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Open Conformation Tissue Transglutaminase Testing for Celiac Dietary Assessment

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Abstract

Objectives—Anti tissue-transglutaminase antibody is the mainstay of celiac disease serologic testing. While it has high sensitivity in patients on an unrestricted diet, sensitivity is poor for evaluation of gluten free diet adherence.

Aim—To assess the utility of a novel assay measuring Immunoglobulin-A antibodies to catalytically active open conformation tissue-transglutaminase in assessment of ongoing gluten exposure in celiac disease patients on an alleged gluten free diet.

Methods—Through prospective assessment, 147 patients with celiac disease were divided into good and poor adherence. Open and closed (conventional) tissue-transglutaminase titers were measured using standard ELISA. 50 patients with inflammatory bowel disease served as disease controls.

Results—Overall 128 patients had been on gluten free diet for more than six months and 19 were found to be poorly adherent on dietary review. Within this group 13 (68.4%) and 10 (52.6%) patients respectively were positive for the open conformation and conventional assay ($p = 0.51$). Two and one control patients tested positive for closed and open assays respectively.

Conclusions—Compared to native assays open conformation tissue-transglutaminase may have higher sensitivity in the poor GFD adherence group and higher specificity in the control population. Larger population studies are warranted to assess whether the open conformation tissue-transglutaminase assay may be superior to the conventional assay.

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Introduction

Celiac disease is increasingly recognized as the most common gastrointestinal disease with autoimmune features.[1] Increased rates of diagnosis worldwide have been in large part attributable to improvements in awareness and serologic testing. IgA anti-tissue transglutaminase (tTG), endomysial antibodies (EMA) and antibodies to deamidated gliadin peptides (DGP) all have sensitivities and specificities above 90% in most populations.[2, 3] However, none of these serologic tests shows a high degree of responsiveness to modest changes in intestinal inflammation or limited amounts/duration of gluten re-exposure.[3–7] This is a major limitation in the evaluation of patients with non responsive celiac disease (NRCD). Non responsive celiac disease is defined as ongoing symptoms or recurrence of symptoms in celiac disease patients despite following a gluten free diet (GFD) for at least 6 months. It has been reported that 30–50% of NRCD cases are secondary to ongoing gluten exposure[8, 9], but due to the inability of conventional tTG assays in detecting low dose gluten exposure and limited availability of skilled celiac dieticians, this becomes a diagnosis of exclusion. While in some patients a dietary source of gluten can be identified through careful interview, in many a broad differential needs to be ruled out before arriving at that diagnosis.

In this study, we assessed the ability of a new test detecting the antibody to the stabilized open (active) conformation tTG (O-tTG) in comparison to the conventional test which detects antibody to the tTG of closed or undefined conformation (C-tTG) to predict gluten free diet adherence. We determined the dietary adherence clinically by an expert dietician, and the specificity of both the tests in a control population with inflammatory bowel disease.

Methods

Serum was obtained from 147 individuals with biopsy proven CD who had previously participated in a study evaluating gluten free diet adherence and its relation to symptoms of celiac disease.[9, 10, 11] Additional tests were run on 50 patients with biopsy confirmed inflammatory bowel disease. (Tables 1 and 2).

Individuals in the celiac disease cohort underwent nutritional evaluation in a standardized fashion as we have previously described.[7] This included analysis of three-day food records, a food ingredient quiz, a dynamic interview and questionnaires evaluating diet adherence, gastrointestinal and non-gastrointestinal symptoms and quality of life, as well as evaluation for gluten exposure by a highly skilled dietician with over 10 years of experience working with celiac disease. Global GFD adherence was recorded on a 6 point Likert scale ranging from 1: 'excellent adherence: consuming gluten less than three times per year' to 6: 'not currently following a gluten free diet' (see appendix 1). For assessment of the utility of the serologic tests to monitor gluten free diet adherence, only participants following the diet for at least six months were included. A subgroup analysis was performed on participants following the diet for at least 12 months as well.

