CASE REPORT

Simultaneous Detection of IgA/IgG Anti-Tissue Transglutaminase/Deamidated Gliadin Peptides inSerodiagnosis of Celiac Disease

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ABSTRACT

Background: Celiac disease is a common autoimmune disorder that is diagnosed based on clinical case identification, serological screening, and duodenal histology. However, the existence of mild clinical forms, such as seronegative cases with patchy atrophy and potential celiac disease, can make it difficult to determine a definitive diagnosis. The seronegative patients with celiac disease can include those with discordant antibody results and false-negative results, due to unknown origins or selective IgA deficiency. **Case presentation:** We present two cases with discordant antibody results in order to evaluate if the simultaneous detection of specific antibodies can improve the serodiagnosis of celiac disease. In both patients, the simultaneous detection of IgA/IgG anti-tissue transglutaminase/deamidatedgliadin peptides gave discordant positive results by the same antibodies assayed individually. **Conclusion:** Although further studies are needed to confirm and extend our findings, the simultaneous detection of specific antibodies seems to improve the serodiagnosis of celiac disease in patients with discordant antibody results.

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Keywords: Antibody, Celiac Disease, IgA, IgG, Serodiagnosis, tTG/DGP

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INTRODUCTION

Celiac disease (CD) is one of the most common autoimmune disorders in the world, with a prevalence of about 1%, triggered by the ingestion of wheat gluten (gliadins + glutenins) and its relative proteins from rye and barley (1). In some genetically susceptible individuals carrying the HLA-DQ2 and/or -DQ8 alleles, the ingestion of these proteins elicits an innate response that, after deamidation of immunodominant peptides by tissue transglutaminase (tTG), promotes both a humoral and cell-mediated adaptive response exhibiting antibody production and intestinal villous flattening, respectively (2,3). Although the small intestine is the primary target organ of CD, this condition is often characterized by a wide variety of symptoms that may be both intestinal and extra-intestinal, such as diarrhea, abdominal pain, bloating (typical form), iron deficiency anemia, dental enamel hypoplasia, and osteopenia/osteoporosis (atypical form) (1).

In both adults and children, the diagnosis of CD is currently based on clinical case identification (or HLA typing for asymptomatic children), serological screening, and duodenal histology (with the exception of symptomatic children showing serum anti-tTG levels exceeding ten times the cut-off value) (4,5). However, the existence of mild clinical forms and seronegative patients, with patchy atrophy and potential CD, can make a correct diagnosis difficult to obtain (6-8). In the present report, we described two cases with discordant antibody results in order to evaluate if the simultaneous detection of specific antibodies may improve the serodiagnosis of CD.

THE CASE

A 44 year old Caucasian female (case 1) and a 29 year old Caucasian male (case 2), both awaiting CD diagnosis, were evaluated and subjected to HLA typing, serum detection of IgA endomysium antibodies (EMA) by indirect immunofluorescence analysis (Eurospital, Trieste, Italy), IgA and IgG anti-tTG, IgA and IgG anti-deamidatedgliadin peptides (anti-DGP) and IgA/IgG anti-tTG/DGP multiplex by enzyme-linked immunosorbent assays (INOVA Diagnostics, San Diego, CA; distributed by Instrumentation Laboratory, Milan, Italy), upper endoscopy with duodenal biopsy sampling, and histological analysis according to the Marsh-Oberhuber classification (9). Serum concentration of total IgA was measured only in case 2. Once diagnosed, both patients were treated with a gluten-free diet (GFD) and strictly monitored for at least one year by means of clinical and serological assessment. All procedures were made for diagnostic purposes and, therefore, were in accordance with the ethical standards of the institutional committee responsible for human experimentation.

Case 1 was admitted to our Gastroenterology Unit due to symptoms including chronic diarrhea (at least 3-4 evacuations per day) with soft or runny stools, abdominal pain, bloating, meteorism, and asthenia that ameliorated in relation to the ingestion of gluten. The HLA typing showed the following outcome: HLA-DQ2 heterozygous in cis. Serum IgA EMA was weakly positive, while IgA anti-tTG, IgG anti-tTG, IgA anti-DGP, and IgG anti-DGP were negative with 1.05, 1.18, 1.30, and 4.26 folds lower than the cut-off value, respectively. The endoscopic evaluation highlighted a reduction in Kerckring folds (<3 folds per endoscopic field) and scalloped folds. Duodenal histology showed

partial villous atrophy, crypt hyperplasia, and intraepithelial lymphocytosis (>40 intraepithelial lymphocytes per 100 enterocytes), compatible with the IIIa type of the Marsh-Oberhuber classification (Table 1).

Table 1. Clinical, serological, and histological parameters of the studied cases.

