Antibodies to Deamidated Gliadin Peptide in Diagnosis of Celiac Disease in Children

Anne Lammi, Pekka Arikoski, Satu Simell, Tuure Kinnunen, Ville Simell, Sari Paavanen-Huhtala, Ari Hinkkanen, Riitta Veijola, Mikael Knip, Jorma Toppari, Outi Vaarala, Olli Simell, and Jorma Ilonen

ABSTRACT

Objectives: Determination of antibodies to synthetic deamidated gliadin peptides (anti-DGPs) may work as an alternative or complement the commonly used test for tissue transglutaminase antibodies (TGA) in the diagnosis of celiac disease (CD). We analyzed the performance of a time-resolved immunofluorometric anti-DGP assay (TR-IFMA) in the diagnosis of CD in children and also retrospectively analyzed the appearance of anti-DGP antibodies before TGA seroconversion.

Methods: The study included 92 children with biopsy-confirmed CD. Serum samples were taken at the time or just before the clinical diagnosis. The control group comprised 82 TGA-negative children who were positive for human leucocyte antigen-DQ2 or -DQ8.

Results: Based on receiver operating characteristic curves, the optimal cutoff value for immunoglobulin (Ig) A anti-DGP positivity was 153 arbitrary units (AU) with a sensitivity of 92.4% and specificity of 97.6% and that for IgG anti-DGP 119 AU, with a sensitivity of 97.8% and specificity of 97.6%. All 92 children with CD were either IgA or IgG anti-DGP positive at the time of diagnosis. Sera from 48 children with CD were also analyzed retrospectively before the diagnosis. Anti-DGP antibodies preceded TGA positivity in 35 of the 48 children with CD and appeared a median of 1 year earlier.

Conclusions: The TR-IFMA assay for detecting anti-DGP antibodies shows high sensitivity and specificity for the diagnosis of CD in children. In a majority of our study population, anti-DGP seropositivity preceded TGA positivity, indicating that earlier detection of CD may be possible by monitoring anti-DGP antibodies frequently in genetically susceptible children.

Key Words: celiac disease, children, deamidated gliadin peptide antibodies

What Is Known

- Determination of deamidated gliadin peptides (anti-DGPs) antibodies performs as an alternative or complements the tissue transglutaminase (TGA) testing in the diagnosis of celiac disease (CD).
- Parallel testing of immunoglobulin G (IgG) anti-DGP and TGA antibodies has the best specificity in screening for CD in pediatric patients

What Is New

- IgG anti-DGP testing alone has been shown to be at least as useful as TGA for detecting CD in children.
- The anti-DGP time-resolved immunofluorometric anti-DGP assay performed high sensitivity and specificity in the pediatric diagnosis of CD. Anti-DGP antibodies, especially of the IgG class, preceded TGA in most children, and thus may allow earlier detection of CD in genetically susceptible children.

Since the identification of tissue transglutaminase antibodies (TGA) as the endomysial autoantigen in celiac disease (CD), serological screening of the disease has been mainly based on measuring immunoglobulin (Ig) A tissue TGA (1,2). Although the sensitivity and specificity of TGA have been reported to be >95%, it may perform at lower sensitivity and specificity, especially in young children (3–6). Antiendomysial antibody testing also has good sensitivity and specificity and is often regarded as the criterion standard for the diagnosis of CD, although it measures the same antigen as the TGA assay (7,8). The final clinical diagnosis of CD has been based on the presence of villous atrophy and crypt hyperplasia in small intestinal biopsy samples. The new criteria proposed by the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN), however, largely omit the
use of biopsy in symptomatic subjects and emphasize the significance of serological testing in the diagnosis of CD (2).

Assays for the detection of antibodies to deamidated gliadin peptides (anti-DGP) have been developed and have largely replaced the conventional gliadin antibody tests with known poor specificity (9,10). Studies have indicated that parallel testing for both IgG anti-DGP and TGA antibodies has the best specificity in screening for CD in both adults and children (11,12). Moreover, IgG anti-DGP testing has been reported to be at least as useful as TGA for detecting CD in young children (4,13). It was shown that anti-DGP antibodies even preceded the appearance of TGA in some children with a genetic risk for CD (11). Anti-DGP assays have also been demonstrated to be useful in monitoring dietary compliance to gluten-free diet in children (4,14,6). In all, these studies demonstrate that anti-DGP antibodies may have an important role in monitoring celiac autoimmunity.

