Original Article

Diagnostic accuracy of IgA anti-tissue transglutaminase in celiac disease in Van-Turkey

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Abstract. Although the IgA anti-tissue transglutaminase test (IgA anti-tTG) has been recommended as the first step in the diagnosis of celiac disease (CD), there are controversial data about the real accuracy of the test in clinical practice. Therefore we evaluated the sensitivity and specificity of the IgA anti-tTG in a group of patients who were suspected of having CD.

The study was performed at Van Training and Research Hospital, Van-Turkey. Details of patients in whom the IgA anti-tTG was requested from January 2009 to April 2012 were obtained from databases. Duplicate requests were excluded. Histopathologic examination of duodenal biopsies and serologic evaluations were compared.

A total of 1614 IgA anti-tTG were requested from different patients. In all, 49.6% of requests were in females and 29.8% from children under the age of sixteen. A total of 192 (11.9%) requests were found to be positive. Duodenal biopsies were performed to 61 (31.8%) of seropositive patients. The overall sensitivity and specificity of IgA anti-tTG were 93.3% and 9.5%.

Our data have revealed that clinicians should be aware of solely relying on the results of the IgA anti-tTG test could result in unnecessary diagnostic procedures and treatments.

Key words: Celiac disease, sensitivity, serologic tests, specificity, tissue transglutaminase

1. Introduction

Celiac disease (CD) is an immune-mediated enteropathy triggered by the ingestion of gluten, which affects genetically predisposed individuals (1). Although the gold standard test to diagnose CD is still the histological examination of the small intestinal mucosa, duodenal biopsy is an uncomfortable and expensive procedure (2,3). Therefore, several serologic tests have been developed and validated against biopsy specimens for the diagnosis of CD. The availability of non-invasive serological tests has dramatically changed the diagnosis of CD (2-4). Over the past few decades, Immunoglobulin (Ig) G and IgA gliadin antibody tests have been replaced by more sensitive and specific IgA endomysial antibodies (EMA) and IgA anti-tissue transglutaminase test (IgA anti-tTG) (5,6). Among those, EMA is considered to be a highly sensitive and specific test for the diagnosis of CD, but is not easily applied for screening and follow-up of CD patients because of its limitations (expensive, qualitative, and subjective) (7-9). Thus, IgA anti-tTG has been recommended as the first step in the diagnosis of CD (6,10-13). However, previous studies have revealed that serologic tests including IgA anti-tTG may not performing as well in the clinical setting as the original research studies suggested they should (14-19). Therefore we determined the sensitivity and specificity of IgA anti tTG in a group of patients who were suspected of having CD.

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2. Material and method

2.1. Patients

Details of all IgA anti tTG requests at Van Training and Research Hospital, Van-Turkey (a referral hospital serving a population of 1 000

000) from January 2009 to April 2012 were obtained from databases. At our hospital, total IgA levels are routinely simultaneously measured and reported with the antibody result to ensure that an apparent negative serological result is not in fact due to IgA deficiency. Data including the number of requests, the age and gender of patients being tested were collected. More than one serological test request within the same individual was excluded. Patients with type 1 diabetes, chronic liver disease, heart failure and psoriatic or rheumatoid arthritis were considered according to patient's medical records and excluded. Details of patients' medical records with positive serological results were searched to ascertain whether they had undergone gastroscopy with duodenal biopsies. Distal duodenal biopsies were judged to be positive for CD if the histological appearances showed any degree of villous atrophy (Marsh III lesion). Milder degrees of mucosal injury such as intraepithelial lymphocytosis alone (Marsh I lesion) or in association with crypt hyperplasia (Marsh II lesion) were also classified as being positive for CD (20).

2.2. IgA anti-tissue transglutaminase antibody assay

IgA anti-tTG antibody was determined by a human recombinant enzyme-linked immunosorbant assay (ELISA) method, using a commercially available Kit Aeskulisa tTG (Aesku Diagnostics, Germany). It was designed for quantitative measurement of IgA autoantibodies directed against tTG. All measurements were made on a Triturus ELISA autoanalyser (Grifols, Spain) according to the manufacturer instructions

2.3. Statistical analysis

Statistical analysis was performed by using $\chi 2$ or Fisher exact tests for categorical variables.

