Value of IgA human recombinant tissue transglutaminase antibody test in diagnosis of symptomatic celiac disease in children

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ABSTRACT

Objective: To identify the value of serological examination in diagnosis of celiac disease in children.
Patients and methods: A prospective case series study was conducted at private clinics in Mosul city during the period from 30th of October 2007 to 30th of October 2009. A total of 40 patients (29 males, 11 females) aged more than 6 months on gluten containing diet presented with symptoms suggestive of celiac disease were screened by serological testing using second generation ELISAs IgA human recombinant tissue transglutaminase antibody. Multiple duodenal biopsies were performed for every patient enrolled in this study regardless of the results of serology. Statistical methods were used to indicate sensitivity, specificity, negative and positive predictive values of serological test in comparison to biopsy results.
Results: A total of 16 (40%) out of 40 symptomatic patients with mean age of 51 months, demonstrated both positive IgA anti-tissue transglutaminase antibody test and biopsy results for celiac disease, the remaining 24 patients (60%) displayed negative results for both serology and biopsy. IgA anti-tissue transglutaminase antibody test had (100%) specificity, sensitivity, positive predictive value, and negative predictive value in relation to biopsy results.
Conclusion: Our results provide additional support to the concept that IgA anti-tissue transglutaminase antibodies can be used as a diagnostic serologic marker for celiac disease.

الخلاصة

هدف الدراسة: معرفة قيمة الفحص المصلي في تشخيص الجواف لدى الاطفال.
طريقة البحث والمشاركون: هذه دراسة مستقبلية لحالات متتالية، أجريت في العيادات الخاصة في مدينة الموصل، خلال الفترة من ٣٠ تشرين الأول عام ٢٠٠٧ ولغاية ٣٠ تشرين الأول عام ٢٠٠٩. العينة المدروسة ضمت (٤٠) مريضا (٢٩ ذكرا،١١ أنثى) تجاوزت أعمارهم أكثر من ستة أشهر. الكل كان يتناول قبل الدراسة طعاما يحتوي على الغروين ولديهم أعراضا موحية بالجواف. خضع كل أفراد العينة للفحص المصلي الذي يمثل الجيل الثاني لفحص الأجسام المضادة من نوع (ELISAs IgA human recombinant tTG). تم إجراء الفحص الناظوري لأعلى الجهاز الهضمي وأخذ خزعات متعددة من الأثني عشري لكل أفراد العينة وبغض النظر عن نتيجة الفحص المصلي. أستخدمت الوسائل الإحصائية لتقييم الفحص المصلي المذكور من ناحية الحساسية والنوعية والقيمة التكهنية الموجبة والسالبة ومقارنتها مع نتائج الفحص النسيجي للخزع.
النتائج: كانت نتيجة الفحص المصلي موجبة لدى (١٦) مريضا والذين شكلوا نسبة ٤٠% من العدد الكلي للمرضى والذي بلغ معدل أعمارهم (٥١) شهرا، وعند إجراء فحص الخزعة لهؤلاء المرضى تبين بأن النتيجة كانت موجبة أيضا. أما بقية أفراد العينة والبالغ عددهم (٢٤) مريضا ونسبتهم ٦٠% من العدد الكلي، فكان كلا الفحصان المصلي والنسيجي لديهم سالبا.

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إن الفحص المصلي المذكور حقق نسبة ١٠٠% فيما يخص الحساسية والنوعية والقيمة التكهنية الموجبة والسالبة بالمقارنة مع نتائج فحص الخزعة.

الاستنتاج: نتائج هذه الدراسة تعطي دعما إضافيا لفكرة أن الفحص المصلي (IgA anti-tTG) من الممكن استخدامه كمؤشر مصلي تشخيصي للجواف.

The North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) recommended that children and adolescents with symptoms of celiac disease (CD) or an increased risk for CD have a blood test for antibody to tissue transglutaminase (anti-tTG) to identify individuals for whom the biopsy is indicated [1]. Despite the increasing importance of serological methods, the diagnosis of CD is still based on histological criteria [1, 2], followed by a therapeutic response to a gluten free diet (GFD) [3].

