Prevalence of IgA antibodies to endomysium and tissue transglutaminase in primary biliary cirrhosis

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HR Gillett, K Cauch-Dudek, E JL Heathcote, HJ Freeman. Prevalence of IgA antibodies to endomysium and tissue transglutaminase in primary biliary cirrhosis. *Can J Gastroenterol* 2000;14(8):672-675. The association between celiac disease and primary biliary cirrhosis has been described in several case reports and small screening studies, with varying prevalence rates. Stored sera from 378 patients with primary biliary cirrhosis were tested for immunoglobulin (Ig) A endomysium and tissue transglutaminase antibodies. Ten patients were positive for both antibodies (2.6%); five of these patients had had small bowel biopsies confirming celiac disease. A further 44 patients (11.6%) had raised titres of IgA tissue transglutaminase antibody but were negative for IgA endomysium antibody. The increased prevalence of celiac-related antibodies in patients with primary biliary cirrhosis suggests that the two conditions are associated, although the reason for the association remains unclear. Patients with primary biliary cirrhosis should be considered to be at high risk for celiac disease. Although liver biochemistry does not improve when these patients are fed a gluten-free diet, the complications of untreated celiac disease warrant the identification and treatment of the condition in this population.

Key Words: Celiac disease; Endomysium antibody; Primary biliary cirrhosis; Tissue transglutaminase

Prévalence des anticorps anti-endomysium IgA et anti-transglutaminase tissulaire IgA dans la cirrhose biliaire primitive

RÉSUMÉ : Le lien entre la maladie cœliaque et la cirrhose biliaire primitive a été décrit dans plusieurs exposés de cas et petites études de dépistage faisant état de taux variables de prévalence. On a analysé le sérum mis en réserve de 378 patients atteints de cirrhose biliaire primitive afin d'y déceler des anticorps anti-endomysium IgA (immunoglobuline A) et anti-transglutaminase tissulaire IgA. Des résultats positifs pour les deux types d'anticorps ont été enregistrés chez dix patients (2,6 %) et cinq d'entre eux avaient subi une biopsie de l'intestin grêle confirmant la présence de la maladie cœliaque. Quarante-quatre autres patients (11,6 %) avaient un titrage élevé d'anticorps anti-transglutaminase tissulaire IgA mais pas d'anticorps anti-endomysium IgA. La prévalence accrue d'anticorps liés à la maladie cœliaque chez les patients atteints de cirrhose biliaire primitive semble indiquer l'existence d'un lien entre ces deux maladies, mais on ne comprend pas très bien la nature de ce lien. Les patients atteints de cirrhose biliaire primitive devraient être considérés comme fortement prédisposés à la maladie cœliaque. Même si la biochimie du foie ne s'améliore pas lorsque les patients suivent un régime sans gluten, les complications d'une maladie cœliaque non traitée justifient les mesures visant à déceler et à traiter la maladie au sein de la population concernée.

The coexistence of celiac disease (CD) and primary biliary cirrhosis (PBC) was first described in four patients by Logan et al in 1978 (1). Since then, numerous case reports have been published, including one from the University Hospital, Vancouver, British Columbia (2-6). Several studies have also examined the prevalence of both conditions oc-

curing together. In general, these studies examined small series of patients and found varying prevalence rates. Kingham and Parker (7) identified 143 patients with CD and 67 with PBC, and found that both conditions occurred in 3% of those in the CD group and 6% of those in the PBC group. Similar results were reported by Dickey et al (8), who found

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Figure 1 Immunoglobulin (Ig) A tissue transglutaminase antibody (tTG) titres in 380 patients with primary biliary cirrhosis (PBC). AU Arbitrary units; EmA Endomysium antibody

that CD and PBC occurred simultaneously in 7% of 57 patients with PBC. In contrast, however, Volta et al (9) found none of 62 PBC patients to have positive CD serology. Bardella et al (10) found no cases of CD in 65 PBC patients and only one case of PBC in 336 with CD.

The aim of this study was to screen a large number of patients with PBC by using immunoglobulin (Ig) A endomysium antibody (EmA) and the newly described IgA tissue transglutaminase antibody (tTG) for CD to determine their detection rates in PBC.

PATIENTS AND METHODS

Stored sera from 378 patients with PBC from The Toronto Western Hospital, Toronto, Ontario, were screened. IgA EmA was detected with the use of indirect immunofluorescence on human umbilical cord by using the method described by Ladinser et al (11); the serum was measured at a 1:5 dilution.

Titres of IgA tTG were measured with the use of an ELISA method devised by Dieterich et al (12), which was modified to account for differences in scientific supplies. In brief, high affinity 96-well microtitre plates (Costar Corporation, USA) were coated with 1/600 U/well of tTG from guinea pig (Sigma-Aldrich Canada Ltd, Canada) in 100 mL 50 mM tris-hydrochloride, 150 mM sodium chloride and 5 mM calcium chloride (13) (pH 7.5) overnight at 4°C. After washing three times with 50 mM tris-hydrochloride, 150 mM sodium chloride, 10 mM EDTA and 0.1% Tween 20 (pH 7.4), the plate was blocked for 10 min at 37°C by using this solution. Samples were then applied to the plates at 1:5 dilution using the same solution as a diluent. The plate was incubated at room temperature for 1 h and then washed three times again. Peroxidase-conjugated rabbit antihuman IgA (Dako Corporation, USA) was applied at 1:3000 dilution for 1 h at room temperature, and the plate was again washed three times. O-phenylenediamine-hydrochloride (Sigma-Aldrich Canada Ltd) 0.4 mg/mL in citric acid 0.05 mM and dibasic sodium phosphate 0.1 mM with hydrogen peroxide 0.06% was applied to the plate and incubated

