

The Usefulness of IgA/IgG DGP/tTG Screen Assay for Celiac Disease Detection among Symptomatic and at Risk Young Children

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Abstract Background: It has been postulated that IgA anti tissue-transglutaminase (tTG) and anti-endomysium (EMA) antibodies can be false negative in young children. ESPGHAN recommended for seronegative children younger than 2 years old with clinical suspicion of celiac disease to perform duodenal biopsies. Recent studies suggested that the combined assay for IgA/IgG deamidated gliadin peptides (DGP) and tTG can detect celiac disease among seronegative young children. Aim: To assess if the new combined assay with synthetic gliadin derived peptides IgA/IgG-DGP/tTG is useful to detect celiac disease in IgA tTG or EMA seronegative children younger than 2 years old. Methods: The authors screened a lot of children aged 6 months to 2 years old that associated characteristic symptoms/risk factors for gluten enteropathy. 368 children were tested for IgAtTG, EMA and IgA/IgG-tTG/DGP combined assay. All children had normal total IgA concentration and were consuming gluten at the time of enrolment. Children with at least one positive serologic test underwent intestinal biopsy, including seronegative infants, DQ2/DQ8 positive, with clinical suspicion of celiac disease that underwent the 2 biopsies protocol. Results: Celiac disease was diagnosed in 22 children based on histology. 19 children were positive for IgA tTG, 20 were positive for EMA and 21 tested positive for IgA/IgG-DGP/tTG. IgA tTG sensitivity was 86.3%, IgA EMA sensitivity was 91% and IgA/IgG-DGP/tTG sensitivity was 95.4% ($p=0.002$). Conclusions: The sensitivity of IgA/IgG DGP/tTG assay was significantly higher than that of IgAtTG in celiac patients younger than 2 years old. The better performance of this new combined test can avoid repeated intestinal biopsies in young children with high clinical suspicion of celiac disease but negative tTG/EMA serology.

Keywords: celiac disease, children, serology, intestinal biopsies

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1. Introduction

Studies regarding atypical or silent forms of celiac disease (CD) have generated a great interest for methods of serologic screening in gluten enteropathy diagnosis. Using different serologic tests permitted a better selection of cases for intestinal biopsy in celiac patients. In clinical practice, serological tests for CD are useful in identifying patients who require intestinal biopsy findings to diagnose this condition. Although anti-reticulin antibodies have historically been used in the evaluation of CD, these assays lack optimal sensitivities and specificities and were eliminated from routine diagnostic use. [1] Besides anti-reticulin antibodies, older markers judged insufficiently accurate like IgA and IgG anti-gliadin antibodies have recently been withdrawn from the list of reimbursed medical expenses in France. [2] Anti-endomysium antibodies (EMA) and anti-tissue transglutaminase antibodies (tTG) assessment are both highly sensitive and

highly specific tests, with values for both parameters exceeding 96% in most studies. [3] The endomysial antigen has been identified as the protein cross-linking enzyme known as tissue transglutaminase. [4] IgA and IgG antibodies against deamidated, synthetic gliadin peptides (DGP) were described as valuable diagnostic parameters in pediatric CD. DGP-antibodies specifically bind the disease-inducing antigen and might therefore be superior in monitoring patients on a gluten-free diet. [5] Recent studies discovered that protein kinase C delta is a substrate of tissue transglutaminase and a novel autoantigen in celiac disease. Post-translational modification of proteins by deamidation or transamidation by tissue transglutaminase enzyme has been suggested as a possible mechanism for the development of autoimmunity. Sequence analysis of protein kinase C delta (PKC δ) identified an amino acid motif that suggested the possibility that PKC δ was a glutamine substrate of tTG. Elevated levels of anti-PKC δ antibodies were detected in sera from patients with coeliac disease ($p<0.0001$) but not from patients with other autoimmune disorders [6].

Serological screening of the general population will identify most cases of previously unrecognized CD, however mass screening for CD is not currently recommended, as the potential cost/benefits of such a strategy have not been determined. An active case-finding strategy targeting both symptomatic and asymptomatic individuals who are at risk for CD is currently considered a more cost-effective approach to diagnosis [7].

Until recent, the gold standard diagnosis tool for gluten enteropathy was represented by intestinal biopsy showing characteristic villous lesions. According to the revised criteria for CD diagnosis proposed by the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), in certain cases with characteristic symptoms, with serum level of auto antibodies higher than 10 times normal values and positive DQ2 or DQ8 HLA, intestinal biopsy is no more mandatory to confirm the disease [8].

