

Assessment of a combination screening assay for celiac disease

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Received: 4 June 2010 / Accepted: 27 April 2011 / Published online: 19 May 2011
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Abstract

Purpose A serological screening assay for celiac disease (CD), designed to simultaneously detect IgA and IgG anti-tissue transglutaminase (a-tTG) and IgA and IgG deamidated gliadin peptide antibodies (a-DGP), was recently developed. In this study, we establish the performance of this assay.

Methods We enrolled 41 CD patients and 18 CD patients on gluten-free diets. The diagnosis of CD was based on histological and serological criteria, including concomitant positive serology tests (a-tTG, IgA anti-endomysial antibodies). As control population, we enrolled 169 subjects: 145 disease controls and 24 blood donors. In all cases, serum samples were tested for: IgA a-tTG, IgG a-tTG, IgA a-DGP, IgG a-DGP, IgA anti-endomysial antibodies (EMA), IgA and IgG for a-tTG and a-DGP in a single assay.

Results The new test, QUANTA Lite™ h-tTG/DGP Screen, detects all IgA and IgG antibodies against a-tTG

and a-DGP present in a sample. In our study, the test showed 100% sensitivity and 91.12% specificity.

Conclusions This study showed additional value of the new h-tTG/DGP Screen assay, which proved superior to more conventional assays and can be considered the best initial test for CD. Further studies are necessary to determine whether combination of h-tTG/DGP Screen with IgA a-tTG or IgA a-DGP can be used to obviate the need for duodenal biopsy in high- and low-risk populations

Keywords Celiac disease · Diagnosis · Deamidated gliadin peptide antibodies · Anti-tissue transglutaminase antibodies · Serological screening assay

Introduction

Celiac disease (CD) is a syndrome characterized by damage to the small intestinal mucosa caused by the gliadin fraction of wheat gluten and similar alcohol-soluble proteins (prolamines) of barley and rye in genetically susceptible subjects [1]. Currently, IgA anti-tissue transglutaminase (a-tTG) antibodies are accepted as the test of first choice, by virtue of their high sensitivity and excellent reproducibility [2]. IgA anti-endomysial antibodies (EMA), measured by immunofluorescence on sections of monkey oesophagus, recognize the same antigen as a-tTG. The EMA test is highly specific (~100%), but less sensitive than IgA a-tTG antibodies, and should therefore preferably be used in a-tTG positive cases as a confirmation test prior to intestinal biopsy [3].

In recent years, IgA anti-gliadin antibodies (AGA), which are found in the serum of CD patients, have lost much of their diagnostic value because they are neither sensitive nor specific and can also be found in healthy

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individuals and patients with other intestinal disorders. Except in pediatric patients, the increased sensitivity and specificity of a-tTG antibodies are a great improvement over the previously available gliadin testing, and the utility of the latter in the diagnosis of CD has been challenged [4]. IgG a-tTG antibodies must only be used as a specific marker in patients with an IgA deficiency, whose risk of developing CD is 10–20 times higher than in the normal population. Searching for these antibodies in patients with normal serum IgA is often misleading, as they can also be found in healthy subjects and in patients suffering from other disorders [5, 6].

Specific ELISA tests for IgA and IgG antibodies against deamidated gliadin peptides (a-DGP) show very promising preliminary results as second-generation AGA assays [7–14]. IgG a-DGP antibodies, in particular, may also be used in patients with IgA deficiency, where they may be the only positive serological marker (sometimes in association with IgG anti-tTG). The recent development of a serological screening assay for CD, that simultaneously detects IgA and IgG a-tTG and IgA and IgG a-DGP, has taken into account all the latest research. In the present study, we investigated the performance of this assay in diagnosed celiac patients and in a control group composed of healthy subjects, subjects with other autoimmune diseases, and subjects with several non-immune diseases.