3. Results

After excluding patients with duplicate requests, there were a total of 1614 requests for IgA anti-tTG during the study period. The mean age of patients tested was 26 years (range 2-87 years). The ratio of female to male testing was 0.99. Overall 481 of 1614 tests (29.8%), were requested in patients less than 16 years old. A total of 192 patients (11.9%) were found to be positive for IgA anti-tTG. None of the subjects with negative IgA anti-tTG results was IgA deficient and none of them were on gluten free diet (GFD) according to the medical records. Sixty-one of those patients (31.8%) were underwent endoscopy for duodenal biopsy confirmation. Main reason for non-biopsy in the 131 patients with positive serology who were never biopsied was patient refusal. Histological evidence of CD was confirmed in 69% (42⁄61) of seropositive patients. In addition, five patients with negative IgA anti-tTG results were underwent gastroscopy and duodenal biopsy according to their clinical presentations. The overall sensitivity and specificity of IgA anti-tTG were 93.3% and 9.5%, respectively (Table 1).

Table 1. Comparison of IgA anti-tissue transglutaminase test (IgA anti-tTG) test results with pathology

		Pathology		Total
		Positive	Negative	
IgA anti-tTG	Positive	42	19	61
	Negative	3	2	5
Total		45	21	66

4. Discussion

The guidelines of the European and North American societies for gastroenterology require a biopsy for diagnosis of CD (21,22). However, because of the inconvenience and high cost associated with jejunal biopsy and the high prevalence of CD in the general population, less-invasive procedures are required (23). The detection of auto-antibodies is often used as a first-line test to identify individuals who might require a duodenal biopsy. Over the period of the last 10 years substantial improvement of the serological testing has occurred and the widespread availability of those tests has permitted any physician to test for CD (24). EMA and IgA anti-tTG are currently the most recommended tests for CD while the patient is on a gluten-containing diet (5,6,24). Although the reported sensitivity (+/- 93.9%) and specificity (96.5%) of the second generation of IgA anti-tTG assays are seemed to be good, there are also controversial data about the sensitivity and specificity of IgA anti-tTG in the clinical practice (5,14-19). Moreover there are reports of positive false IgA anti-tTG in the absence of CD which can be seen in those with type 1 diabetes, chronic liver disease, heart failure and psoriatic or rheumatoid arthritis (25). Nevertheless tTGA has remained the test of choice for initial testing (6,10-13). Our data have revealed a reliable sensitivity (93.3%) for IgA anti-tTG but the specificity of the test was found to be low (9.5%). This is considerably lower than the value of 70-90% reported by Lewis et al (6) in their systematic review. The lower specificity could be partly due to the differences of laboratory test systems. However, this does not account for the

marked difference in specificity. Although it is difficult to translate the results obtained by one manufacturer test-systems to other manufacturers' products without a comparative analysis, our finding is supporting the hypothesis which has been suggested that in some patients, proteins other than tissue transglutaminase (tTG) may act as antigens for anti-tTG antibodies (26).

Regarding the fact, interpretation of the positive and negative predictive values should be done with caution, because these values are influenced by disease prevalence in the population being studied. We did not analyse the positive predictive value (PPV) and negative predictive value (NPV). However, the fact that this study was carried out at a referral center should have no impact on the sensitivity and specificity, because these values are independent of disease prevalence.

Duodenal biopsies have been recommended to be performed in all individuals with positive celiac serological results (13,22). Our study has revealed that a significant proportion of seropositive individuals were not undergoing duodenal biopsy in our hospital. However the overall biopsy rates (33.8%) for positive serological results in our study is in consistency with previous reports (4,27,28). Unfortunately we did not have a chance to learn how those unbiopsied seropositive patients, were being managed subsequently. A total of 31% of our patients with positive serology who did undergo biopsy were found to have normal histology. The same rate of false positive results would be expected in those unbiopsied individuals and some of these patients might now be needlessly following a GFD.

In conclusion, our study have revealed that physicians should be aware of the low specificity of IgA anti-tTG in the diagnosis of CD in clinical setting which could lead to unnecessary diagnostic procedures and treatments.

