as antigen. The cut-off was fixed at 7 arbritary units (AU) for both antibodies.

IgA and IgG AGA

IgA and IgG AGA were detected by indirect immunofluorescence using unfixed cryostat sections (4 μm) of rodent kidney, liver, and stomach pretreated with crude gliadin (Sigma), as previously described (20).

Total Serum IgA Levels

Serum IgA levels were assayed by nephelometry (21) in subjects with an isolated IgG AGA positivity. Those with serum IgA concentrations <0.05 g/L were regarded as being affected by selective IgA deficiency.

Intestinal Biopsy

Patients who were positive for IgA EmA, IgA h-tTGA, gp-tTGA, or IgA AGA were informed of their high probability of having CD and were invited to undergo biopsy of the small intestine. Informed consent for this invasive procedure was requested. Intestinal biopsy was taken from the second part of duodenum during fiberoptic upper GI tract endoscopy. Samples of small intestinal mucosa were graded according to the modification of Marsh's classification (22, 23). Histological evaluation was performed by a pathologist who was unaware of antibody test results. A second confirmatory duodenal biopsy was performed after 6–12 months of a gluten-free diet in patients with small intestinal mucosal damage consistent with CD. Because of the low specificity of an isolated IgG AGA positivity for CD (20), patients with this antibody pattern had histological evaluation only when IgA deficiency was proved.

Statistical Analysis

A two-tailed Fisher's exact test was used to assess the significance of the different prevalence of CD in PBC, PSC, and AIC patients. The significance of the different CD prevalence found in Italian and Spanish patients was also investigated.

RESULTS

CD-Related Antibodies

Nine (3.5%) of the 255 patients with autoimmune cholestatic liver diseases tested were positive for both IgA h-tTGA and EmA, whereas IgA gp-tTGA and IgA AGA were found in six and in five patients, respectively (Table 1). In these nine patients, EmA titers ranged from 1:5 to 1:160 and h-tTGA from 10 AU to 28 AU. Seven of these nine patients were affected by PBC (six Spanish and one Italian women, mean age 56, range 43–70 yr); the remaining two patients were a 54-yr-old Italian woman with AIC and a 32-yr-old Italian man with PSC. An isolated positivity for IgA gp-tTGA with borderline values (7–9 AU) was observed in five other cases (three PBC and two PSC), whereas three patients and 15 patients had only IgA and IgG AGA, respectively. All patients who were positive for IgG AGA showed normal values of total serum IgA, thus excluding a condition of IgA deficiency.

Correlation Between Antibody Positivity and Small Intestinal Disease

All nine patients with IgA EmA and h-tTGA underwent duodenal biopsy, which showed small intestinal mucosal damage consistent with CD (Table 1). Five patients showed mild (IIIA), three marked (IIIB), and one total villous atrophy (IIIC). In all cases, the intraepithelial lymphocyte count was $>40/100$ epithelial cells. A second intestinal biopsy performed after 6 months of gluten withdrawal confirmed CD diagnosis in all nine cases, showing a complete regrowth of small intestinal villi. Six of the eight patients with

Table 1. Antibody, Clinical, and Histological Findings in the Nine Celiac Patients Identified Among the 255 Patients With Autoimmune Cholestatic Liver Disorders

Patient	Age (yr)	Sex	GI or Other CD-Related Signs	IgA EmA >1:5	IgA h-tTGA >7 AU	IgA gp-tTGA >7 AU	IgA AGA >1:10	Duodenal Histology*	Associated Disorders
1 PBC (Spain)	58	F	None	1:80	21	15	1:40	Grade IIIA	None
2 PBC (Spain)	59	F	None	1:160	9	Neg.	Neg.	Grade IIIC	None
3 PBC (Spain)	47	F	None	1:80	16	10	Neg.	Grade IIIB	None
4 PBC (Spain)	66	F	Diarrhea, hypocalcemia, sideropenic anemia	1:10	13	9	1:10	Grade IIIA	None
5 PBC (Spain)	52	F	None	1:5	8	Neg.	Neg.	Grade IIIA	None
6 PBC (Spain)	70	F	None	1:5	16	Neg.	Neg.	Grade IIIA	None
7 PBC (Italy)	43	F	Sideropenic anemia	1:80	25	10	1:10	Grade IIIB	Autoimmune thyroiditis
8 AIC (Italy)	54	F	None	1:40	24	18	1:10	Grade IIIA	None
9 PSC (Italy)	32	M	Diarrhea, hypocalcemia, sideropenic anemia	1:160	29	15	1:10	Grade IIIB	Ulcerative Colitis

Neg. = negative.
*Duodenal histology: Marsh's revised criteria (IIIA mild, IIIB marked, and IIIC total villous atrophy).

an isolated positivity for IgA gp-tTGA or IgA AGA underwent duodenal biopsy, which excluded a condition of CD.