Materials and methods

We enrolled 41 recently diagnosed CD patients: 31 adults (7 males, mean age 36; range 19–59 years; 24 females, mean age 38; range 18–77 years) and 10 children (3 males, mean age 7; range 6–9 years; 7 females, mean age 7; range 3–13 years). We also included 18 previously diagnosed CD patients on gluten-free diets for 8–24 months: 8 adults (1 male, age 37 years; 7 females, mean age 27; range 18–42 years) and 10 children (3 males, mean age 8; range 4–11 years; 7 females, mean age 11; range 3–16 years).

The diagnosis of CD was based on histological and serological criteria, including concomitant positive serology tests (a-tTG, EMA). Intestinal biopsies were performed in the same period as CD serological tests and were classified by a modified version of the Marsh classification [15] (Table 1). Examination of all biopsies was performed blindly by the same operator.

We enrolled 169 subjects (60 males, mean age 50; range 17–75; 109 females, mean age 51 range 18–85 years) as control population: 145 disease controls (15 with autoimmune hepatopathies, 12 with hepatitis/cirrhosis, 35 with viral hepatitis/cirrhosis, 83 with other gastrointestinal diseases) and 24 blood donors as normal controls. In all cases, serum samples were tested for:

Table 1 Histological characteristics of patients at the time of the diagnosis

Histological characteristics of CD patients	No. of patients (total cases: 41)
Type 3c	26
Type 3b	11
Type 3a	4
Histological characteristics of CD patients on treatment with the gluten-free diet	No. of patients (total cases: 18)
Type 3c	9
Type 3b	7
Type 3a	1
Type 1	1

- IgA a-tTG: ELISA (QUANTA Lite™ h-tTG IgA, INOVA Diagnostics Inc., San Diego, CA, USA)
- IgG a-tTG: ELISA (QUANTA Lite™ h-tTG IgG, INOVA Diagnostics Inc., San Diego, CA, USA)
- IgA a-DGP: ELISA (QUANTA Lite™ Gliadin IgA II, INOVA Diagnostics Inc., San Diego, CA, USA).
- IgG a-DGP: ELISA (QUANTA Lite™ Gliadin IgG II, INOVA Diagnostics Inc., San Diego, CA, USA).
- IgA anti-endomysial antibodies (EMA): IFI (Eurospital, Trieste, Italy).
- IgA and IgG for a-tTG and a-DGP in a single assay (QUANTA Lite™ h-tTG/DGP Screen, INOVA Diagnostics Inc., San Diego, CA, USA). QUANTA Lite h-tTG/DGP Screen is an enzyme-linked immunosorbent assay (ELISA) which uses purified synthetic DGPs and native human tissue transglutaminase (h-tTG) bound to a polystyrene microwell plate under conditions that preserve the antigen in its native state.

Sensitivity, specificity, positive and negative predictive values were calculated for each assay using cut-offs provided by the manufacturer. 95% confidence intervals were also computed for sensitivity, specificity, positive and negative predictive values.

Results

Table 2 reports the number (and percentage) of subjects positive for each parameter. The h-tTG/DGP Screen assay was positive in all CD patients and 14 out of 18 CD patients on gluten-free diets; it was also positive in 15 controls, specifically 14 out of 145 disease controls and 1 out of 24 normal controls.

Table 3 shows the number (and percentage) of the subgroup of disease controls positive for each parameter. Only one patient with hepatitis/cirrhosis was positive with

Table 2 Positive subjects in relation to parameters analyzed

	Total cases	IgA a-tTG ^a	IgG a-tTG ^a	IgA EMA ^a	IgA a-DGP ^a	IgG a-DGP ^a	h-tTG/DGP Screen ^a
CD patients	41	41/41 100%	32/41 78.04%	41/41 100%	37/41 90.24%	36/41 87.80%	41/41 100%
CD patients on treatment with the gluten free-diet	18	11/18 61.11%	10/18 55.55%	9/18 50.00%	5/18 27.77%	6/18 33.33%	14/18 77.77%
Disease controls	145	2/145 1.37%	29/145 20.00%	0/145 0%	5/145 3.44%	7/145 4.82%	14/145 9.65%
Normal controls	24	0/24 0%	1/24 4.16%	0/24 0%	0/24 0%	0/24 0%	1/24 4.16%