We have earlier described a time-resolved immunofluorometric anti-DGP assay (TR-IFMA), which is able to detect IgA- and IgG-class antibodies in a single well using 2 different lanthanide labels (15). The fluorescence of the lanthanides is long-lived and allows for differentiation of the short-lived background fluorescence of biological material, plastics, and optics (16). It also has a superior range of linearity compared with enzyme-based immunoassays. Performing the assay is essentially similar to a routine enzyme-linked immunosorbent assay (ELISA), and the specific reagents needed, such as streptavidin plates and lanthanide-labeled antibodies, labeling kits, and labeling services, are widely available and their costs are reasonable. Multilabel counters able to detect TR fluorescence are routine equipment in many clinical laboratories. In the present study, we analyzed the performance of this assay using sera from children at the time of diagnosis of CD. We further analyzed whether anti-DGP antibodies precede TGA positivity and thus may allow earlier detection of CD development.

METHODS

Study Population

The study included 92 Finnish children with CD confirmed by a duodenal biopsy based on the histological changes of crypt hyperplasia and villous atrophy of the intestinal mucosa (Table 1) (17). Although the Marsh classification was not used routinely for the evaluation of biopsy results, namely, the number of intraepithelial lymphocytes was not recorded in the pathology report, based on villous atrophy, it is clear that all of our patients belong to Marsh classes 3A–C. Forty-four of the children were analyzed for TGA positivity because of a clinical suspicion of CD, and the duodenal biopsy was taken with a Watson capsule at the Kuopio University Hospital (23 boys and 21 girls). Forty-eight of the children were participating in the Finnish Type 1 Diabetes Prediction and Prevention study (DIPP) at the Turku and Tampere university hospitals (31 boys and 17 girls). Children in the DIPP follow-up study have been selected based on the HLA class II–conferred genetic risk for type 1 diabetes (T1D) and are monitored prospectively for the development of T1D-associated autoantibodies. A subset of children positive for DQA1*05 and DQB1*02 alleles encoding the CD risk–associated DQ2 molecule or DQB1*03:02 encoding the DQ8 molecule were also monitored for the development of TGA positivity, and if TGA was found positive in 2 consecutive samples, they were biopsied to verify the diagnosis of CD. Serum samples from children in the DIPP study were collected every 3 to 6 months from birth until 2 years of age and every 6 to 12 months thereafter. The serum sample used for the analysis of anti-DGP assay sensitivity and specificity was taken either at the time of biopsy (n = 60) or at the last available follow-up visit before the biopsy (n = 32, median 31 days, range 1–252 days). The median age at the time of CD diagnosis was 5.6 years (range 1.2–15.0 years). There were altogether 54 boys and 38 girls in the CD study group. Fifty-one of the 89 patients genotyped (57.3%) were positive for HLA-DQ2 (DQA1*05 and DQB1*02), 15 (16.9%) were positive for both HLA-DQ2 and -DQ8 (DQB1*03:02), and the remaining 23 of them (25.8%) were positive for HLA-DQ8. HLA-typing was not performed in 3 patients because of a missing sample. Because of the original screening for genetic risk for T1D in the DIPP study, the proportion of HLA-DQ8 positive children with CD in this study is higher than in clinical studies.

The control group comprised 82 age- and sex-matched healthy children (49 boys) carrying the CD risk–associated HLA-DQ2 or -DQ8 molecules. The control samples were obtained independently from the Finnish Dietary Intervention Trial for the Prevention of Type 1 Diabetes (FINDIA) follow-up study in which otherwise healthy children have been screened for T1D HLA risk alleles (18). The median age of the control children was 6.0 years (range 3.0–6.8 years) at the time of sampling. Twenty-seven (32.9%) of the controls were HLA-DQ2 positive, 8 (9.8%) were positive for both HLA-DQ2 and -DQ8, and 47 (57.3%) were HLA-DQ8 positive. All of the control children were negative for TGA at the time of sampling.