References

1. Green PH, Jabri B. Coeliac disease. Lancet 2003; 362: 383-391.
2. Lock RJ, Stevens S, Pitcher MC, Unsworth DJ. Is immunoglobulin A anti-tissue transglutaminase antibody a reliable serological marker of coeliac disease? Eur J Gastroenterol Hepatol 2004; 16: 467-470.
3. Akbari MR, Mohammadkhani A, Fakheri H, et al. Screening of the adult population in Iran for coeliac disease: comparison of the tissuetransglutaminase antibody and anti-endomysial antibody tests. Eur J Gastroenterol Hepatol 2006; 18: 1181-1186.
4. Evans KE, Malloy AR, Gorard DA. Changing patterns of coeliac serology requests. Aliment Pharmacol Ther. 2009 May 15; 29: 1137-1142.
5. Hill I. What are the sensitivity and specificity of serologic tests for celiac disease? Do sensitivity and specificity vary in different populations? Gastroenterology 2005; 128: 25-32.
6. Lewis NR, Scott BB. Systematic review: the use of serology to exclude or diagnose coeliac disease (a comparison of the endomysial and tissue transglutaminase antibody tests). Aliment Pharmacol Ther 2006; 24: 47-54.
7. Rostom A, Dube C, Cranney A, et al. The diagnostic accuracy of serologic tests for celiac disease: a systematic review. Gastroenterology 2005; 128: 38-46.
8. Lepers S, Soula F, Fily S, et al. Relevance of anti-tissue transglutaminase antibodies in coeliac disease diagnosis. Ann Biol Clin (Paris) 2003; 61: 337-343.
9. Rashtak S, Ettore MW, Homburger HA, Murray JA. Comparative usefulness of deamidated gliadin antibodies in the diagnosis of celiac disease. Clin Gastroenterol Hepatol 2008; 6: 426-432.
10. Volta U, Fabbri A, Parisi C, et al. Old and new serological tests for celiac disease screening. Expert Rev Gastroenterol Hepatol 2010; 4: 31-35.
11. Lewis NR, Scott BB. Meta-analysis: deamidated gliadin peptide antibody and tissue transglutaminase antibody compared as screening tests for coeliac disease. Aliment Pharmacol Ther 2010; 31: 73-81.
12. Rashtak S, Ettore MW, Homburger HA, Murray JA. Combination testing for antibodies in the diagnosis of coeliac disease: comparison of multiplex immunoassay and ELISA methods. Aliment Pharmacol Ther 2008; 28: 805-813.
13. Rostom A, Murray JA, Kagnoff MF. American Gastroenterological Association (AGA) Institute technical review on the diagnosis and management of celiac disease. Gastroenterology 2006; 131: 1981-2002.
14. Abrams JA, Brar P, Diamond B, Rotterdam H, Green PH. Utility in clinical practice of immunoglobulin a anti-tissue transglutaminase antibody for the diagnosis of celiac disease. Clin Gastroenterol Hepatol 2006; 4: 726-730.
15. Rostami K, Kerckhaert J, Tiemessen R, et al. Sensitivity of antiendomysium and antigliadin antibodies in untreated celiac disease: disappointing in clinical practice. Am J Gastroenterol 1999; 94: 888-894.
16. Dickey W, McMillan SA, Hughes DF. Sensitivity of serum tissue transglutaminase antibodies for endomysial antibody positive and negative coeliac disease. Scand J Gastroenterol 2001; 36: 511-514.
17. Dickey W, Hughes DF, McMillan SA. Reliance on serum endomysial antibody testing underestimates the true prevalence of coeliac disease by one fifth. Scand J Gastroenterol 2000; 35: 181-183.
18. Abrams JA, Diamond B, Rotterdam H, Green PH. Seronegative celiac disease: increased prevalence with lesser degrees of villous atrophy. Dig Dis Sci 2004; 49: 546-550.
19. Emami MH, Karimi S, Kouhestani S, Hashemi M, Taheri H. Diagnostic accuracy of IgA anti-tissue transglutaminase in patients suspected of having coeliac disease in Iran. J Gastrointestin Liver Dis. 2008; 17: 141-146.

20. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. Eur J Gastroenterol Hepatol 1999; 11: 1185-1194.
21. Report of a working group of the united European gastroenterology week in Amsterdam: When is a coeliac a coeliac? Eur J Gastroenterol Hepatol 2001, 13: 1123-1128.
22. Hill ID, Dirks MH, Liptak GD, et al. Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology Hepatology and Nutrition. J Pediatr Gastroenterol Nutr 2005; 40: 1-19.
23. Bürgin-Wolff A, Mauro B, Faruk H. Intestinal biopsy is not always required to diagnose celiac disease: a retrospective analysis of combined antibody tests. BMC Gastroenterol 2013; 23: 13-19.
24. Geboes K, Geboes KP. Diagnosis and treatment of coeliac disease. F1000 Med Rep 2009; 1: 32.
25. Hummel S, Hummel M, Banholzer J, et al. Development of autoimmunity to transglutaminase C in children of patients with type 1 diabetes: relationship to islet autoantibodies and infant feeding. Diabetologia 2007; 50: 390-394.
26. Santaolalla R, Fernández-Bañares F, Rodríguez R, et al. Diagnostic value of duodenal antitissue transglutaminase antibodies in gluten-sensitive enteropathy. Aliment Pharmacol Ther 2008; 27: 820-829.
27. McGowan KE, Lyon ME, Loken SD, Butzner JD. Celiac disease: are endomysial antibody test results being used appropriately? Clin Chem 2007; 53: 1775-1781.
28. Pearce AB, Sinclair D, Duncan HD, Saas M, Poller DN. Use of the anti-endomysial antibody test to diagnose celiac disease in clinical practice. Clin Lab 2002; 48: 319-325.