The control population consisted of 24 patients with Crohn's disease (Mean age 35 years St Dev 9.6 years) and 26 patients with Ulcerative colitis (Mean age 44 years St Dev 15 years). Twenty four (100%) of Crohn's disease and 3 (11.5%) of Ulcerative colitis patients had active disease at the time of assessment. 18 (75%) of Crohn's disease patients were on immunomodulator therapy at the time of assessment as compared to 5 (19.23%) of Ulcerative colitis patients. Overall Male: Female ratio was 1:1.

Analysis of closed and open conformation anti-tTG IgA titers was done by enzyme linked immunosorbent assay (ELISA) with recombinant human antigen (Product No. E001, E002, E006 and E007, Zedira, Darmstadt, Germany) Recombinant tTG was produced in insect

cells and purified to >95% purity and a specific activity of >2000 U/mg, measured by dansylcadaverine incorporation in dimethylated casein (T036, Zedira). This tTG formulation (closed conformation) was then used to coat plates for ELISA-kits E001 and E002. For the production of open conformation tTG, the same antigen was used and incubated with the tTG specific irreversible inhibitor ZED754 which locks the enzyme in the open conformation. After removal of surplus inhibitor the remaining activity was documented at <10%. The open conformation tTG was proven by crystallization and X-ray structure resolution, (Lindemann et al., 20th International Symposium on Medicinal Chemistry; EFMC-ISMIC 2008, Vienna, Austria, August 31 -September 4, 2008). The coatings for both the assays were performed under identical conditions. Serum at a dilution of 1:100 was incubated with the wells for 30 minutes, followed by human IgA coupled to horseradish peroxidase for 30 minutes and color development with 5, 5'-tetramethylbenzidine (TMB) and hydrogen peroxide. The open and closed tTG-kits are identical with the exception of the antigen utilized. The cut-off in these assays has been set to 3 U/ml using analysis of blood donor sera.

Results were compared using Student's T test, ANOVA, Fisher's exact test and Wilcoxon signed-rank test where appropriate. Informed consent was obtained from all study subjects to use biological specimen. The study was approved by the Beth Israel Deaconess Medical Center (BIDMC) Investigational Review Board.

Results

147 patients with biopsy proven celiac disease were tested. None of the participants had IgA deficiency. Time on gluten free diet ranged from 3 to 576 (mean 56.5) months. The demographic characteristics of the study population were not significantly different from that of overall celiac disease population seen at our hospital (BIDMC) (Table 1). 85% of the subjects studied under the celiac disease group had 'excellent' or 'good' GFD adherence as measured by the skilled dietician. (Figure 1)

For assessment of the utility of the serologic tests to monitor gluten free diet adherence, only participants following the diet for at least six months were included (n =128). In this group, 19 were found to have inadequate dietary adherence and 109 had either good or excellent adherence. The two groups were similar in terms of age at enrollment (50 vs. 51 yrs p=0.8), female predominance (76% vs. 68% p=0.5) and time on gluten free diet 62 vs. 70 months (p=0.7). Within the poorly adherent group 10 (52.6%) were positive for C-tTG and 13 (68.4%) tested positive for O-tTG (p= 0.5). Closed and open conformation IgA anti-tTG titers were highly correlated.(Figure 2) In the 109 individuals with excellent or good gluten free diet adherence, 31 (28.4%) and 47 (43%) were C-tTG and O-tTG positive, respectively (p=0.034). Within the good adherence group patients who were positive for either test were found to be on the GFD for a significantly shorter duration than the patients with negative tests. (O-tTG positive, 41.8 months vs. O-tTG negative, 77.5 months vs., p = 0.032 and C-tTG positive, 37.5 months vs. C-tTG negative, 71.9 months, p = 0.057). These findings along with the dietetic survey findings supported the fact that positive assays in the well adherent population were a marker for slow immune recovery and not ongoing gluten exposure.

