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Role of Anti-Transglutaminase (Anti-tTG), Anti-Gliadin, and Anti-Endomysium Serum Antibodies in Diagnosing Celiac Disease: A Comparison of Four Different Commercial Kits for Anti-tTG Determination

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The aims of this study were: (1) to compare the diagnostic efficacy for celiac disease (CD) diagnosis of serum determination of anti-gliadin (AG) (IgA and IgG) and anti-endomysium (AE) with that of anti-transglutaminase (AtTG); and (2) to compare the accuracy of four different assays to measure AtTG. We studied 72 children: the histological diagnosis of CD was made in 38 cases and excluded in the remaining 34 children. In fasting sera we measured AE, AG-IgA and IgG, and AtTG, the latter with four different commercial kits (Eurospital, Medipan, Inova, Arnika). Moreover AtTG was measured in a group of 58 CD children after a gluten-free diet. AE was positive in all but 1 case of CD patients (sensitivity = 97%); false positive results were found in 1/34 controls (specificity = 97%). When a specificity of 95% was fixed, the sensitivities were 97% for AE, 83% for AG-IgA, and 63% for AG-IgG; the sensitivities of anti-tTG were 90, 84, 84, and 75% when measured with Eurospital, Medipan, Inova, and Arnika kits respectively. The new AtTG seems to be accurate enough to be proposed as a noninvasive diagnostic tool for CD diagnosis; the 4 kits analyzed showed similar diagnostic efficacy. J. Clin. Lab. Anal. 15:112–115, 2001.

Key words: gluten; ELISA; children

INTRODUCTION

Tissue transglutaminase (tTG) recently has been claimed to play a fundamental role in the pathogenesis of celiac disease, which is thought to be an HLA-DQ2 and/or -DQ8 associated immunological disease, mediated by T lymphocytes (1–4). Tissue transglutaminase is a Ca++-dependent enzyme that catalyzes several biochemical reactions, mainly the formation of covalent linking between glutamine and lysine residues and the deamidation of glutamine to glutamic acid (5,6). When gluten-derived peptides from pepsin/trypsin digestion are subjected to tTG action, molecules with high affinity for HLA-DQ2 and/or -DQ8 as well as for tTG-gluten-derived peptides complexes originate (5–7). These latter complexes are thought to behave as autoantigens determining the formation of antibodies elicited against gliadin peptides and tTG (8,9). The latter recently has been suggested to correspond to anti-endomysium antibodies, which are considered the most sensitive and specific serologic tool for the diagnosis of celiac disease (1,8). However this determination, which is immunofluorimetric, suffers three limitations: (1) it is costly, because the epithelial monkey cells of the third part of the esophagus are required; (2) the results partly depend on the ability of the operator; and (3) the results are qualitative and not quantitative, thus limiting their use in the follow-up. The serum determination of anti-gliadin both of IgA and IgG classes, which is less costly, does not depend on the operator's ability, is quantitative, and can improve the just-cited limits of anti-endomysium antibodies determination for celiac disease diagnosis. However, anti-gliadin IgA and IgG assays do not possess sensitivities and specificities as high as those of anti-endomysium (10,11). The recent availability of the serum ELISA determination of anti-tTG antibodies might give sensitive and specific information as anti-endomysium antibodies with the advantages of being quantitative, operator independent, and of limited cost (12–17).

The aims of this study were to assess the diagnostic efficacy of the serum determinations of anti-tTG, anti-endomysium, and anti-gliadin antibodies in the assessment of children

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with celiac disease and to compare the performances of four different commercial assays to measure anti-tTG.

MATERIALS AND METHODS

We studied a total of 130 children. Thirty-eight (10 males, 28 females, age range 2–16) were affected by celiac disease, diagnosed on the basis of histology and confirmed by the ameliorative effect of a gluten-free diet. Thirty-four children (15 males, 19 females, age range 1–14) were controls. A group of 58 children with celiac disease (24 males, 34 females, age range 3–16) were studied after a gluten-free diet. All children consecutively underwent upper-gastrointestinal endoscopy either for the clinical suspicion of celiac disease in 72 cases or for monitoring the efficacy of a gluten-free diet in the remaining 58 children. Before endoscopy a serum sample was obtained from all children. The samples were stored at –20°C until the biochemical determinations for no more than one year.

