Can High Titres of Anti Tissue Transglutaminase Antibodies Reduce the Need for Intestinal Biopsy for Diagnosis of Celiac Disease?

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Received: 27 April 2017 / Accepted: 7 September 2017 / Published online: 13 September 2017
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Abstract Traditionally small intestinal biopsy has been considered a gold standard for the diagnosis of celiac disease (CD). But now data has shown that serological markers like anti-tissue-transglutaminase antibodies (tTGA) can be used to make the diagnosis with great sensitivity and specificity. The objective of the present study was to evaluate whether patients with high probability of CD and high titre of tTGA, have a high probability of intestinal damage and may not require biopsy for final diagnosis. All the cases with tTGA levels ≥ 15 IU/ml and who subsequently underwent biopsy from July 2010 to June 2013 were selected. Histopathological findings graded as per Marsh classification were correlated with serum tTGA levels. Grade 3 lesions were considered diagnostic for the disease. Out of total 731 patients 470 had serum tTGA levels >100 IU/ml and 261 patients had <100 IU/ml. Highest levels of tTGA (219.3 IU/ml) were seen in grade 3c which was >12 times the normal cutoff value. Mean serum tTGA in higher histological grade i.e. 3 (3a, 3b, 3c) was 186.7 IU/ml (>12 times the normal cut off value) as compared to grade 1 which was 108.9 IU/ml (>7 times the normal cut off value). Using a tTGA cutoff value of 70 IU/ml, sensitivity was found to be 83.9% while specificity was 56.10% with an overall accuracy of 77.7%. This study confirms that a small intestinal biopsy is not always necessary for the diagnosis of CD in symptomatic patients with high tTGA levels (>70 IU/ml).

Keywords Celiac disease · Diagnosis · Serology · Anti-tissue-transglutaminase antibody

Introduction

Celiac disease (CD), also known as gluten-sensitive enteropathy (GSE), is an immune-mediated disease primarily affecting the small bowel. In genetically susceptible individuals, consumption of gluten triggers an inflammatory reaction with intraepithelial lymphocyte infiltration, crypt hyperplasia, and villous atrophy of the small bowel mucosa [1]. These changes might cause malabsorption, diarrhoea, abdominal distention, and abdominal pain, a broad range of non-classical findings, or be clinically silent [2, 3]. Down syndrome, family history of GSE, and autoimmune disease such as type 1 diabetes mellitus (DM) and thyroiditis are associated with CD [4]. If left untreated, patients are at increased risk for gastrointestinal lymphoma and other malignancies [5]. Treatment with a gluten-free diet (GFD) achieves complete reversal of disease and eliminates increased risk of malignancy in most patients [5, 6]. The gold standard for the diagnosis of CD has been considered to be a small intestinal biopsy since the histological lesions of CD were discovered in 1954 [7]. However, a small intestinal biopsy is not only expensive, time-consuming and stressful but may also provide inconclusive or even false results, due to patchy disease or to inadequate quality or orientation of the biopsy specimen [8–10]. Therefore, there has long been research focused on finding non-invasive markers to diagnose CD. For this purpose, the disease-associated auto-antibodies, especially anti-endomysium antibodies (EMA) and anti-tissue-transglutaminase antibodies (tTGA), have proven to be highly sensitive and specific [11–14]. In fact, according to the new

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European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) guidelines for the diagnosis of CD, a confirmatory small intestinal biopsy is no longer necessary in genetically predisposed individuals who are symptomatic and who have a tTGA of at least 10 times the upper limit of normal, a positive EMA and good clinical response to gluten free diet [15]. In view of the paucity of literature in this regard from our region, the present study was conducted to correlate the levels of tTGA with biopsy in North Indian population and to estimate a cut off level at which biopsy can be avoided.

Materials and Methods

This study was conducted in a tertiary level hospital over a duration of 3 years from July 2010 to June 2013. All the cases who were sent to department of biochemistry for the estimation of tTGA whose level was more than >15 IU/ml were included in the study. These cases further underwent biopsy and histopathological findings were graded as per Modified Marsh classification [16, 17].

tTGA levels were measured by BIO RAD autoimmune ELISA anti tissue transglutaminase IgA test kit. The Bio Rad tTG microplate is coated with recombinant human tTG crosslinked with gliadin specific peptides. Value of 15 IU/ml was taken as cut off as per manufacturer's guidelines. The sensitivity of this kit is 96.9% while the specificity is 95.0%.