Second generation ELISAs that detect anti-tTG2 IgA using human recombinant or human purified tTG2 antigen have sensitivity and specificity values ranging from 91% to 97% with the manufacturer-recommended cut-off values and are easy to perform [4-7]. As this type of analysis can be automatized, it is a valid tool in screening programs, and also is recommended for monitoring CD patients on GFD [4]. Failure of the anti-tTG level to decline over a period of 6 months after starting the GFD suggests continued ingestion of gluten or related products [1]. IgA anti-tTG antibody test can be falsely negative with IgA deficiency, which is associated with an increased incidence of CD. Measurement of serum IgA concentration is mandatory to assure that false-negative results in IgA-deficient individuals are excluded [8]. Newer assays incorporating synthetic deamidated gliadin-related peptides or other TG isoenzymes as antigen, enhances the sensitivity for detecting gluten sensitivity among non-IgA- deficient, anti-tTG seronegative patients with CD-like enteropathy [9]. A positive serological test in an individual with normal small intestinal histology may represent a false positive serological test, milder disease or a more sensitive test that identifies latent CD before mucosal injury [1]. It is important to set the lower limit of antibody titers high enough to avoid false-positive results [8]. In children with CD (87%) younger and (96%) older than 2 years showed high serum levels of anti-tTG2 [10].

Definitive diagnosis of CD requires small intestinal biopsy [8]. The mucosal involvement can be patchy and varies in severity, so multiple biopsies must be obtained [1, 3,11]. The histologic findings in celiac disease are characteristic but not specific; indeed, celiac disease is not the only cause of villous atrophy [12]. Marsh classified the histologic changes of CD as Type 0 (normal), Type 1 (increased intraepithelial lymphocytes), Type 2 (Type 1+ hyperplastic crypts), Type3 (Type 2 + variable degree of villous atrophy) and Type 4 (total villous atrophy with crypt hypoplasia)[1].

Based on these facts investigators inquired the possibility of obviating the need for small intestinal biopsy which is invasive, time consuming, not free of complications, and not accepted by all patients, by assessing the value of serological test in diagnosis of CD.

Studies concerning different aspects of CD are rare in our locality and to the best of our knowledge no similar study has been conducted in Mosul.

Patients and methods

This study was approved by ethical committee in Nineveh College of Medicine and Local Health Authority. This prospective study has been conducted at private clinics in Mosul city during the period from 30th of October 2007 to 30th of October 2009. Patients with suspected CD presenting with anorexia, failure to thrive, abdominal distention, and chronic diarrhea (in various combinations) who were aged more than 6 months and on gluten containing diet were selected.

A total of 40 patients (29 males, 11 females) with mean age of 51 months, were serologically screened by measuring IgA

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antibody to human recombinant tTG which was done by commercially available kit (AESKULISA tTG-A 3503/ Germany) a new generation of a solid phase enzyme immunoassay employing human recombinant tTG cross linked with gliadin-specific peptides display neo-epitopes of tTG. The cut-off value of the kit for a positive result is more than 15 U/ml.

For the purpose of achieving the objective of evaluation of serological data in comparison to biopsy results, and also because of unavailability of IgA level measurement for those with negative IgA anti- tTG2, all patients were subjected to duodenal biopsy regardless of the results of serology. Consents of parents of all patients were taken prior to laboratory and endoscopic examination. Upper gastrointestinal endoscopy was done in Al-Salam General Hospital in Mosul city where three biopsies from different sites of duodenum were taken from every patient. Histopathologic reports were analyzed according to Marsh criteria [6].

The validity of the serologic test and the mean age were computed through using Statistical Package for Social Sciences (SPSS, version 14).

Results

IgA anti- tTG2 test of the used kit was positive in 16 patients (40%), all of them displayed a positive biopsy suggestive of CD, including three patients aged ≤ 2 years, where as the remaining 24 patients (60%) manifested negative serology test and normal biopsy results at the same time (Table 1). All patients with positive serology displayed March 3 histopathologic grading. In this study the lowest level of IgA anti- tTG2 that was associated with positive biopsy of CD was 15.05 U/ml, whereas the maximum level associated with negative biopsy was 12.75 U/ml.

Positive biopsy reports of all patients with CD showed variable degree of villous atrophy consistent with the definition of Marsh type 3 histological grading.

Follow up of CD patients after starting GFD showed that IgA anti-tTG2 levels declined to normal in 14(87.5%) patients after 6 months of starting GFD. The remaining 2(12.5%) female patients, in spite of stressing on importance of strict adherence to GFD, their IgA-anti tTG2 levels remained positive after 6 months and their repeated biopsy was positive too (Table 2).

Table (1): Sensitivity, specificity, and predictive values of IgA anti- tTG2 in comparison to results of duodenal biopsy.

IgA anti- tTG2	Positive biopsy	Negative biopsy
Test positive >15U/ml	16	0
Test negative≤15U/ml	0	24
Sensitivity = 100%		
Specificity = 100%		
Predictive value of positive test result = 100%		
Predictive value of negative test result = 100%		

Table (2): Clinical, serological, and histological follow up of 2 CD patients with persistent abnormal IgA-anti tTG2 level.