Anti-tTG IgA was measured by means of four commercial ELISA procedures provided by Medipan Diagnostica (Selchow, Germany), Eurospital (Trieste, Italy), Inova Diagnostics Inc. (San Diego, CA), Arnika (Milan, Italy). Anti-endomysium antibodies were identified by immunofluorescence (Eurospital). Anti-gliadin IgA and IgG were measured using ELISA (Pharmacia and Upjohn, Sweden). Anti-tTG in the group of 58 children on a gluten-free diet were measured only with the Eurospital kit. In the majority of controls and of children with celiac disease sera, the measurement of anti-tTG was made with all four commercial kits; however this was not possible for all cases due to the low amount of available sera. All the biochemical determinations were performed in duplicate. The statistical analysis was made using Student's *t*-test, receiver operating characteristic (ROC) curves, and the Kendal–Tau test.

RESULTS

Negative results for anti-endomysium antibodies were found in 1/38 children with celiac disease (sensitivity = 97%), while positive findings were recorded in 1/34 controls (specificity = 97%). The child with celiac disease and a negative result for anti-endomysium also had a documented IgA deficiency, negative results for anti-tTG, and anti-gliadin of the IgA and IgG classes.

Figure 1 shows the results for anti-tTG measured with the four different assays in children with and without celiac disease. The cut-off values, calculated on the basis of mean values + 2 SD of control values, were 5 AU, 40 U/mL, 20 Units, and 0 U/mL for the Eurospital, Medipan, Inova, and Arnika assays, respectively. The mean values of anti-tTG were significantly higher in children with celiac disease compared to controls considering the values obtained with all four methods ($t = 11.3$, $P < 0.001$ for Eurospital; $t = 5.4$, $P < 0.001$ for Medipan; $t = 8.2$, $P < 0.001$ for Inova and $t = 5.13$, $P < 0.001$ for Arnika).

