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Alimentary Tract

The anti-deamidated gliadin peptide antibodies unmask celiac disease in small children with chronic diarrhoea

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ABSTRACT

Objectives: To assess the usefulness of a new class of antibodies, the anti-deamidated gliadin peptides, in the diagnostic approach to children less than 2 years with suspected celiac disease.
Patients and methods: We investigated 40 children (median age: 16.8 months; age range: 4–24 months), with symptoms and signs of chronic enteropathy and high serum levels of conventional anti-gliadin antibodies, but normal values of anti-transglutaminase and anti-endomysial antibodies; all underwent measurement of anti-deamidated gliadin peptides serum levels, upper gastrointestinal endoscopy with biopsies and HLA typing; 40 subjects served as controls.
Results: In 29 patients (group A) serum levels of anti-deamidated gliadin peptides were normal and duodenal histology showed a spectrum of abnormalities ranging from mucosal inflammatory infiltrates to villous damage (in almost all cases compatible with Marsh 1-to-2 lesions). All improved on a cow's and soy milk free diet containing gluten. In 11 patients (group B) there were high serum levels of anti-deamidated gliadin peptides and histology showed features suggestive of celiac disease (Marsh 2-to-3 lesions) in all; furthermore, human leucocyte antigen typing was consistent with a celiac disease genetic pattern in all. Group B patients significantly improved on a gluten free diet containing cow's and soy milk proteins. None of the control group was anti-deamidated gliadin peptides positive.
Conclusions: In children younger than 2 years with signs of chronic enteropathy and normal values of classical serum markers of celiac disease, the latter can be predicted by high serum levels of anti-deamidated gliadin peptides.

1. Introduction

Celiac disease (CD) is an autoimmune chronic enteropathy triggered by the ingestion of wheat gliadins and related cereal proteins in genetically susceptible individuals [1]. In the pathogenesis of the disease an altered T-cell response and the involvement of tissue transglutaminase (tTG), an enzyme engaged in several biological processes, seem quite evident [2–5].

The gliadin is a good substrate for tTG, which deamidates its residues, making gliadin peptides suitable for human leucocyte antigen (HLA)-mediated antigen presentation and able to trigger a Th1 immunological response [1,6,7]. In fact, cross-linking of gliadin epitopes to tTG has been hypothesised to induce the production of antibodies to gliadin–tTG complexes [8]. It has been demonstrated that deamidated gliadin peptides play a pivotal role in the development of the autoimmune process [9,10].

Currently, even though many serological tests have been shown to be highly predictive for CD diagnosis, intestinal biopsy is still considered essential [1], although duodenal histological lesions showing villous damage and inflammatory infiltrate cannot be specific for CD and are also detectable in other enteropathies [11–15].

Anti-tTG IgA and anti-endomysial IgA (EMA) are the serum gold standard tests for CD screening and diagnosis; however, they are not detected in IgA deficient patients and are not uncommonly negative in celiac children aged less than 2 years [16–19]. On the other hand, high serum levels of anti-gliadin antibodies (AGA) IgA and IgG are often detected in very young celiac children, but they lack specificity since they are found in other diseases also with increased intestinal permeability, such as food allergy and, more rarely, in asymptomatic individuals [20]. Amongst paediatric gastroenterologists, differentiating between CD and other chronic enteropathies (i.e. food allergy, protracted diarrhoea, toddler diarrhoea, giardiasis) in very young children is not uncommonly

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Table 1
Demographic data and clinical features of the enrolled patients of group A (food allergies and other enteropathies) and group B (celiac disease).

	Group A	Group B
Number of patients	29	11
Male sex	19 (66%)	4 (36%)
Median age (age range)	14.0 months (4–24 months)	16.0 months (7–23 months)
Main presenting symptoms; number of patients (%)	Chronic diarrhoea: 27 (93%); failure to thrive: 9 (31%)	Chronic diarrhoea: 9(31%); failure to thrive: 8 (31%)
Minor symptoms	Bloating: 8 (28%); colics: 15 (52%); vomiting: 11 (38%)	Bloating: 5 (17%); colics: 8 (28%); vomiting: 3 (10%)

a clinical challenge, also because small intestinal mucosal damage can be non specific [13].

Recently, a new enzyme-linked immunoadsorbent assay (ELISA) test for antibodies to deamidated gliadin peptides (aDGP) IgA and IgG has been developed. These antibodies, especially IgG, reveal high sensitivity and specificity, and they seem to be very useful for CD screening [21–25], mostly when anti-tTG IgA antibodies are lacking [26].

The aim of our study was to assess the role of aDGP in differentiating between CD and other causes of chronic diarrhoea and failure to thrive in children ageing less than 24 months with high levels of serum AGA, and negative values of serum EMA and anti-tTG.