Results

The age ranged widely, with the youngest patient being 1 year old and the oldest being 85 years old. Majority of the cases (25.2%) were in the age range of 21–30 years with the mean age of 28 ± 16.3 years. 423 patients (57.9%) were males and 308 were (42.1%) were females. Out of total 731 patients 470 had serum tTGA levels >100 IU/ml and 261 patients had <100 IU/ml. Majority of the patients that is 32% had tTGA more than 250 IU/ml (Table 1). On comparing mean serum levels of tTGA with the histological grade it was found that as tTGA levels increased, the histological grade also increased. Maximum level was seen in grade 3c which was 14 times the normal value. Mean serum tTGA level of all patients was 176.2 IU/ml which is >10 times the normal value of 15 IU/ml. (Table 2).

Correlation of tTGA levels with the different histological grades on biopsy showed that mean serum tTGA in higher histological grade i.e. 3 (3a, 3b, 3c) was 186.7 IU/ml (>12 times the normal cut off value) as compared to lower histological grade 1 which was 108.9 IU/ml (>7

times the normal cut off value. In histological grade 1 majority of the patients had serum tTGA levels <100 IU/ml, in grade 3a number of patients with tTGA level <100 and >100 IU/ml were equally divided whereas in grade 3b and 3c majority of patients had tTGA level >100 IU/ml. The correlation between serum tTGA levels and histological grade was statistically highly significant with the *P* value of zero (0.000) (Table 3).

Sensitivity, specificity and predictive values were calculated taking 70, 100, 120 and 140 as cutoff values for tTGA with histological evaluation as gold standard. It was seen that as the cut off increased from 70 to 140, sensitivity decreased while the specificity increased. Maximum sensitivity of 83.9% was found at a cut off value of 70. Positive and negative predictive values were also reasonably much better at this cut off with the highest overall accuracy of 77.7% (Table 4).

Receiver Operating Curve (ROC) analysis showed the area under the curve to be 0.774 (standard error 0.064 and 95% confidence interval 0.649–0.900). Cut off value of 77.4 derived through ROC showed a sensitivity of 82.3%, specificity of 60.37%, positive predictive value of 87.78%, negative predictive value of 49.75%. Overall accuracy was found to be 77.43% (Fig. 1).

Discussion

The diagnosis of CD has traditionally depended on intestinal biopsies though according to recently published guidelines, serologic testing is of primary importance in screening for CD.

Table 1 Distribution of patients at different tTGA levels

tTGA levels (IU/ml)	Number of patients (731)	Percentage
16–50	137	18.7
51–100	124	16.9
101–150	52	7.1
151–200	108	14.7
201–250	76	10.4
>250	234	32.0

Table 2 Serum tTGA levels (mean $\pm$ SD) at each histological grade

Histological grade	Mean serum tTGA (IU/ml) $\pm$ SD
1	108.9 $\pm$ 99.5
3a	147.4 $\pm$ 106.1
3b	193.5 $\pm$ 96.8
3c	219.3 $\pm$ 88.3
Total	176.2 $\pm$ 104.1

Table 3 Correlation between tTGA levels and histological grade

Histological grade	Number of patients	tTGA level ranges (IU/ml)					
		16–50	51–100	101–150	151–200	201–250	>250
1	164	75	33	11	11	5	29
3a	154	36	41	15	15	5	42
3b	90	9	16	6	19	9	31
3c	323	17	34	20	63	57	132
Total (731)		137	124	52	108	76	234

P value = 0.000

Table 4 Sensitivity, specificity and predictive values at different cut off levels of tTGA

tTGA cut off levels (IU/ml)	70	100	120	140
True positive (no.)	476	430	406	386
True negative (no.)	92	107	112	116
False positive (no.)	72	57	52	48
False negative (no.)	91	137	161	181
Sensitivity (%)	83.9	75.84	71.6	68.08
Specificity (%)	56.1	65.24	68.29	70.7
Positive predictive value (%)	86.8	88.3	88.6	88.9
Negative predictive value (%)	50.2	43.8	41.0	39.0
Overall accuracy (%)	77.70	73.46	70.86	68.67

Fig. 1 Receiver operating curve (ROC) depicting maximum area under the curve for Marsh 3 histopathology at tTGA titer of 77.4 IU/ml