The mean values of anti-gliadin IgA and IgG were signifi-

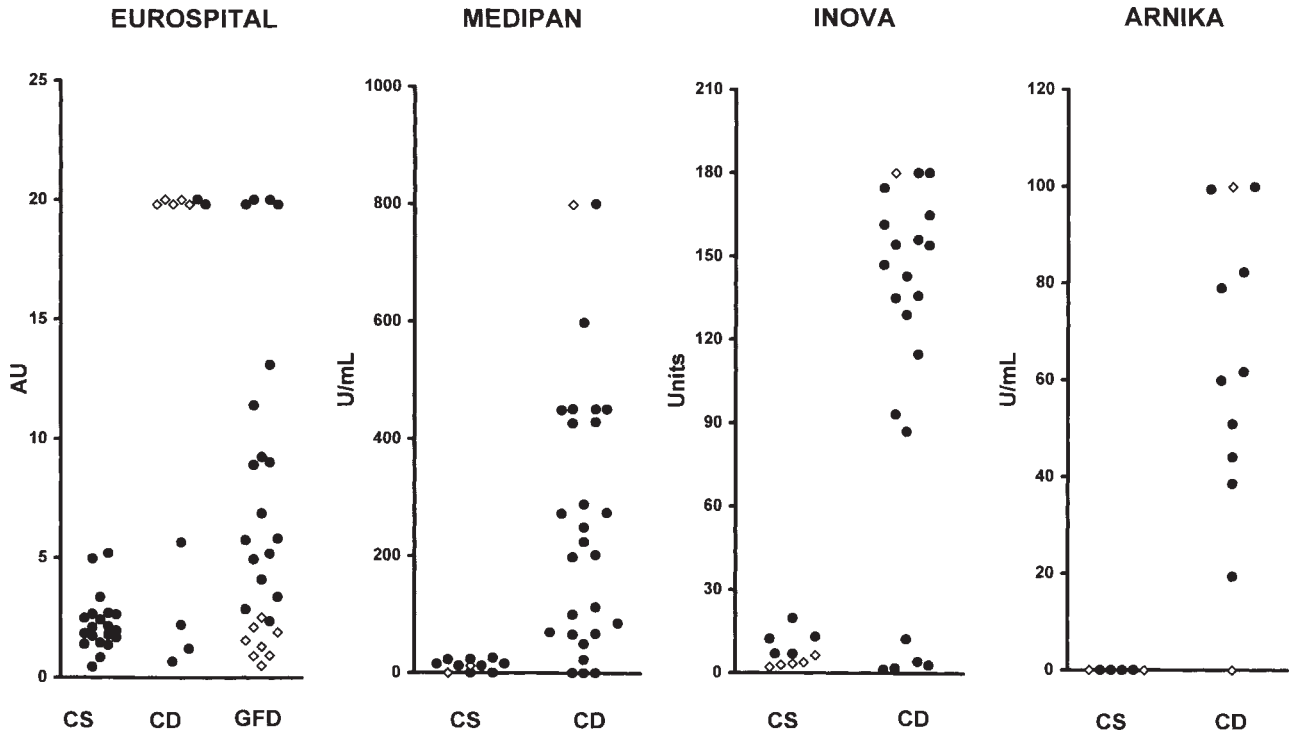

Fig. 1. Individual values of anti-tissue transglutaminase (Anti-tTG) in children with celiac disease. Anti-tTG was measured with four different commercial kits. CS, control children; CD, children with celiac disease; GFD, children with celiac disease after a gluten-free diet; □, five cases; ●, one case.

cantly higher in celiac children compared to controls (t = 8.36, $P < 0.001$ and t = 7.29, $P < 0.001$).

Table 1 shows the results of sensitivity, specificity, diagnostic accuracy, and corresponding cut-off values for anti-tTG measured with the four different commercial kits. Figure 2 illustrates the ROC curves for anti-tTG measured with the four different commercial kits in distinguishing children with or without celiac disease. The under-curve areas were 93, 92, 91, and 80% for Eurospital, Medipan, Inova, and Arnika kits, respectively. The Kendal–Tau test did not show any statistically significant difference between these under-curve areas.

Table 2 illustrates the sensitivity of the different methods in diagnosing celiac disease when a specificity of 95% was fixed.

DISCUSSION

Tissue transglutaminase seems to be the self-antigen for anti-endomysium antibodies, and the serum determination of anti-tTG recently has been suggested for diagnostic purposes in celiac disease (1,8–17). This determination has been demonstrated to possess high sensitivity and specificity in detecting untreated patients and was suggested as useful in monitoring the success of therapy (12). In this study we evaluated the diagnostic accuracy of the serum determination of anti-tTG antibodies made with four different commercially provided systems. Overall the assay had a high sensitivity, a high specificity, and a high diagnostic accuracy in diagnosing patients with celiac disease, which were closed to those of anti-endomysium antibodies. Anti-gliadin IgA and IgG were significantly higher in children with celiac disease compared to controls; however, when a specificity of 95% was fixed, their sensitivities were lower than those obtained with the serum determination of anti-tTG or anti-endomysium antibody, suggesting their limited usefulness in diagnosis of celiac disease.

Five patients with celiac disease had false-negative findings for anti-tTG measured with Medipan, Inova, or Arnika assays; three of these five patients also had false-negative results with the Eurospital assay, while another had a value only slightly elevated (5.65 AU). All five of these patients also had low levels of anti-gliadin IgA, but had high levels of anti-gliadin IgG. These data suggest that the limits in sensitivity of these assays are not correlated with a specific antigenic preparation for any kit, but rather to a lack in serum increase of IgA antibodies specific for single patients and possibly due to total or partial IgA deficiency. IgA deficiency also accounted for the false-negative result of anti-endomysium antibodies recorded in one child with celiac disease, which could be identified neither with the determination of anti-tTG nor with anti-gliadin. We suggest therefore a screening for total IgA levels in those patients having low levels of anti-tTG in order to exclude false-negative results due to IgA deficiency.