Clinical Findings of Small Intestinal Disease and Associated Autoimmune Disorders

In seven of the nine patients identified as having CD, no GI symptoms were reported (Table 1). A malabsorption syndrome characterized by diarrhea, weight loss, and severe weakness was found in one patient with PBC and in one patient with PSC. Both of these patients also showed impairment of biochemical absorption, with low levels of calcium and sideropenic anemia. Iron-deficiency anemia with decreased ferritin levels was also found in another PBC patient. Six of the nine celiac patients showed no CD-related symptom. With regard to associated autoimmune disorders, only one PBC patient with iron-deficiency anemia had Hashimoto thyroiditis with increased TSH levels, as well as high titers of thyroid peroxidase or thyroglobulin antibodies. Two other patients with PBC showed positivity for thyroid peroxidase antribodies with normal thyroid function.

Characteristics of Liver Disease in CD Patients

Of the nine patients with CD, seven patients (all women) were affected by PBC, with a histological picture (when liver disease had been diagnosed) of liver cirrhosis in two cases (stage IV) and florid bile duct lesions or ductular proliferation in the remaining five (stage I to II) (Table 2). The duration of liver disease at the time of CD diagnosis varied from 1 to 72 months (mean duration 33 months). Only two of these seven patients complained of pruritus or had significantly raised serum levels of bilirubin and alkaline phosphatase (AP) (>2 times upper limit of normal). The mean age (56 yr) of the seven PBC patients with CD did not differ from that of the other PBC cases without CD.

The two remaining patients were a 32-yr-old man with PSC and a 54-yr-old woman with AIC, diagnosed 39 and 27 months before CD identification, respectively. The PSC patient showed jaundice, pruritus, high levels of AP, positivity for perinuclear neutrophil cytoplasmic antibodies, and associated ulcerative colitits (histological diagnosis by colonoscopy). Liver histology gave evidence of severe cirrhosis. The AIC patient was completely symptomless, and her liver involvement was recognized based on the occasional finding of nuclear antibodies with a multinuclear dot pattern. She showed normal liver enzymes and a histological picture of florid bile duct lesions.

All nine CD patients were treated with bile acid (ursodeoxycholic acid) when CD was diagnosed.

Impact of Gluten Withdrawal on Course of Liver Disease

In the three celiac patients with severe liver disease (two PBC and one PSC cases), gluten restriction did not change the clinical and biochemical picture of cholestasis. Similarly, no change could be observed after gluten withdrawal in the other six patients with mild liver disease, because of the absence of clinical and biochemical signs of cholestasis (AP between 1.1 and 1.8 upper limit of normal, bilirubin in

Table 2. Clinical, Histological, Biochemical, and Immunological Features of the Autoimmune Cholestatic Liver Disease in the Nine Identified Celiac Patients

Patient	Clinical Signs	Disease Duration (mo) at CD Diagnosis	Liver Histology	ALT (×nl) nl 40 μ/L	AP (×nl) nl 280 μ/L	Bilirubin (×nl) nl 1.1 mg/dl	Autoantibodies
1 PBC	None	21	Florid bile duct lesion (stage I)	×0.9	×1.2	×0.5	AMA
2 PBC	None	25	Ductular proliferation (stage II)	×0.6	×1.6	×0.7	AMA
3 PBC	None	54	Ductular proliferation (stage II)	×1.1	×1.2	×0.5	AMA
4 PBC	Pruritus	72	Cirrhosis (stage IV)	×1.0	×4.8	×2.6	AMA
5 PBC	None	9	Ductular proliferation (stage II)	×0.5	×1.1	×0.6	AMA
6 PBC	Pruritus	52	Cirrhosis (stage IV)	×3.6	×3.1	×2.1	AMA
7 PBC	None	1	Florid bile duct lesion (stage I)	×0.9	×1.1	×0.7	AMA
8 AIC	None	27	Florid bile duct lesion	×1.0	×1.8	×0.5	ANA (MND)
9 PSC	Jaundice Pruritus	39	Cirrhosis	×0.8	×3.1	×4.8	p-ANCA

AMA = mitochondrial antibodies; ANA = nuclear antibodies; MND = multiple nuclear dots; nl = normal limits; p-ANCA = perinuclear neutrophil cytoplasmic antibodies.

the normal range) when they were on a gluten containing diet.