Interpretation of serological assays in CD must consider IgA selective deficiency as potential source of false negative results for IgA serology based tests. It has also been postulated that measuring IgA or IgG EMA and tTG is less accurate than IgA or IgG anti-gliadin antibodies (AGA) to detect CD in young children, leading therefore to false negative results. [9] Thereby, in the past years ESPGHAN recommended for the group of age under 2 years old to apply the protocol of the two intestinal biopsies (considering gluten challenge procedure) in order to diagnose CD at this group of age. The revised criteria elaborated by ESPGHAN in 2012 stated that for CD diagnosis the gluten challenge is not mandatory, except under unusual circumstances. These circumstances include situations in which there is doubt about the initial diagnosis, including patients with nonspecific CD antibodies before starting a gluten free diet or children younger than 2 years old with characteristic symptoms, positive HLA DQ2 or DQ8 and negative tTG or EMA serology. [8] Several recent studies suggested that IgA/IgG DGP antibodies assays can detect CD among tTG seronegative celiac patients, decreasing the need for repeated biopsies in young children [10,11].

2. Aim

The objective of this study is to assess if new combined assay incorporating synthetic gliadin derived peptides (IgA/IgG DGP/tTG) is useful to detect gluten sensitivity in IgA tTG and EMA seronegative children younger than 2 years old. A better performance of this new combined test could avoid repeated intestinal biopsies in young children with high clinical suspicion of CD but negative tTG/ EMA serology.

3. Material and Methods

We developed a prospective active case finding study over a period of 3 years between May 2010 and June 2013. The randomized prospective study was based on screening strategy in a pediatric group aged 6 months to 2 years old that linked symptoms or several risk factors for CD such as: chronic diarrhea, malabsorption syndrome, weight deficit, short stature, failure to thrive, iron deficiency

anaemia, idiopathic hyper-transaminasemia, association of autoimmune diseases, chromosomal disorders (Down syndrome, Williams syndrome, Turner syndrome), or presence in the family of a first degree relative with CD. The lot of study consisted of 368 children. All infants enrolled in this study have received complementary food with gluten previously. Children included in this study were all IgA sufficient. We obtained the informed consent from legal guardians for all children included in this study. All children were tested for IgA tTG (ImmuLisa™ anti-hu tTG antibody IgA, Immco Diagnostics), IgA EMA (ImmuGlo™ Anti-Endomysial Antibody, Immco Diagnostics) and IgA/IgG h-tTG/DGP combined assay (QUANTA Lite). Children with at least one positive serologic test underwent intestinal biopsy, including seronegative infants HLA DQ2/DQ8 positive, with high clinical suspicion of CD who underwent the 2 biopsies protocol. All biopsy samples were classified using Marsh criteria (1992) modified by Oberhuber (1997): type I infiltrative (infiltrative lympho-plasmocytic lesions in villous corion), type II hyperplastic (infiltrative lympho-plasmocytic lesions in villous corion, associated by glandular crypt enlargement) and type III destructive (including partial and subtotal villous atrophy – type IIIa, IIIb respectively and total villous atrophy – type IIIc).

Statistical analysis was performed using a specific informatics application - R statistic soft program version 2.7.1. Data were analyzed by chi-square test. For all statistical analyses, a two-tailed p value <0.05 was considered significant.

4. Results

From 368 patients enrolled in this study and screened for CD, 19 were positive for IgA tTG, 20 were positive for EMA and 21 tested positive for IgA/IgG DGP/tTG. All tTG and EMA positive cases were also positive when tested by combined DGP/tTG Screen test. 2 infants with negative results for IgA tTG were also detected positive by using combined IgA/IgG DGP/tTG assay and one child with negative result for EMA was detected positive by using combined IgA/IgG DGP/tTG assay. The intestinal biopsies showed characteristic villous injuries in all 21 patients with at least one positive serologic test, with lesions scores ranging from Marsh II to Marsh IIIc.

In this study, the authors excluded the diagnosis of celiac disease in exceptional cases with conditions causing non-celiac enteropathy and villous atrophy such as: parasitic infection (Giardiasis), immune-mediated enteropathy, eosinophilic gastroenteritis, HIV enteropathy, combined immunodeficiency states s.a. Non-celiac enteropathy is suggested by a normal initial tTG, villous atrophy with lack of intraepithelial lymphocytosis, absence of histological response to a gluten free diet and can be confirmed by negative HLA-DQ2/DQ8.