TABLE 1
Demographic data of patients with primary biliary cirrhosis

	Sex (F:M)	Age at time of study, median years (range)	Duration of serum storage, median years (range)
EmA positive (n=10)	10:0	56.1 (34.1-68.5)	7.9 (0.3-9.7)
EmA negative, tTG >40 AU/mL (n=44)	37:7	58.7 (32.6-91.9)	7.0 (0.1-10.3)
EmA Negative, tTG ≤140 AU/mL (n=324)	295:29	56.0 (23.6-82.8)	7.1 (0.1-10.5)

No significant differences between patient age or duration of serum storage were found among the three groups. AU Arbitrary units; EmA Endomysium antibody; F Female; M Male; tTG Tissue transglutaminase antibody

for 1 h in the dark at room temperature before reading using 450 nm wavelength light. A sample from a patient with biopsy-proven, untreated CD was tested in doubling dilutions until the linear portion of the standard curve was obtained. This sample was designated to have 400 arbitrary units (AU)/mL during the initial validation of the assay and was used for the standard curve of each plate. The titre, in AU, of each test sample was then calculated from this curve. Samples with optical densities above the upper limit of the standard curve were repeated at increasing dilutions until final titres were calculated. During the initial validation of the assay, a reference range of up to 140 AU/mL was calculated from titres from gastrointestinal disease control subjects with normal small bowel biopsies.

Samples with titres of less than 5 AU/mL were tested for IgA deficiency using NOR-Partigen Total IgA kit (Behring Diagnostics Inc, USA).

RESULTS

Of the 378 samples tested, 90.4% were from females. The median age of patients was 56.3 years (range 23.6 to 91.9 years), and sera were stored for a median length of 7.2 years (range 0.1 to 10.5 years).

Ten (2.6%) samples (all from females) were found to be positive for IgA EmA. All of these samples had raised titres of IgA tTG (median 2400 AU/mL; range 230 to 17,440 AU/mL), with a reference range of up to 140 AU/mL.

Forty-four (11.6%) samples (84.1% female) were negative for IgA EmA but had elevated titres of IgA tTG (median 200 AU/mL; range 142 to 505 AU/mL). The remaining 324 samples were negative for IgA EmA and had IgA tTG titres within the reference range (median 35 AU/mL; range 3 to 140 AU/mL). Three samples had tTG titres of below 5 AU/mL, but none demonstrated IgA deficiency.

The tTG titres of the EmA-positive and -negative samples are shown in Figure 1, and the demographic data are shown in Table 1.

Of the 10 patients who were positive for both antibodies,

five had had a small bowel biopsy, and all showed evidence of CD, with a confirmed prevalence of 1.3%. None of the patients from the group with high tTG titres and negative EmA had been biopsied at the time of writing.

DISCUSSION

The true nature of the association between PBC and CD has not yet been established, probably due to the lack of screening studies in large series of patients. Our study is the largest reported group of patients with PBC screened for CD. As yet, only a small number of patients have undergone small bowel biopsy, but in all biopsied patients, the diagnosis of CD was confirmed. We plan to biopsy as many of the seropositive patients as possible. CD is still diagnosed histologically, and although the majority of studies testing IgA EmA on human umbilical cord have quoted sensitivities and specificities close to 100% (11,14,15), cases of false-positive IgA EmA in patients with chronic liver disease have been reported (10,16), further emphasizing the importance of biopsy in this population. The prevalence of IgA EmA positivity in our study group was 2.6%, which corresponds to the average prevalence of the studies quoted above (7-10).

The value of tTG in screening such a population has not been validated. Early reports of the sensitivity and specificity of IgA tTG ELISAs have quoted figures slightly lower than those of the IgA EmA assays (12,17). The clustering of the titres in our samples that were negative for IgA EmA suggests that the titres in PBC may be generally higher than those in the control population used to establish the assay. Increased titres of IgA antigliadin antibodies occurring in patients with chronic liver disease, including those with PBC, have been reported (18). In this study, higher titres tended to occur in samples stored for longer periods of time or in patients

with higher levels of Ig. The authors suggested that the raised titres may simply be part of a nonspecific immunological reaction, or may be artefactual due to prolonged storage. The raised titres of tTG in our patients may also have been due to similar processes occurring within the ELISA system, but are unlikely to be due to prolonged serum storage because no difference in storage duration was found between the groups with high tTG titres and those with normal titres.

The cause of an association between PBC and CD remains unclear. Whereas CD has been strongly linked to the human leukocyte antigen (HLA) DQ2 heterodimer DQA1*0501/DQB1*0201 (19), HLA associations in PBC are less clear cut and vary among reporting centres and ethnic populations (20). However, one study that compared 23 Danish PBC patients with healthy Danish control subjects found a higher frequency of HLA B8, DR3, DQA1*0501 and DQB1*0201 in those with PBC (21). In a group of patients referred to the Toronto Hospital, an association was found with the extended haplotype HLA DRβ1*0801 DQβ*0402 (22), confirming previous reports of this association (23). This haplotype is known to be in linkage disequilibrium in the white population (24).

Treatment of CD with gluten-free diet has failed to improve liver biochemistry in patients with coexistent PBC (1,4,7). The risk of malignant and nonmalignant complications in untreated CD is, however, well documented (25,26), making treatment of identified cases imperative. In addition, complications of CD such as osteoporosis may compound similar complications in PBC.

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