Within the poorly adherent group, patients with elevated and normal tTG titers were on the gluten free diet for similar lengths of time (O-tTG positive; 76.1 months vs. O-tTG negative; 76.8 months, p = 0.98 and C-tTG positive; 91.8 months vs. C-tTG negative; 59.8 months, p = 0.38). Significantly more participants with poor GFD adherence (13/19 68.4%) were positive for O-tTG compared to participants with good GFD adherence (47/109 43.2%) (p=0.048). A similar but lesser difference was seen with C-tTG (10/19 vs. 31/109, p=

0.059). No other differences were significant. See Table 3 for a summary of the test characteristics of the two assays. We also looked at the subset following GFD for at least 12 months. Both positive and negative predictive value increased slightly in this selected group, however this did not reach statistical significance. See Table 3

In the six individuals who fell outside of the 95% confidence interval for correlation between closed and open conformation IgA anti-tTG, four (67%) had been on the gluten free diet for less than one year compared to 28% of the overall study population.(see figure 2) However, this did not reach statistical significance ($p = 0.06$). No other demographic or clinical differences were noted between these two groups.

Among the 50 patients with inflammatory bowel disease either active or in remission (24 with Crohn's disease, 26 with ulcerative colitis) all but two individuals showed antibody titers within the normal range for both open and closed antibodies (range undetectable to 3.0). Of the individuals with positive titers, all were less than two times the upper limit of normal. These included one patient with ulcerative colitis with a C-tTG of 3.4 and an O-tTG of 3.6, and a patient with Crohn's disease with a C-tTG of 3.0.

Discussion

While diagnostic methodologies for celiac disease are highly developed, monitoring strategies remain limited and refinements in serologic tests are a viable option. Analogous to the improvement made to anti-gliadin testing by deamidated gliadin testing, open conformation tTG tests may offer benefits beyond conventional tTG assays.

The rationale for the novel assay is based on the fact that in healthy tissue, tTG is largely confined to the intracellular compartment and in an inactive closed conformation. However, in inflammation, increased amounts of tTG are secreted into the extracellular space where the concentration of ionized calcium is much higher. [12] The calcium binds to the beta-barrel domains of tTG causing a significant change in conformation to the open form necessary for catalytic activation.[13, 14] Once in the open form, tTG is able to mediate deamidation of glutamine residues in certain gluten peptides generating peptides with higher affinity for T cell receptors and increased immunogenicity.[15–17] It is therefore hypothesized that auto-antibodies to the open conformation of tTG may have a higher specificity for the inflammatory intestinal process active in celiac disease, and yield more otherwise cryptic epitopes for auto-antibody formation, resulting in higher antibody titers and thus higher sensitivity in patients with lesser degrees of inflammation.

To date there are very limited data regarding the utility of antibodies to open-conformation tTG as a test of dietary compliance in celiac disease.[18] Compared to closed-conformation antibodies, our data show an increased sensitivity of the open-conformation antibody test to detect dietary non-compliance. Also significantly more participants with poor GFD adherence (13/19 68.4%) were positive for O-tTG compared to participants with good GFD adherence (47/109 43.2%) ($p=0.048$). A similar but non-significant difference was seen with C-tTG (10/19 vs. 31/109, $p= 0.059$). This may mean that the open conformation TTG assay may be more effective at differentiating between good and poor adherence.

As expected, neither antibody showed a very high sensitivity (68.4% for O-TTG and 58.6% for C-tTG) to detect dietary non-compliance. This is in line with past reports which suggest that serologic tests alone are not accurate measures of gluten free diet adherence.[7]

In the 109 individuals with excellent or good gluten free diet adherence, 31 (28.4%) and 47 (43%) had elevated titers of C-tTG and O-tTG, respectively ($p=0.034$). We also note that comparative titers for the O-tTG were significantly higher than for the C-tTG assay (10.3 vs.

6.2 p <0.001), suggesting that O-tTG may be more sensitive in individuals with low titer celiac auto-antibodies. These findings are in line with the hypothesis that auto-antibodies to the open conformation of tTG may have higher sensitivity in patients with lesser degrees of inflammation or as in our case, resolving inflammation, making it a valuable tool in workup of non responsive celiac disease.