2. Patients and methods

Amongst patients referred to our Paediatric Gastroenterology Unit, we enrolled 40 children (17 females) less than 2 years (median age: 16.0 months; age range: 4–24 months), with normal serum IgA levels, who presented gastrointestinal symptoms and signs suggesting a chronic enteropathy (i.e. chronic diarrhoea, colics, failure to thrive, abdominal distension and bloating). Serum IgA, AGA (IgA and IgG), EMA IgA, anti-tTG IgA had been previously measured to screen for CD; skin prick tests had also been carried out to evaluate sensitisation to the most common food antigens. In these patients, the only remarkable laboratory finding was conventional AGA positivity. Subsequently, we searched for serum aDGP (IgA and IgG) and performed upper gastrointestinal endoscopy with duodenal biopsies under general anaesthesia. Duodenal histology was graded according to Marsh classification, modified by Oberhuber et al. [27].

The aDGP (IgA and IgG) were also measured in 40 control children, who had been matched for age and sex to the studied patients. Controls were represented by children referred to our Paediatric Surgical Unit for minor surgical procedures and without chronic gastrointestinal diseases.

Conventional AGA had been tested by a sandwich type enzyme immunoassay. It can be used for the quantitative determination of IgA/IgG specific antibodies directed against the α-fraction of wheat gluten gliadin in the human serum. The cut-off values, provided by the manufacturer, were 16.0 UA/ml and 50.0 U/ml for AGA IgA and IgG, respectively. Anti-tTG IgA/IgG had been analysed by a sandwich type enzyme immunoassay. The cut-off values, provided by the manufacturer, were 16.0 UA/ml and 30.0 U/ml for anti-tTG IgA and IgG, respectively. EMA had been analysed by an indirect immunofluorescence assay on sections of monkey oesophagus. All assays listed above had been tested by Kits Eurospital, Trieste, Italy. aDGP IgA/IgG, then, were analysed by an ELISA for the semi-quantitative detection of anti-deamidated gliadin peptides IgA and IgG antibodies in human serum. The sample were classified as positive if >20 U/ml (Kit QuantaLite™ Gliadin IgA/IgG II-INOVA Diagnostics) [28].

HLA typing was based on polymerase chain reaction (PCR) with allele-specific primers identifying HLA DQ2 and DQ8. In celiac disease, 90–95% of patients carry HLA DQ2-haplotype and most of the remainder carry HLA DQ8 [29].

Written informed consensus from all children's parents was obtained. The study was approved by the Ethical Committee of our Hospital.

Statistical data were analysed using the SPSS software version 16. Antibodies titres are reported as mean, standard deviation (SD) and 95% confidence interval (95% CI). A serologic test was considered positive if the serum concentration of the antibody was above the cut-off value. Comparisons between groups were performed using the Student's *t* test; differences were considered statistically significant when their p value was <0.05.

3. Results

Demographic data and clinical features of enrolled patients are summarised in Table 1. In 29 of 40 children (group A) serum levels of aDGP were normal, and skin tests for the most common food antigens negative. All of them exhibited a spectrum of histological duodenal abnormalities ranging from a mild-to-moderate inflammatory infiltrate of the lamina propria to various signs of villous abnormalities; in particular, 27 of 29 had histological lesions compatible with the Marsh–Oberhuber criteria (10 were graded as Marsh-2 and 17 as Marsh-1), whilst 2 of them only showed an increased number of lymphocytes in the lamina propria. All these patients exhibited a successful response to a cow's milk- and soy-free diet containing gluten, with disappearance of intestinal symptoms. HLA typing in this group of patients revealed that 8 of 29 had alleles at risk for developing CD, resembling slightly the same prevalence of this genetic pattern in the general population. An oral food challenge was performed after 4-to-6 weeks and 20 of 29 patients had a clinical worsening whilst the return to the previous diet was once more beneficial: thus, diagnosis of FA was proved. In addiction, the values of serum conventional AGA markedly decreased upon the oligoantigenic diet after 6 and 12 months ($p < 0.01$) (Figs. 1 and 2). Of the 9 children with negative cow's milk and soy challenge, only 3 carried CD-related HLA haplotypes; anyhow, all remained symptomless and negative for AGA, aDGP, anti-transglutaminase and anti-endomysial antibodies at follow-up on a gluten-containing diet.

In 11 of 40 patients (group B) there were high serum levels both of conventional AGA and aDGP; amongst the latter, high values of aDGP IgG were detected in all of them, whereas high values of aDGP IgA in 9. All patients of this group had duodenal histopathological features suggestive of CD (2 were graded as Marsh-2, 1 as Marsh-3a, 2 as Marsh-3b and 6 as Marsh-3c), and exhibited a noticeable improvement on a gluten-free diet containing cow's and soy milk proteins. HLA typing in these patients was consistent with a CD genetic pattern (DQA1*05/DQB1*02 in 9 patients; DQA1*03/DQB1*0302 in 2 patients). Moreover, serum aDGP significantly decreased at 6 and 12 months after the beginning of diet ($p < 0.01$) (Figs. 3 and 4).

None of the control group (group C) was positive for aDGP; 8 children of this group showed an increased level of AGA IgG, but AGA IgA, which are more specifically associated with CD, were not detected in any of them (Table 2).