^a Positivity is expressed as number of positive cases/total number of cases and as % of positive cases

Table 3 Positive subjects for each parameter analyzed in the subgroup of disease controls

	Total cases	IgA a-tTG ^a	IgG a-tTG ^a	IgA EMA ^a	IgA a-DGP ^a	IgG a-DGP ^a	h-tTG/DGP Screen ^a
Patients with autoimmune hepatopathies	15	0/15 0%	2/15 13.33%	0/15 0%	0/15 0%	0/15 0%	0/15 0%
Patients with hepatitis/cirrhosis	12	1/12 8.33%	3/12 25.00%	0/12 0%	1/12 8.33%	0/12 0%	1/12 8.33%
Patients with viral hepatitis/cirrhosis	35	1/35 2.85%	6/35 17.14%	0/35 0%	0/35 0%	0/35 0%	1/35 2.85%
Patients with other gastrointestinal diseases	83	0/83 0%	18/83 21.68%	0/83 0%	4/83 4.81%	7/83 8%	12/83 14.45%

^a Positivity is expressed as number of positive cases/total number of cases and as % of positive cases

Table 4 Sensitivity, specificity, PPV and NPV of tests

	h-tTG/DGP Screen	IgA a-tTG	IgG a-tTG	IgA a-DGP	IgG a-DGP	EMA IgA
% Sensitivity (95% CI)	100	100	78.05 (76.78–79.32)	90.24 (89.34–91.15)	87.80 (86.80–88.81)	100
% Specificity (95% CI)	91.12 (90.70–91.55)	98.82 (98.65–98.98)	82.25 (81.67–82.82)	97.04 (96.79–97.30)	95.86 (95.56–96.16)	100
% PPV (95% CI)	73.21 (72.05–74.37)	95.35 (94.72–95.98)	51.61 (50.37–52.86)	88.10 (87.12–89.07)	83.72 (82.6–84.82)	100
% NPV (95% CI)	100	100	93.92 (93.53–94.30)	97.62 (97.31–97.85)	97.01 (96.75–97.26)	100

the h-tTG/DGP Screen assay, confirmed by individual IgA a-tTG, IgG a-tTG and IgA a-DGP tests. In the viral hepatitis/cirrhosis group, only one subject was weakly positive to the h-tTG/DGP Screen test, confirmed by individual IgA a-tTG and IgG a-tTG tests. In the other gastrointestinal diseases patient group, 12 subjects were positive to h-tTG/DGP Screen, 9 of whom were positive for at least one individual parameter.

Table 4 shows the sensitivity, specificity, PPV and NPV of all tests. Cut-off >20 U/ml was used for a-tTG, a-DGP and h-tTG/DGP Screen tests. The diagnostic sensitivity of h-tTG/DGP Screen was 100% in CD patients, which was

similar to or higher than the other tests evaluated (EMA 100%, IgA a-tTG 100%, IgG a-tTG 78.05%, IgA a-DGP 90.24%, IgG a-DGP 87.80%). The specificity of the assay was 91.12% with respect to the control population.

Discussion and conclusions

Few studies on the h-tTG/DGP Screen assay have been published in the literature [11, 16–19]. Jaskowski et al. [11] studied 111 pediatric patients suspected of having CD, 130 adults diagnosed with dermatitis herpetiformis (DH), and