It is also notable that in the disease control population, two individuals were positive for C-tTG, while one individual was positive for O-tTG. The combination of a higher percentage of patients with active celiac disease who have a positive O-tTG, and lower percentage of disease controls with a positive titer in this assay is suggestive that this novel test may truly have a higher sensitivity and specificity than the commonly used C-tTG. Although the current C-tTG assays are highly accurate, on a population level positive predictive value is typically less than 80%.[2, 3] For this reason, even modest improvements in sensitivity and specificity of this cost-effective and reproducible test would make celiac disease testing significantly more efficient.

While our study suggests a difference between the sensitivity of two tests in detecting dietary compliance some limitations are notable. First and the most important limitation of this study was that, adherence and serological status was not compared with concurrent histological evaluation, although it is unclear whether, for assessment of GFD adherence, biopsy is superior to an expert celiac dietician's assessment.[19, 20] Second, this study was performed at a single center and limited sample size may have contributed to lack of statistical significance.

In conclusion, we present data on a novel open conformation anti-tTG assay. Because the test performance characteristics were different between open and closed tTG, these tests may have different utility in different clinical settings and further study is necessary. Our data also suggest that the open tTG assay has the potential to perform better than traditional closed conformation anti-tTG assays in detecting ongoing gluten exposure in celiac disease patients on a reported gluten free diet. Larger studies are needed to assess whether these differences in test characteristics translate into meaningful improvement in predictive value for assessment of adherence to a gluten free diet and diagnosis of celiac disease.

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Figure 1. Distribution of standardized dietician evaluation scores

Standardized Dietician Evaluation Scores in 147 Celiac Disease patients.

1: Excellent Gluten Free Diet Adherence, 2: Good Gluten Free Diet Adherence, 3: Fair Gluten Free Diet Adherence, 4: Poor Gluten Free Diet Adherence; 5: Very Poor Gluten Free Diet Adherence, 6: Not on a Gluten Free Diet

Figure 2. Scatter Plot of IgA anti-unfolded tTG vs IgA anti-native tTG. Dashed lines represent 95% confidence intervals for the correlation. Dotted lines mark cut-offs of the respective assays.

Table 1

Characteristics of the celiac disease study population.

	Study Group (n=147)	Overall BIDMC* Celiac population (n=601)	p value
Mean age at diagnosis (Standard deviation)	44.8 (16)	43.7 (16.4)	45
Females	113 (76.8%)	431 (71.7%)	23
Ethnicity White Other	145 (98.6%) 2 (1.4%)	598 (99.5%) 3 (0.5%)	58
Other autoimmune disorders ^a	47 (30.5%)	163 (27%)	42

^a predominantly thyroid disease > type 1 diabetes mellitus >> Raynaud's phenomenon > inflammatory bowel disease > sarcoidosis > psoriasis

* Beth Israel Deaconess Medical Center

Table 2

Characteristics of the control cohort

	Ulcerative Colitis (n=26)	Crohn's disease (n=24)	Overall IBD (n=50)	CeliacGroup (n=147)	P value Celiac vs overall IBD
Mean Age (yrs)at Enrollment & SD *	43.9 (15)	35 (9.6)	39.5 (13.5)	50 (16)	.0001
Females	12 (46 %)	13 (54 %)	25 (50%)	113 (76.8%)	.0006
Active	3 (11.5 %)	8 (33 %)	11 (22%)	NA	NA
On Immunomodulator	5 (19 %)	18 (75%)	23 (46%)	NA	NA

Designated active if Crohn's Disease Activity Index score 150 for patients with Crohn's disease or Simple Clinical Colitis Activity Index score 5 for patients with ulcerative colitis

* SD = Standard Deviation

Table 3

Test Characteristics of Study Assays for Determination of Poor Dietary Adherence

Anti IgA Tissue transglutaminase	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Gluten free diet duration 6 months and more				
Closed Conformation	52.6%	71.5%	24.4%	89.6%
Open conformation	68.4%	56.8%	21.6%	91%
Gluten free diet duration 12 months and more				
Closed conformation	56.2%	75%	28%	91%
Open conformation	68.7%	63.4%	24%	92%