Frozen serum samples from 48 children with confirmed CD from the DIPP study were also analyzed retrospectively in order to monitor the development of anti-DGP antibodies before the TGA positivity.

The study protocol was approved by the ethical committees of the participating university hospitals. Written informed consent was obtained from parents/guardians and from children >7 years of age.

Anti-DGP Antibody Assay

All of the serum samples were kept frozen at −80°C until analyzed for TGA and anti-DGP antibody reactivity. IgA- and IgG-class anti-DGP antibodies to a synthetic peptide comprising amino acids 86 to 103 of γ gliadin of wheat were measured by a solid-phase lanthanide–based, TR-IFMA, as previously described (15). Total IgA was not separately tested in our study population. The TR-IFMA anti-DGP assay, however, allows simultaneous determination of IgA and IgG antibodies, and extremely low background reactivity in the IgA assay indicates the possibility of IgA deficiency.

Tissue TGA Autoantibody Assay

Commercial recombinant human Celikey kit was used to measure IgA tissue TGA antibodies in patients and controls (Phadia, Freiburg, Germany). Values >8 U/mL were considered positive, as suggested by the manufacturer.
HLA Typing

The presence of HLA-DQA1 and -DQB1 alleles in all of the samples were analyzed using sequence-specific oligonucleotide hybridization reactions, as described earlier (19,20).

Statistical Analysis

All of the statistical analyses were performed by GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA). Mann-Whitney U test was used to compare distributions of anti-DGP antibodies between patients and controls and median ages of children with CD and controls. The cutoff values for IgA and IgG anti-DGP antibody reactivity with the highest sensitivity and specificity were determined by using receiver operating characteristic (ROC) curves. Fisher exact test was used to compare the sensitivity and specificity of anti-DGP IgA and IgG tests. Spearman correlation test was used to assess the correlation between antibody levels of IgA and IgG and severity of villous atrophy in the intestinal mucosa. Wilcoxon matched-pairs signed-rank test was used to compare the antibody levels of anti-DGP in the first and last positive serum samples. P values < 0.05 were considered significant.

RESULTS

Comparison of Antibody Levels in Patients With CD and Controls and Determination of the Cutoff Values for the Anti-DGP Antibody Assay

In order to evaluate the performance of our anti-DGP assay, we analyzed serum samples from children with a clinical diagnosis of CD. We used either a serum sample taken at the time of the diagnostic biopsy or the latest available serum sample before the diagnosis. Serum samples from healthy age- and sex-matched control children positive for CD-associated HLA-DQ2 or -DQ8 molecules were used as controls. All of the serum samples from patients with CD were positive for TGA and those from healthy controls were negative.

The median value of IgA anti-DGP in children with CD was 943 arbitrary units (AU) (range 27–7607 AU) and that of IgG anti-DGP was 718 AU (range 72–5138 AU). In controls, the median value of IgA anti-DGP was 22 (range 4–227 AU) and IgG anti-DGP 16 (1–225 AU). The levels of both IgA and IgG anti-DGP antibodies were significantly higher in patients than in controls (P < 0.0001, Mann-Whitney U test) (Fig. 1A). Moreover, the levels of both IgA and IgG anti-DGP antibodies correlated with the severity of villous atrophy in small intestinal biopsies (Fig. 1B).

ROC curves were used to analyze the sensitivity and specificity of the assays and for determining the optimal cutoff values for seropositivity. The area under the curve was 0.99 (95% CI 0.98–1.00) for IgA anti-DGP and 0.99 (95% CI 0.99–1.00) for IgG anti-DGP assay (Fig. 2). Based on the ROC curves, the optimal cutoff value for IgA anti-DGP positivity was 153 AU with a sensitivity of 92.4%, specificity of 97.6%, and likelihood ratio (LR) of 37.9. The optimal cutoff value for IgG anti-DGP positivity was 119 AU, with a sensitivity of 97.8%, specificity of 97.6%, and LR of 40.1. The sensitivity of anti-DGP IgG was significantly higher than that of anti-DGP IgA assay (P = 0.04, Fisher exact test). There was no statistical difference when comparing the specificity of anti-DGP IgA and IgG assays (P = 0.38, Fisher exact test). In conclusion, the anti-DGP assay used showed high sensitivity and specificity for both IgA- and IgG-class antibodies.