From the rest of 347 seronegative children, one infant aged 11 months old with chronic diarrhea, failure to thrive and severe iron deficiency anemia presented total villous atrophy on intestinal biopsy sample. HLA typed in this case was DQ2. We established the diagnosis of CD based on the 2 biopsies protocol. After the first biopsy, the infant was put on gluten free diet for 6 months, showing symptoms remission. The second biopsy was performed

after another 6 months of gluten challenge showing histological relapse with villous atrophy and also symptoms relapse. The original protocol of 3 biopsies for diagnosis of CD in children younger than 2 years old was modified into “the 2 biopsies protocol”, excluding the biopsy recommended initially after 6 months of gluten free diet, in order to decrease the invasive pattern of this protocol in infants. Only clinical and serological survey is recommended after 6 months of gluten free diet.

All 22 children with intestinal injuries classified Marsh II-IIIc were framed as celiac patients. HLA typing was performed in all these patients and all these children associated DQ2 or DQ8 alleles. The rest of 346 children were considered the control lot. Their digestive symptoms were due to different conditions other than celiac disease: gastro-esophageal reflux disease, cow’s milk protein allergy, secondary lactose deficiency after rotavirus or adenovirus gastroenteritis etc.

Figure 1 shows the distribution of seropositive and seronegative children.

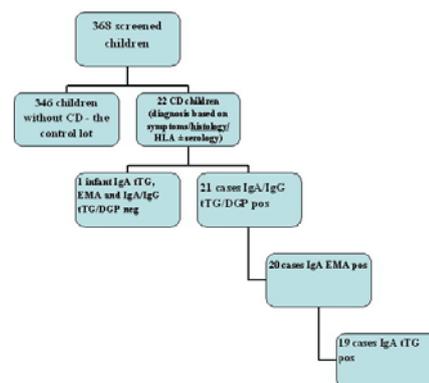


Figure 1. The distribution of seropositive and seronegative children

Table 1 presents clinical, histological and genetic characteristics of the study lot consisting in 22 celiac patients aged less than 2 years old with histological based diagnosis. Positive IgA/IgG tTG/DGP included positive IgA tTG and EMA patients.

Table 1. The clinical, histological and genetic characteristics of CD patients

Serologic pattern	Pos IgA tTG	Pos IgA EMA	Neg IgA tTG	Neg IgA EMA	Pos IgA/IgG tTG/DGP	Neg IgA/IgG tTG/DGP
No of Patients	19	20	3	2	21	1
Clinical Forms Of Celiac Disease						
Typical	14	15	3	2	15	1
Atypical	5	5	0	0	5	0
Silent	0	0	0	0	1 (Down Sdr)	0
Marsh Classification Of Villous Injury Severity						
Marsh I	0	0	0	0	0	0
Marsh II	1	1	0	0	1	0
Marsh IIIa	0	0	0	0	0	0
Marsh IIIb	2	2	1	0	2	0
Marsh IIIc	16	17	2	2	18	1
HLA in Sero negative Patients and Silent Form			1 Case DQ2 Heterozygous 1 Case DQ2 Homozygous 1 Case DQ8	1 Case DQ2 Heterozygous 1 Case DQ2 Homozygous	DQ2 Heterozygous (For Patient With Silent CD)	DQ2 Homozygous

In this study, among those 22 celiac children aged less than 2 years old, the main form of presentation at diagnosis was the classic type of gluten enteropathy, including chronic diarrhea, weight loss, failure to thrive, malabsorption syndrome. Only 5 cases (23%) presented atypical forms of disease as chronic constipation, iron deficiency anemia non responsive to oral iron therapy, idiopathic hyper-transaminasemia or dental defects. All 5 patients with atypical forms of CD had positive results for all tested antibodies. One infant with negative IgA tTG and EMA but positive IgA/IgG tTG/DGP test, presented the silent form of disease. He was asymptomatic, with Down syndrome, total villous atrophy and positive heterozygous DQ2 HLA. All 3 patients with negative IgA tTG antibodies associated typical forms of CD and villous flattening, one of them presenting Marsh IIIb and 2 of them Marsh IIIc injuries. Those 2 infants with Marsh IIIc lesions had also negative tests for EMA. These seronegative children associated DQ2 or DQ8 haplotypes.

Assessing these data, we calculated the sensitivity (Sn), specificity (Sp), positive predicted value (PPV) and negative predicted value (NPV) of IgA EMA, IgA tTG and combined IgA/IgG DGP/tTG Screen test in young children aged less than 2 years old.