Age (year)	Symptoms (6 months after GFD)	IgA anti-tTG2 (U/ml)			Biopsy 6 months after GFD
		Initial	6 months after GFD)	7 months after GFD)	
9	Abdominal pain	25.45	29.50		positive
12	Asymptomatic	38.70	43.75	86.76	positive

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Discussion

The newly developed ELISA tests for IgA anti-tTG antibodies are now available and are easier to perform, less expensive than the immunofluorescence assay that is used to detect anti-endomysium IgA antibody (anti-EMA); it is not subjected to inter-observer variation being investigator-independent [13-16]. The diagnostic accuracy of anti-tTG immunoassays has been improved by the use of human tTG instead of nonhuman tTG preparations [13]. It has a high sensitivity and specificity in CD, comparable to (anti-EMA) antibodies [17]. The serologic tests with the highest overall diagnostic accuracy were the tTG and the EMA, addition of HLA-DQ typing did not add to increase the diagnostic accuracy of these two tests [18].

Our study confirms the excellent specificity (100 %) of the IgA anti-tTG2 test reported by previous studies [4, 14, 19-21], and the excellent sensitivity (100%) found in other studies [13, 19, 22-24]. Up to 100% positive predictive value was also registered [4, 14], which is identical to our finding. In the current study, the negative predictive value of IgA anti-tTG2 test was 100%, which was similar to Carroccio A et al study result [22]. The fact that IgA anti-tTG2 titer has a good relationship with the severity of the mucosal damage of the small bowel[23,25], may explain the 100% sensitivity of this test in relation to Marsh 3 histopathological grading in our patients. The sensitivity of this test appears to be lower than reported when milder histologic grades are used to define CD [26].

In some studies high positive tTG level antibody results has not always been associated with final diagnosis of CD [27-29]. This may be attributed to false negative duodenal biopsy, probably due to patchy histopathological lesion or using guinea pig tTG which lacks specificity, and although tTG antibody positivity may appear in gastrointestinal and liver inflammatory disorders, to date, strong positive results have not been described for such conditions; in addition many of these patients may have coexistent CD [30].

The human tTG-based ELISA is the method of choice for easy and noninvasive screening and diagnosis of CD [19] .The presence of human anti-tTG is a reliable indicator for the diagnosis and follow-up of CD [2]. In patients with symptomatic CD, the presence of circulating anti-EMA or anti-tTG antibodies is highly predictive (97%–100%) of biopsy changes of CD [31]. Serologic testing is important not only for screening but also for confirmation of CD [32].

In children <2 years of age, milk protein-sensitive enteropathy can produce changes similar to CD; confirmation of diagnosis after a gluten challenge and biopsy is sometimes required [8]. IgA anti-tTG2 measurements show higher sensitivity for the diagnosis of CD in children older than 2 years compared with younger children [10]. In this study three patients aged ≤ 2years with biopsy proven CD all had positive IgA anti-tTG2 levels, indicating that IgA anti-tTG2 test below two years of age may also have a high sensitivity in diagnosing CD comparable to its sensitivity above 2 years of age.

In this study the lowest level of anti-tTG that was associated with positive biopsy of CD was 15.05 U/ml, indicating that the cut-off value of the used kit for a positive result which was more than 15 U/ml was appropriate value that detected all studied patients with CD. The choice of an upper cut-off limit of tTG antibody to predict accurately CD or Marsh type 3 lesions may depend on the commercial kit used for tTG IgA ELISA and the cut-off value should probably be standardized in each laboratory based on experience with different kits[30].

Dietary non-adherence is the most common cause of unresponsive CD [12]. In the current study adherence to a GFD was observed in 87.5% (14/16) of CD patients, comparable results ranges from 50% to 100% were found in Middle East and North African countries [33]. The remaining12.5% of CD patients (2/16) who manifested seropositivity after 6 months of follow up were possibly non-adherent to a GFD either intentionally or unintentionally. Histologic recovery in patients who have CD usually takes several months but can take up

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to 1 year, even if the patient remains on a strict GFD [34]. Refractory CD occurs in approximately 5% of patients with CD [12] and is defined by persistent or recurrent malabsorptive symptoms and villous atrophy despite strict adherence to a GFD for at least 6-12 months in the absence of other causes of non-responsive treated CD and overt malignancy, and require additional laboratory and therapeutic intervention besides a GFD [35-38].

Though our study is limited by its relatively small sample size and being a private clinic based rather than hospitals or community based, the study clearly showed that anti-tTG antibody test is a highly sensitive and specific marker for CD diagnosis and biopsy might not always be needed to confirm it.

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