CD is usually diagnosed when the duodenal and jejunal mucosa display villous atrophy, crypt hyperplasia, and an increase in intraepithelial lymphocytes [18]. However, different diseases not related to gluten—sensitive enteropathy can induce a flat mucosa, thus mimicking CD. Moreover patients with gluten sensitive enteropathy and normal bowel architecture have also been described [19]. Most likely because of lack of technical proficiency with grasping biopsy forceps or endoscopic procedure, biopsy specimens have been shown to be sufficient for diagnosis of CD in only 90% of cases [20]. CD may also be missed owing to variation in different pathologists assessments during histological examination [8]. Therefore low cost, less invasive tests like serological markers are required for the diagnosis of this disease.

Earlier anti gliadin antibody and endomysial antibody (EMA) were used as serologic tests for diagnosis and screening of celiac disease. Although the specificity of EMA was very high but inadequate sensitivity of these tests resulted in some seronegative celiac cases, making them undesirable in clinical practice [21]. tTG antibody was recognized by Dieterich and colleagues in 1997 as the major endomysial autoantigen and has been used as a sensitive and specific ELISA based test in celiac disease [22, 23]. During the previous decade, studies have shown that tTGA levels are different in various degrees of intestinal damage and there is a correlation between titers of tTG antibody and marsh grading [24–29]. Literature regarding a reliable cutoff in the Indian population which may obviate the need for biopsy is lacking. We therefore conducted this study to decide a reliable cutoff level with specificity and reliability which may correlate with a Marsh score of 3 or higher.

In our study the mean tTGA levels correlated well with the degree of histological grade and the villous atrophy and the mean tTGA levels were highest in grade 3c and lowest in grade 1. This is in agreement with previous study by Vivas et al. [27]. Barker et al. [24] showed that 48 of 49 mostly symptomatic children with a tTGA level ≥ 100 U/ml had at least Marsh II enteropathy. Comparably, Donaldson et al. [25] showed that 38 of the 38 pediatric patients with tTGA

≥ 100 U/ml had Marsh III histopathology. Similarly, in a study conducted in a mixed adult/pediatric population, it was shown that a tTGA ≥ 100 U/ml occurs almost exclusively in the setting of Marsh III (73 of 76 patients) and that the 3 patients without villous atrophy had either a Marsh II ($n = 2$) or a Marsh I ($n = 1$) lesion [26]. Likewise, a study performed in adults showed that 91 patients with a tTGA level of at least 10 times the upper limit all had at least Marsh II enteropathy [11].

In patients with high tTGA levels, there is increasing evidence that a small intestinal biopsy is not needed to confirm the diagnosis of CD, as these increased levels are highly suggestive of the disease. This conclusion was also stated in the new ESPGHAN guidelines for the diagnosis of CD in the pediatric population [10]. Briefly, these guidelines suggest that in symptomatic individuals who have tTGA levels of at least 10 times the upper limit of normal and who respond well to the gluten free diet, histological confirmation is unnecessary. This diagnostic approach has been confirmed by Mubarak et al. [29]. They found that small intestinal biopsy can be avoided in symptomatic patients with tTGA ≥ 100 U/ml. Recently Allesio et al. [30], have concluded that, in both adults and children, there is a high probability of duodenal damage in patients with positive anti-tTG serology ≥ 7 times the cut-off, along with positive EMA. They found that tTGA > 20 U was more specific for identification of patients with Marsh grade 3 lesions. The choice of an upper cut off limit of anti tTGA to predict accurately CD or Marsh type 3 lesion may depend on the commercial kit used for tTG IgA ELISA. We found highest sensitivity and good positive and negative predictive values at a cutoff level of 70 U/ml. Other authors have considered a cut off value > 100 U for a better Marsh type 3 prediction [24–26]. The cut off needs to be standardized in each laboratory based on experience with different kits.

It must be mentioned that since we did not estimate total IgA levels we could have missed patients with IgA deficiency.

Overall our results confirm the high predictive value and diagnostic accuracy of tTGA. We suggest that duodenal biopsy may not always be necessary in symptomatic patients. This low cost approach may be useful in a less resourceful country like India.

Compliance with Ethical Standards

Conflict of interest Authors Ekta Bansal, Navpreet Kaur & Naveen Mittal declare that we have no conflict of interest.

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