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Ten Times Normal Anti Tissue Transglutaminase Level Detected By Enzyme Linked Immunosorbent Assay (ELISA), But Normal Level by Chemiluminescent Assay (CLIA)

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Abstract According to the new guidelines of the European Society for Pediatric Gastroenterology Hepatology and Nutrition [1], it is mandatory to test for anti tissue transglutaminase IgA (anti TTG IgA) and serum IgA in first step in the diagnosis of celiac disease. A diagnosis of celiac disease can be made when the anti-TTG IgA titer is ten times normal (with normal Ig A level) and anti-endomysial antibody (EMA) is positive in the second blood sample, without requiring biopsy. In this article, we present cases of anti TTG IgA levels more than ten times the upper limit of normal determined by ELISA (enzyme-linked immunosorbent assay). In some patients, EMA was checked and in all of these cases upper endoscopy and duodenal biopsy was performed for confirmation, Although biopsy was performed from the bulb and the second part of the duodenum, and the number of biopsies were adequate; no evidence of celiac disease was observed on histological examination. These patients were recruited, and another blood sample was taken and anti TTG IgA was checked by a chemiluminescence immunoassay (CLIA) and the levels were reported normal.

Keywords: *Celiac Disease, Serology, Anti Tissue Transglutaminase, Enzyme Linked Immunosorbent Assay (ELISA), Chemiluminescent Immunoassay (CLIA)*

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1. Introduction

Celiac disease is a lifelong autoimmune condition requiring strict avoidance of gluten containing foods.

In low- and middle-income countries, bread is a major food item for families, including their children. It is hard to share information with families that their child is not able to eat bread with other family members. So the diagnosis of celiac disease should be declared after finding strong evidence.

Based on ESPAGHAN 2020, eligible children for no biopsy approach for celiac disease diagnosis are those with high serum IgA class antibody concentration against transglutaminase 2 values (more than 10 times the upper limit of normal) with appropriate tests and positive endomysial antibodies (EMA-IgA) in a second serum sample.

The method for evaluating anti TTG IgA level is not

mentioned in the guidelines and it is only mentioned that only calibration curve – based immunoassays should be used. Although most western countries have greater access to standardized and high tech instruments for the evaluation of anti TTG IgA level, however the method mostly used in our country is ELISA.

Endoscopic studies are frequently performed in our country, especially in the pediatric age group, either following the suspicion of a pediatric gastro - enterologist or because the family insists on doing so. We encountered cases in which elevated anti TTG IgA level (ten times higher than normal) were reported in recent years, in whom a biopsy was taken from the bulb and duodenum and the sample was sent to our laboratory.

Later, we checked the anti TTG IgA level using a chemiluminescent assay, and the level was reported as normal.

This has led to confusion among most clinicians and raised anger in families about faults and flaws in laboratory tests.

Here, we discuss the matter just to mention that in some cases, ten times normal anti TTG IgA level by ELISA method maybe false and the level should be confirmed by CLIA method on a second blood sample.

2. Materials and Methods

Between 2018 and March 2024, we encountered 6 children with elevated anti TTG IgA levels ten times normal, they all underwent upper endoscopy and biopsy.

Anti TTG IgA levels were measured using ELISA in different laboratories (some are the best in our country) and with different types of kits. EMA was measured on second blood sample in some of the patients and some had negative result.

In all of these cases, biopsy was performed due to the insistence of the parents or in some cases due to suspicion of the pediatric gastro-enterologist.

At least four biopsy samples obtained from the bulb and distal duodenum in each patient (except patient number 4: one piece from bulb and two pieces from D2) and sample was sent to our laboratory (Roshan Azma Pathobiology Laboratory, Tehran, Iran). New Marsh grading system was used for histopathology report [2].

Our patients approve that the information can be used in scientific processes before submitting the sample. Besides this is a retrospective study and cases presented here are anonymous and personal information is well preserved.

After processing and embedding, two slides were taken from each sample and stained with Hematoxylin & Eosin.

The slides were observed by a pathologist with approximately 27 years of experience in pediatric gastrointestinal pathology.

In all cases, another blood sample was analyzed using the chemiluminescence method (Liaison Instruments) [3].

It is important to state that none of these children were on gluten free diet at the time of upper endoscopy and biopsy.

3. Results

Six patients with anti TTG IgA antibody levels more than 10 times the upper limit of normal detected.

Four patients were male and the median age was 8.4 years (5-11.5 years). The demographic details are depicted in attached table (Table 1).

EMA was checked and in patients number 5 and 6, it was positive (other patients had negative EMA level)

Two patients underwent repeat ELISA tests on a second blood sample in another laboratory and the results were the same (patients number 1 and number 5).

All of the above mentioned cases had normal histology and normal anti TTG IgA levels by chemiluminescence method. In addition, we had patients with elevated anti TTG IgA with ELISA who had normal histology, but patients did not referred for assessment of anti TTG IgA level with chemiluminescent method and were not included in this study.