Statistical Evaluation of CD Prevalence in Autoimmune Cholestatic Liver Disorders

The difference of CD prevalence in PBC (4.0%), PSC (1.6%), and AIC (4.8%) was not significant. Moreover, although a higher prevalence of CD was found in Spanish (5.7%) than in Italian patients (2.0%), this difference also did not reach statistical significance (Table 3).

DISCUSSION

The association between CD and primary biliary cirrhosis has been debated in recent years, because conflicting results have been obtained in different European countries. A high CD prevalence ranging from 3% to 7% has been found in PBC patients from Northern Europe (1, 3, 4), whereas CD has only sporadically been found to be associated with PBC in Italy (5). As for the correlation between CD and other autoimmune cholestatic liver disease such as PSC and AIC, only one study systematically evaluated a large number of patients with PSC (11), and only one case report dealt with the combined finding of CD and AIC (12).

Table 3. CD Prevalence in Autoimmune Cholestatic Liver Disorders

	Total Cases (CD %)	Spanish Cases (CD %)	Italian Cases (CD %)
PBC	173 (4%)	86 (7%)	87 (1.1%)
PSC	61 (1.6%)	13 (0)	48 (2.1%)
AIC	21 (4.8%)	6 (0)	15 (6.6%)
PBC+PSC+AIC	255 (3.5%)	105 (5.7%)	150 (2.0%)

Statistical analysis by Fisher's exact test. CD in: A-PBC vs A-PSC, A-PBC vs A-AIC, A-AIC vs A-PSC (p = ns); B-PBC+PSC+AIC vs C-PBC+PSC+PSC+AIC (p = ns).

Our serological screening for gluten-sensitive enteropathy in Southern Europe sheds light on the close association between CD and autoimmune cholestatic liver disorders. IgA EmA and h-tTGA confirmed a higher sensitivity and specificity for CD than IgA gp-tTGA and AGA, being positive only in nine patients (seven PBC, one PSC, and one AIC) whose duodenal biopsy displayed small intestinal damage consistent with untreated CD. Instead, IgA gp-tTGA and AGA allowed identification of six and five patients, respectively; moreover, their positivity was associated with the finding of a normal small intestinal mucosa in six patients.

All but two of the nine celiac patients were without GI symptoms. In the two symptomatic patients (one PBC and one PSC), a malabsorption syndrome characterized by diarrhea, weight loss, hypocalcemia, and iron-deficiency anemia was recorded. The existence of hidden CD could be suspected only in one of the other seven CD patients because of the presence of an isolated iron-deficiency anemia and autoimmune thyroiditis, which are generally regarded as conditions at risk for CD (24, 25).

The impact of gluten restriction on the course of autoimmune cholestatic liver disorders is difficult to evaluate. In the two patients with severe liver disease and symptomatic CD, a gluten-free diet produced a stable clinical and biochemical improvement, resulting in resolution of the malabsorption syndrome, weight loss, and anemia, whereas biochemical cholestasis test results and liver disease symp-

toms such as pruritus did not improve after 1 yr of gluten withdrawal. Clinical and biochemical signs of cholestasis also remained unchanged in the third PBC patient with severe liver disease but without CD-related symptoms. In the other six patients, a gluten-free diet obviously could not bring any improvement because of the absence of clinical and biochemical liver impairment before gluten restriction. Longer follow-up of these patients with short duration of hepatic disorder at the time of CD diagnosis will clarify whether gluten restriction, together with ursodeoxycholic treatment, can contribute to slowing down the progression of liver disease.

Our study demonstrates that the prevalence of CD in patients with autoimmune cholestatic liver disorders in Southern Europe overlaps that found in Northern Europe (1–4). The prevalence of biopsy-proven gluten-sensitive enteropathy was one in 25 cases of PBC, one in 21 cases of AIC, and one in 61 cases of PSC, with an overall frequency of 3.5%. This prevalence is far higher than those found in the general population of Spain (2.6/1000, 95% CI = 0.7–8.2) and Italy (5.7/1000, 95% CI = 3.5–8.8) (26, 27).

In summary, serological screening for CD must be routinely performed in patients with autoimmune cholestatic liver disorders. For this purpose, the performance of IgA h-tTGA is far higher than that of IgA gp-tTGA and can be considered a valid alternative to IgA EmA. Although gluten withdrawal does not seem to influence the clinical and biochemical course of the associated liver disease, early recognition of the intestinal disease is mandatory, as gluten restriction improves the symptomatic forms of CD and can also reduce the risk of developing neoplastic complications such as intestinal lymphoma in patients with either silent or symptomatic CD (28).

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