Anti-DGP Seropositivity at Diagnosis

According to the cutoff values for the anti-DGP assay, all of the children with CD were either IgA or IgG anti-DGP positive at the time of diagnosis or the latest sampling before the diagnosis. Eighty-five of the 92 children with CD (92.4%) were IgA anti-DGP positive, and 91 of the 92 children (98.9%) were IgG anti-DGP positive, and 84 of the 92 children (91.3%) were both IgA and IgG anti-DGP positive. Instead, only 2 of the 82 control children (2.4%)
were positive for IgA anti-DGP and also 2 of the 82 controls (2.4%) were IgG anti-DGP positive. None of the controls were positive for both IgA and IgG anti-DGP.

IgG-Class Anti-DGP Positivity Precedes TGA Positivity in Patients With CD

Forty-eight of the children who developed CD were participating in the follow-up program of the Finnish DIPP study in which serum samples were collected at regular intervals from birth. In this cohort, frozen serum samples taken before the TGA positivity and diagnosis of CD were analyzed retrospectively also for the presence of IgA and IgG anti-DGP antibodies (Table 2). Using the criteria for positivity described above, anti-DGP preceded the TGA positivity in 35 of the 48 (72.9%) children with CD. In almost half of the cases (21 of 48, 43.8%) only IgG anti-DGP was positive before TGA. Thirteen of the children were both IgA and IgG anti-DGP positive before TGA positivity, and only 1 of the children was IgA anti-DGP positive preceding TGA positivity. IgA anti-DGP positivity was first detected a median 0.9 years earlier (range 0.3–6.6 years) and IgG anti-DGP a median 1.0 year earlier (range 0.2–7.6 years) than TGA positivity. In 16 of these 35 children (33.3%), anti-DGP was positive at least 2 time points before TGA positivity. Nineteen of the children with CD (39.6%) had anti-DGP positive at the same time as the TGA was positive for the first time.

In general, the levels of both anti-DGP IgA and IgG antibodies increased during the follow-up after their first appearance. The levels of both IgA and IgG anti-DGP antibodies were significantly higher in the last positive serum sample before CD diagnosis than at the first positive serum sample ($P = 0.0013$ and $P = 0.0004$, respectively, Wilcoxon matched-pairs signed-rank test) (Fig. 3A and B).

DISCUSSION

In this study, we analyzed the performance of a TR-IFMA in the diagnosis of CD in children. For this purpose, we first used serum samples from children taken just at the time of diagnosis of CD or the latest sample available before the diagnosis. An ROC curve analysis demonstrated that the optimal cutoff value for IgA anti-DGP was 153 AU and for IgG anti-DGP 119 AU. All 92 children with CD were either IgA or IgG anti-DGP positive. The cutoff values derived in this study are actually similar to the 150 AU for both Ig classes defined by adult serum samples in an earlier study (15). Only 1 of the children with CD was not IgG anti-DGP positive 35 of the 79 (44.3%) children with CD were IgA or IgG DGP positive by using the Inova’s combined IgA and IgG anti-DGP assay. In contrast, Olen et al (22) reported a 91% sensitivity by using the GAF3X (Euroimmun Medizinische Labordiagnostika AG, Luebeck, Germany) and QUANTA Lite Celiac DGP Screen ELISA tests (Inova Diagnostics, San Diego, CA). The ESPGHAN working group has published a systematic review and meta-analysis on serological tests used in CD diagnostics (5). The sensitivity of IgA anti-DGP ranged between 80.7% and 95.1% and that of IgG anti-DGP between 80.1% and 98.6%. Taken together, the poor performance of some tests may give a spurious picture on the performance characteristics of the anti-DPG tests as a whole.