77 pediatric and 49 normal adult controls. They reported that the assay achieved 92.6% sensitivity in the cohort of pediatric patients with CD and its specificity was 96.1% in normal pediatric controls. The authors concluded that the new test was highly sensitive for pediatric CD. Agardh [17] analyzed 119 children with CD, 57 children with other disorders, and 398 blood donor samples. Treatment with a gluten-free diet was evaluated in 20 children with CD who were followed up for 6 months after diagnosis. 100% sensitivity was obtained. The assay's specificity in disease controls and blood donors was 89 and 97%, respectively. The author recommended the assay as a front-line screening test for the identification of childhood CD; it could also be used as a marker of dietary compliance. Sugai et al. [19] prospectively analyzed duodenal biopsy and serology in 679 adults at high ($n = 161$) or low risk ($n = 518$) for CD. They obtained high sensitivity and high specificity with the h-tTG/DGP Screen assay. The authors concluded that the assay was the best initial test for CD and its use in combination with IgA a-tTG or IgA a-DGP could diagnose CD accurately in different clinical situations, avoiding biopsy in a high proportion of subjects.

In order to evaluate the sensitivity and specificity of the h-tTG/DGP Screen assay, we selected a well-characterized population. In the group of celiac patients, we sought to recruit both children and adults to observe the performance of the test in different age groups which may have different variations in IgA and IgG isotypes. In selecting the control population, we decided to enrol a greater number of subjects with pathologies than normal controls (145 disease controls and 24 blood donors), with respect to previous studies, in order to assess the specificity of the test in critical conditions, such as diseases that could be confused with or occur together with CD: autoimmune hepatopathies, hepatitis/cirrhosis, viral hepatitis/cirrhosis, other gastrointestinal diseases. 14 out of 145 disease controls were false positive with the h-tTG/DGP Screen assay, but some of these could be true positive: in fact, 11 of the 14 "false positive" specimens were found to be positive when tested with one or more single assays for IgA or IgG a-tTG and a-DGP, so it appears that the screening assay, which detects up to four different antibodies (IgG and IgA antibodies reactive with a-tTG and a-DGP), revealed real antibodies, and some of these screen-positive disease controls may have CD associated with some other disease.

We found a much higher sensitivity of IgA a-tTG than recently reported by Vermeersch et al. [13], probably due to the different commercial assay used in the study. We also found higher sensitivity of IgA a-tTG and h-tTG/DGP Screen than recently reported by Jaskowski et al. [11]. Our higher sensitivity was not surprising because all the patients we selected had a well-defined CD diagnosis, whereas in the Jaskowski paper only 54 of the 111 pediatric

patients suspected of having CD had really CD and only 90.7% of these patients had a Marsh grade of 3a–c, while five patients had a Marsh 1 biopsy. Moreover, despite the sensitivity of the serological testing, the authors explain that the diagnosis of CD was unclear in some patients. We obtained a specificity slightly lower than Jaskowski et al. [11] for the h-tTG/DGP Screen. The reason could be the particular selection of the control group, especially composed of disease controls. The choice of control group is very important, because specificity depends on it: for example Agardh [17], who also selected patients with other disorders, obtained specificities for disease controls (89%) and blood donors (97%) more similar to ours.

The results obtained here not only demonstrate high sensitivity of the QUANTA Lite™ h-tTG/DGP Screen test, but also its high specificity, which is difficult to find in screening tests as they generally have good sensitivity at the expense of specificity.

This excellent analytical performance was confirmed in all the situations evaluated in our study (groups of subjects selected, adults/children). In addition, the test gave positive results in various CD patients on gluten-free diets, being positive for at least one of the single tests, confirming suspicions about correct compliance with the diet. The test also performed very well in comparison with the single tests produced by the same company (INOVA Diagnostic Inc.) and in relation to single tests produced by other companies (data not reported).

These results show the additional value of the new h-tTG/DGP Screen assay, which proved superior to more conventional assays and can be considered the best initial test for CD. Further studies are necessary to determine whether combination of the h-tTG/DGP Screen assay with IgA a-tTG or IgA a-DGP tests as proposed by Sugai et al. [19] can be used to avoid duodenal biopsy in high- and low-risk populations.

Acknowledgments The authors thank INOVA Diagnostics (San Diego, CA, USA) for donating the IgA/IgG h-tTG/DGP EIA screen and the single a-tTG and a-DGP EIA kits for this study.

Conflict of interest None.

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