Parameters	Case 1 (♀, 44 yrs)	Case 2 (♂, 29 yrs)	Normal Values
Clinical picture	Typical[a]	Atypical[b]	
HLA typing	DQ2 Heterozygous (cis)	DQ2 Heterozygous (trans)	
IgA EMA	Weakly positive	Doubtful	
IgA anti-tTG	3.8 kU/L	3.3 kU/L	<4.0 kU/L
IgG anti-tTG	5.1 kU/L	10.8 kU/L	<6.0 kU/L
IgA anti-DGP	15.4 AU	14.1 AU	<20.0 AU
IgG anti-DGP	4.7 AU	26.9 AU	<20.0 AU
Total IgA	nd	2.98 g/L	0.9-4.0 g/L
Upper Endoscopy	↓↓ Kerckring folds Scalloped folds	↓ Kerckring folds	
Duodenal Histology	IIIa type[c]	IIIb type[c]	

DGP, deamidatedgliadin peptides; EMA, endomysium antibodies; HLA, human leucocyte antigen; Ig, immunoglobulin; nd, not detected; tTG, tissue transglutaminase.
[a] Patient with intestinal symptoms. [b] Patient with systemic manifestations without evidence of intestinal symptoms. [c] In reference to the Marsh-Oberhuber histological classification (9).

Case 2 was admitted because of an iron deficiency anemia refractory to oral iron supplementation [red blood cells (RBC) = $3.95 \cdot 10^{12}$/L, hemoglobin (Hgb) = 127 g/L, hematocrit (Hct) = 37.8%, mean corpuscular volume (MCV) = 82.0 fL, iron = 10.2 µmol/L, ferritin = 42.7 pmol/L], dental enamel hypoplasia, clubbing, and asthenia without an evidence of intestinal symptoms. The absence of rectal bleeding, as well as the negative results of the fecal occult blood test and urea breath test excluded the main gastroenterological causes of anemia other than CD. The HLA typing showed the following result: HLA-DQ2 heterozygous in trans. Serum IgA EMA was doubtful. IgA anti-tTG and IgA anti-DGP were negative with values 1.21 and 1.42 times lower than the cut-off, respectively. Contrastingly, IgG anti-tTG and IgG anti-DGP were positive with values 1.80 and 1.35 folds higher than the cut-off, respectively. Serum concentration of total IgA fell within the normal range. The endoscopic evaluation highlighted a mild reduction in the Kerckring folds (2-3 folds per endoscopic field). Duodenal histology showed subtotal villous atrophy, crypt hyperplasia, and

intraepithelial lymphocytosis (>40 intraepithelial lymphocytes per 100 enterocytes), compatible with the IIIb type of the Marsh-Oberhuber classification (Table 1).
As shown in Figure 1, IgA/IgG anti-tTG/DGP serum levels were 24.1 AU (1.21 times higher than the cut-off) in case 1 and 32.3 AU (1.62 fold higher than the cut-off) in case 2 (n.v. <20.0 AU). Therefore, on the basis of all the clinical, serological, and histological data, case 1 was diagnosed with typical CD and case 2 with having atypical CD. In both patients, the clinical and serological picture progressively improved after a GFD was started.