IgA EMA Sn was 91%, IgA tTG Sn was 86,3% and IgA/IgG DGP/tTG Sn was 95,4%. The Sp was 100% for IgA EMA, 100% for IgA tTG and 100% for IgA/IgG

DGP/tTG Screen. PPV was 100% for EMA, 100% for tTG and 100% for IgA/IgG DGP/tTG Screen. NPV was 99,4% for EMA, 99,1% for tTG and 99,7 % for IgA/IgG DGP/tTG Screen.

We used chi square test in order to compare Sn for IgA tTG and IgA/IgG DGP/tTG. The sensitivity of IgA/IgG DGP/tTG Screen single assay was significantly higher than that of IgA tTG in children with CD younger than 2 years old. We obtained a statistical significant difference $p = 0,002$. There were no statistical differences between IgA EMA, IgA tTG and IgA/IgG DGP/tTG Sp, PPV and NPV for diagnosis of CD in children younger than 2 years old ($p > 0,05$). The statistic parameters for CD serologic assays described in this study are presented in Table 2.

Table 2. Statistic parameters for celiac disease serologic assays in infants and children younger than 2 years old

Test Test	Sn	Sp	PPV	NPV
IgA EMA	91%	100%	100%	99,4%
IgA tTG	86,3%	100%	100%	99,1%
IgA/IgG DGP/tTG	95,4% ($p = 0,002$ versus IgA tTG)	100%	100%	99,7%

5. Discussions

Recent studies have revealed that gliadin reactive antibodies from celiac patients bind a very limited number of specific epitopes on the gliadin molecule. [12] These studies further revealed that selective deamidation of gliadin by the celiac-associated enzyme tissue transglutaminase results in enhanced binding by AGA. [13] Based on the observations mentioned above, assays using deamidated and defined peptides have been shown to have higher diagnostic accuracy for CD when compared to standard AGA and tTG assays. [14] Conventional or native gliadin antibody tests have, in general, low specificity and sensitivity. Some evidence exists, however, that their sensitivity may be higher in children younger than 2 years in comparison with EMA and anti-tTG tests. Unfortunately, the specificity is low in this age group and makes AGA tests unreliable in clinical practice. It is thus advisable to obtain a small-intestine biopsy sample in young children with severe symptoms suggestive of CD, even when their serology is negative. [15] If villous atrophy is found in children who are negative for CD-specific antibodies, then a later gluten challenge procedure followed by biopsy always should be performed to confirm CD as a cause of the enteropathy [8].

Quanta Lite h-tTG/DGP Screen is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of IgA and IgG antibodies to synthetic DGP and human tTG in serum. The presence of these antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of IgA sufficient and IgA deficient celiac disease. According to recent studies as PreventCD cohort, testing for AGA-IgA, DGP-IgG or DGP-IgA in competent young children was not superior for CD finding compared to tTG-IgA testing. [16]

A new sensitive single screening assay was developed for the combined assessment of IgA and IgG isotypes of both DGP and tTG (DGP/tTG Screen). This test attempts to identify the potential presence of four different antibodies, including IgA deficient cases.

There are few studies on pediatric populations that assess the accuracy of this new combined test for CD diagnosis in children younger than 2 years old. Further studies on larger cohorts are needed on this topic.

6. Conclusions

IgA/IgG DGP/tTG Screen is a useful tool for guiding the diagnosis of gluten enteropathy in IgA tTG or EMA seronegative children with clinical suspicion of gluten sensitivity aged less than 2 years old.

The sensitivity of IgA/IgG DGP/tTG assay was significantly higher than that of IgA tTG in children with celiac disease younger than 2 years old. The improved performance of this new combined test can prevent repeated intestinal biopsies in young children with high clinical suspicion of CD, genetic predisposition HLA DQ2 or DQ8 and negative tTG/ EMA serology.

Taking into consideration the immunological particularities of this age group and the possibility of false negative serologic results for CD, this combined assay with high accuracy can be proposed as a screening tool in

young pediatric patients. The role of this combined assay for CD diagnosis in children younger than 2 years requires further assessment in larger prospective studies.

Serologic markers of gluten enteropathy are still negative in an important proportion of cases of any ages which associate intestinal alterations and genetic background for CD - DQ2 or DQ8 HLA. These facts suggest that further research should be directed at discovering even more sensitive serological tests.

Disclosure

The authors have no competing interests.

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