Several studies have demonstrated that IgG anti-DGP has high specificity for CD in both adult and pediatric patients (4,6,23,24). Volta et al (6) reported a 98.9% specificity for IgG anti-DGP in adult patients with CD by using 2 commercial ELISA tests (Quanta Lite Gliadin II IgG, Inova Diagnostics, and Eurospital DGP-AGA IgG, Trieste, Italy). Basso et al (4) observed even a
In several studies, it has been suggested that the best sensitivity and specificity for diagnosis of CD, especially in young children, can be achieved by combining assays detecting IgA TGA and IgG anti-DGP (12,23–25). In the present study, we further analyzed whether anti-DGP antibodies appear before TGA positivity and demonstrated that in up to 72.9% of children with CD, anti-DGP antibodies preceded TGA. We demonstrated that in particular, IgG-class anti-DGP antibodies were detectable earlier than TGA. Of the children with CD and IgG anti-DGP antibody reactivity preceding TGA reactivity, 13 had also IgA-class antibodies to DGP. Only 1 of the children examined had IgA, but not IgG anti-DGP antibodies preceding TGA positivity. The first IgA or IgG anti-DGP seropositivity could be detected a median 0.9 or 1.0 year earlier than TGA seropositivity, respectively. In the only prospective study previously published, anti-DGP positivity preceded the appearance of TGA in only 19% of the subjects studied (11). Although both this and our present study demonstrate an earlier appearance of anti-DGP antibodies, the percentage of anti-DGP positivity before TGA positivity was much higher in our study. More important, in the previous study, a considerable number of TGA-positive children with CD were negative for anti-DGP antibodies at diagnosis, suggesting a lower sensitivity for the anti-DGP assay they have used (11).

Transient TGA seropositivity has been reported in young children in a large prospective Finnish study (27). Similarly, it is possible that seropositivity for anti-DGP may be a transient phenomenon in young children and does not necessarily predict the clinical onset of CD in a similar way as transient seropositivity for TGA (11). Based on our present data, however, we assume that this is a rare phenomenon because there were only 4 anti-DGP positive children in the control group. It is also notable that the level of the DGP antibodies in these control children was significantly lower than the median level of IgA and IgG anti-DGP in patients with CD, even at their first appearance (Figs. 1 and 3).

In line with previous studies (4,6,26,28), we demonstrate here that the sensitivity of IgG anti-DGP antibodies in the diagnosis of CD is higher than that of IgA anti-DGP. Moreover, both at the time of the CD diagnosis (Fig. 1) and in retrospective samples preceding TGA positivity (Table 2), IgA anti-DGP positivity without IgG anti-DGP positivity appears to be extremely rare. Because the specificity of IgA anti-DGP has also been reported to be inferior to IgG anti-DGP (4,6,24), it remains questionable
whether IgA anti-DGP positivity without concurrent IgG anti-DGP positivity provides additional benefit in the diagnosis of CD.

It is well known that the T-cell–mediated inflammation and villous atrophy of small intestine have already begun when CD-associated antibody positivity in serum samples develops. It has been hypothesized that immunization to deamidated gliadin peptides initiates the process, and antibodies to tissue TGA are generated later on (29). The earlier appearance of anti-DGP may offer an important tool for research purposes in addition to enhancing clinical diagnostics. It is apparent that there are environmental factors affecting the development of CD in genetically susceptible subjects, and in order to identify these it is important to detect the CD-related immune process as early as possible (30–32).

In conclusion, we have shown that the TR-IFMA assay for detecting antibodies to a synthetic deamidated gliadin peptide performs with a high sensitivity and specificity in the diagnosis of CD in pediatric patients. The TR-IFMA is also cost-effective because it allows the simultaneous detection of IgA- and IgG-class anti-DGP antibodies. Moreover, we demonstrated retrospectively that anti-DGP antibodies, in particular those of the IgG class, appear earlier than IgA antibodies to tissue TGA in the majority of young children. This indicates that earlier detection of CD may be possible by monitoring anti-DGP antibodies frequently in genetically susceptible children.

Acknowledgments: The authors thank Terttu Laurén for excellent technical assistance.

REFERENCES