Figure 1

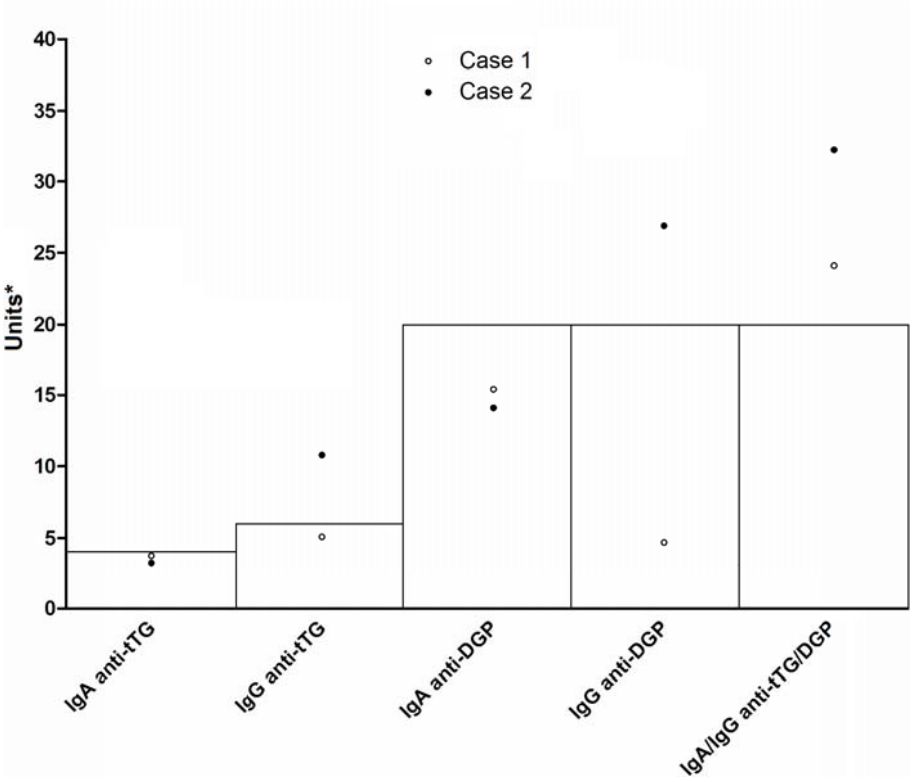

Figure 1. Serum antibody levels of the cases being studied. The levels of serum antibodies are plotted in the graph, where each box is as high as the corresponding cut-off value,making the antibody levels falling within each box negative, and those outside positive.
Legend: DGP, deamidatedgliadin peptides; Ig, immunoglobulin; tTG, tissue transglutaminase.
* kU/L for IgA anti-tTG and IgG anti-tTG, AU for IgA anti-DGP, IgG anti-DGP and IgA/IgG anti-tTG/DGP.

DISCUSSION

According to the latest guidelines from the British Society of Gastroenterology (BSG) (4), as well as those from the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) (5), a proper diagnosis of CD requires a clinician's evaluation of a wide spectrum of symptoms (clinical case identification) and the presence of specific antibodies in the patient's serum (serological screening). If the

clinical picture and serological tests are suggestive of CD, the histological evidence of intestinal villous atrophy is sufficient for confirming the diagnosis. The clinical remission and disappearance of circulating antibodies on a GFD further confirms the diagnosis. However, the existence of mild clinical forms such as seronegative patients, with patchy atrophy and potential CD, can make a correct diagnosis difficult to determine (6-8).

Serum detection of specific antibodies is used to screen cases with suspected CD as well as to monitor the adherence and response to GFD in patients with a definitive diagnosis. For these purposes, some antibodies against non-self (IgA and IgG anti-DGP) and self (IgA EMA and anti-tTG) proteins are normally used. IgG EMA and anti-tTG are specifically used for patients with selective IgA deficiency (SIgAD), in whom the screening program for CD may give false-negative results if performed with only the IgA isotype (10,11). In addition to cases with false-negative results due to SIgAD, the seronegative CD patients can include those with discordant antibody results and false-negative results of an unknown origin (6). New antibody tests, such as IgA/IgG anti-DGP dual, IgA/IgG anti-tTG/DGP multiplex, and IgA/IgG anti-tTG-gliadin complexes, have been recently developed to improve the screening program for CD, especially in patients with seronegative results (12-15).

Bearing in mind these observations, we studied two cases with discordant antibody results to evaluate if the simultaneous detection of specific antibodies may improve the serodiagnosis of CD. The first case is a 44 year old female with intestinal symptoms, HLA predisposition to CD, and a histological outcome compatible with intestinal damage in the absence of serum antibody positive results, with the exception of IgA EMA that were weakly positive. The second case, case #2, is a 29 year old male with extra-intestinal symptoms, CD predisposing HLA type, and a histological outcome compatible with intestinal damage in the absence of serum antibody positive results, with the exception of IgG anti-tTG and anti-DGP which were above the cut-off value. In both cases, the IgA/IgG anti-tTG/DGP serum test provided positive finding, thus, contributing to the final diagnosis of typical and atypical CD, respectively. The improvement in the clinical and serological picture obtained in both patients within one year from a GFD start date supports the reliability of the IgA/IgG anti-tTG/DGP simultaneous detection in the serodiagnosis of CD.

Another finding that emergesfrom the assessment of case 2 is the positive result for specific antibodies of IgG isotype, but not IgA, in the absence of SIgAD, since the serum concentration of total IgA fell within the normal range. Although this finding is consistent with two previous studies describing, respectively, 9 and 49 IgA EMA-negative cases with IgG anti-tTG and/or IgG1 EMA positive results in the absence of SIgAD (16,17), further investigations are needed to understand the importance of this antibody pattern in CD patients. However, It has been hypothesized that the antibody response to gluten exhibit mucosal IgA as its major component, while systemic IgG represent a longer-term reaction,which is probably related to the occurrence of extra-intestinal manifestations (18,19). Consistently, case 2, as described in our report, had systemic manifestations without an evidence of intestinal symptoms.

Regarding all this data, the simultaneous detection of IgA/IgG anti-tTG/DGP seems to have the potential to improve the serodiagnosis of CD in patients with discordant antibody results and, as it also seems, its use as a first-level screening test could help save economical, temporal, and human resources, as previously suggested (12,14). However, given that CD-specific anti-tTG antibodies can be detected in those suffering

from Crohn's disease (CrD) (20), and CrD-specific anti-glycoprotein 2 antibodies have been recently described in CD patients (21), a differential diagnosis between these two conditions should be performed, especially in cases with discordant antibody results. Furthermore, the two cases described in our report are not sufficient for assessing the diagnostic performance of the IgA/IgG anti-tTG/DGP serum test in terms of sensitivity, specificity, accuracy, and positive/negative predictive values. Further studies are therefore needed to confirm and extend our observations ina greater number of cases before the employment of this new serological test in the diagnostic work-up of CD can be fully suggested.

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