The four methods evaluated in this study were confirmed to possess similar discriminatory capacity between children with or without celiac disease by the ROC curve analysis. In fact no statistically significant differences were found between the under-curve areas, even if the lowest one was that of the Arnika assay, which also shows the lowest sensitivity and diagnostic accuracy. This may, however, be due to the fact that we evaluated the lowest number of patients with celiac disease in this assay.

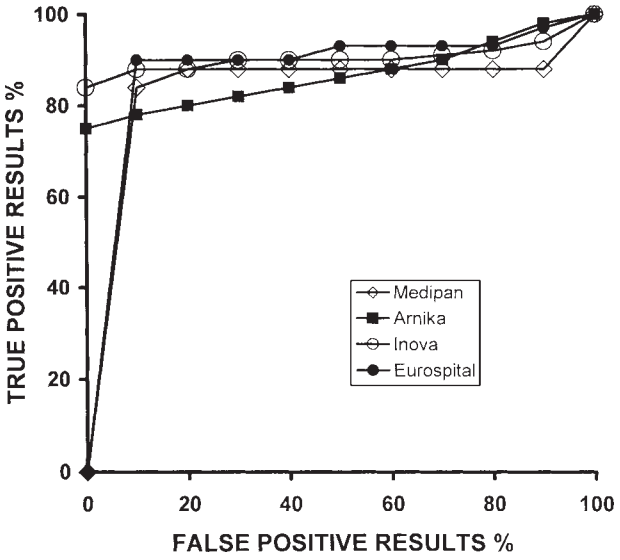

Fig. 2. Receiver operating characteristic (ROC) curves of anti-tissue transglutaminase measured with four different commercial kits in distinguishing children with from those without celiac disease.

TABLE 1. Sensitivity, specificity, diagnostic accuracy, and corresponding cut-offs for anti-tTG measured with the four commercial kits studied

	Cut-off	Sensitivity (%)	Specificity (%)	Diagnostic accuracy (%)
Eurospital	5.5 AU	90	100	94
Medipan	26 U/mL	84	100	90
Inova	20 Units	84	100	92
Arnika	0 U/mL	75	100	85

TABLE 2. Cut-off values and corresponding sensitivity of anti-transglutaminase (AtTG) (measured with the four different commercial kits evaluated), anti endomysium (AE), anti-gliadin IgA (AG-IgA), and IgG (AG-IgG) used in diagnosing celiac disease when a specificity of 95% was fixed

	Cut-off	Sensitivity (%)
AE		97
AtTG (Eurospital)	5 AU	90
AtTG (Medipan)	25 U/mL	84
AtTG (Inova)	15 Units	84
AtTG (Arnika)	0 U/mL	75
AG-IgA	3.66 U/mL	83
Ag-IgG	40 U/mL	63

Anti-tTG was measured by means of only one commercial kit, as an example of anti-tTG behavior in the follow-up of celiac disease, in a group of patients after a gluten-free diet. The percentage of cases with low values (74%) was significantly higher than that found in children with an active celiac disease (9.6%) and further indicates the potential role for this determination in monitoring treatment of celiac disease (12).

We suggest that the serum determination of anti-tTG might be a substitute for that of anti-endomysium for screening purposes to diagnose celiac disease, because of its high sensitivity and specificity, because it is quantitative, it is not subjected to interobserver variation, it is easy to perform, and it is less expensive than the determination of anti-endomysium antibodies. The choice of a commercial kit should be guided by its diagnostic performance; among the four kits analyzed in this study only minimal differences were observed in the diagnostic accuracy, even if that provided by Arnika was the least accurate. In this context the system choice should be dictated by cost effectiveness.

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