Despite the highly elevated anti TTG IgA levels detected by ELISA, these cases were regarded as false positive and no gluten free diet was administered. Seven other patients had anti TTG IgA levels between 5 and 10 times normal. All patients had normal histology. The second blood sample was checked by the chemiluminescent method and the level was reported as normal.

Table 1. Demographic Characteristics And Serologic Markers of Our Patients

N	Age (Years)	Gender	Anti-TTG IgA (ELISA)	EMA Level	Anti-TTG IgA (CLIA)	Number of Biopsies
1	11.5	Male	366 (Positive >18) Second Blood Sample: > 300 (Positive > 18)	<1/5 (Negative, IF)	0.27 (Negative <7.2)	Four pieces including bulb
2	5	Male	>300 (Positive >18)	<1/5 (Negative, IF)	2.3 (Negative <7.2)	Four pieces including bulb
3	10.5	Female	230 (Positive >18)	<1/5 (Negative, IF)	0.44 (Negative <7.2)	Four pieces including bulb
4	7.5	Female	270 (Positive >18)	4.8 (Negative <20, ELISA)	0.31 (Negative <7.2)	Three pieces (1 from bulb, 2 from D2)
5	8.5	Male	>300 (Positive >18) Second Blood Sample: > 300 (Positive > 18)	74.8 (Positive >20, ELISA) / <1/5 (Negative, IF)	3.81 (Negative <7.2)	Five pieces including bulb
6	7.5	Male	345 (Positive >20)	311.5 (Positive >20, ELISA) / <20 (Negative, ELISA)	<0.2 (Negative <7.2)	Six pieces including bulb

4. Discussion

Tissue transglutaminase (TTG or TG2) is a 78-kDa, calcium-dependent enzyme belonging to the glutamyl - transferases family [4,5]. TTG acts as an autoantigen in celiac disease [6]. Diagnostic Serological tests for anti TTG antibodies have been claimed to have strong sensitivity (99%) and specificity (> 90%) for identifying celiac disease and has superseded other older tests, such as the anti gliadin antibody test [7].

Modern anti-TTG assays use a recombinant human protein as antigen [8].

Based on a recent study there is no significant differences in diagnostic accuracy among ELISA, CLIA, and fluorometric enzyme-linked immunoassay (FEIA) for detecting anti-TTG Ig A, but while different methods exist, ELISA remains highly accurate [9].

Based on ESPGHAN guidelines only antibody tests with proper calibrator curve-based calculation should be used [1].

Although, the ESPGHAN guideline recommends that celiac disease can be diagnosed without biopsy in cases with anti TTG IgA levels ten times above normal and if EMA is positive in second blood sample, we had cases in which the assessment was performed using ELISA, but the biopsy was normal, and later, anti TTG IgA levels were reported to be normal by the CLIA method.

This could be due to the effect of sanctions on the supplementation of kits in Iran. Surprisingly, there have been cases in which anti TTG IgA levels were reported to be very high in the above mentioned laboratories and in biopsy, celiac disease was approved.

Therefore, the problem seems to be a random error that cannot be traced to a specific laboratory and/or kit.

In two of our patients (number 5 and 6) even EMA was positive however the clinician wanted to confirm celiac disease by biopsy and upper endoscopy was performed despite ESPGHAN guidelines. In patient number 5 first EMA was done by ELISA method and was positive but another sample was checked with immunofluorescent method and was reported as negative. In patient number 6, EMA was checked by ELISA and was highly elevated but test was repeated on another blood sample in a different laboratory and was reported as negative. This shows that ELISA method for assaying of EMA is not flawless.

We believe that in countries such as Iran, where anti TTG IgA is mostly checked by ELISA, it is prudent to check anti TTG IgA in the second sample by another method (in two of our cases, the second sample was checked by ELISA in another laboratory and the result was the same) or to perform biopsies, especially when there is any suspicion of the diagnosis of celiac disease.

The main ingredient in the diet of Iranians is bread and gluten- free products are usually very expensive.

In this regard accurate diagnosis is very important.

Unfortunately access to chemiluminescence or fluorescent immunoassays is difficult due to sanctions and rare laboratories, such as the Sophia Pathobiology Laboratory, perform the test using the Liaison instrument in Iran [3].

However, this laboratory has many difficulties maintaining the supply of instruments and kits in Iran.

Pediatric gastro - enterologists in Iran are willing to perform upper endoscopy for all patients with elevated levels of anti TTG IgA despite the ESPGHAN guidelines due to mistrust of test results. Sometimes they put the patient on a gulten free diet despite normal biopsy findings and interpret it as a hidden type of celiac disease.

However, we believe that in cases where anti TTG IgA levels are reported to be normal by the CLIA method, it is useless to suffer families by this laboratory fault, and it is prudent not to recommend gluten free diet for these kids.

The above findings may lead to further studies comparing ELISA with other methods for the assessment of anti TTG antibodies, and we hope that the test method will be mentioned in upcoming guidelines.

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