

Immunodiagnosis of celiac disease among children with chronic diarrhea in Gaza Strip, Palestine

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To cite this article:

Ahmed Mahmood Ruby, Randa Al-Khodary, Mohammad Shubair, Mahmoud Sirdah. Immunodiagnosis of Celiac Disease among Children with Chronic Diarrhea in Gaza Strip, Palestine. *American Journal of BioScience*. Vol. 2, No. 6, 2014, pp. 192-195.

doi: 10.11648/j.ajbio.20140206.11

Abstract: Celiac disease (CD) is a permanent intolerance to gluten that results in damage to the mucosa of the small intestine. The prevalence of CD in developing countries may be undervalued due to different factors, but lack of awareness and low suspicion of the disease could be the main factors. The aim of the present work was to estimate the occurrence of CD among children suffering from chronic diarrhea in Gaza Strip and to adopt dependable non-invasive immunological techniques for diagnosis of CD in the laboratories of the Ministry of Health. This study was conducted on children (6-96 months) suffering from frequent (>3times/day) chronic diarrhea that not caused by infection. The study population comprised 123 symptomatic Palestinian children. Five ml peripheral blood were collected, sera were separated and stored at -70 °C until performing the following assays: IgA Anti-endomysial antibodies (EMAs) using indirect immunofluorescence technique (IF), anti-tissue transglutaminase enzyme antibodies (tTG, IgG, IgA) and (tTG, IgA) using ELISA technique, anti-smooth muscle antibodies (ASMA) using indirect immunofluorescence and total IgA using radial immunodiffusion (RID). The prevalence of CD using EMAs test was 3.25% but 12.2% when (tTG IgG, IgA) assay was applied. However, the prevalence of ASMA was 28.5% which may mask the EMAs antibodies and hence giving false negative results of EMAs. Our results showed comparable sensitivity of both (tTG IgG, IgA) and EMAs. Deficient or low IgA represented 33.3% of all (tTG IgG, IgA) positive samples. It was concluded that EMAs and (tTG IgG, IgA) tests could be used as noninvasive techniques on children suffering from CD. However for those having low or IgA deficiency, the class IgG of EMAs and tTG should be performed.

Keywords: Celiac Disease, Tissue Transglutaminase Antibodies, Anti-Endomysial Antibodies, Antismooth Muscle Antibodies, Gaza Strip.

1. Introduction

Celiac disease (CD) is a long-lasting intolerance to gluten that harms the small intestine mucosa due to mucosal inflammation and loss of absorptive surface area and exhibited a broad spectrum of symptoms and nutritional deficiencies [1]. Untreated CD is also associated with a humoral immune response which is directed against the reticulin and endomysium of connective tissue i.e endomysial antibodies (EMAs) and against various peptides which are derived predominantly from wheat, antigliadin antibodies (AGAs) [2]. The susceptibility to CD, its activation and continuance encompass blend of environmental, genetic factors and immunological pathways [3]. The genetic

determinants which are associated with CD could be found in the majority of CD patients, but they are not enough by themselves to cause the disease. At least seven known HLA-DQ variants (DQ2 and DQ4-DQ9) are associated with the CD, however, isoform of DQ2 or DQ8 are reported in more than 95% of Celiac patients families and increased the risk of CD as the receptors formed by these genes bind to gliadin peptides more tightly than other forms of the antigen-presenting receptors. Consequently, these forms of receptors are more likely to trigger T lymphocytes and start an autoimmune response [4].

The prevalence of CD in the USA was reported as 0.75% in not-at risk group, increasing to 1.8% in symptomatic cases, 2.6% in second-degree relatives of a patient with CD and 4.5% in the very close family members like first degree

relatives [5]. Unfortunately, the prevalence of CD in developing countries may be underestimated due to different factors, most of which, the lack of awareness and low suspicion of the disease. A systematic review of the prevalence of CD in developing countries is well documented in the 2010 and 2012 studies of Barada and his coworkers [6,7]. However, no previous studies were conducted to estimate the prevalence of CD in Gaza Strip, Palestine.

The combinations of clinical, immunologic and histologic finding plus response to a gluten-free diet confirm the diagnosis of CD in most patients. The laboratory tests are divided into two different types of antibodies; anti-gluten and anti-self. The anti-gluten antibodies are the anti-gliadin IgG and IgA. The anti-self antibodies are EMAs IgA and anti-tissue transglutaminase (TTG) IgA [8].

The National Institute of Health (NIH) recommends two test; TTG and EMAs [9]. These tests could be used for preliminary diagnosis, monitoring response to a gluten-free diet and screening first degree relatives of individuals with CD [10]. Recently anti-partially deaminated gliadin peptides (anti-DPG) has been introduced, while other laboratories depend on molecular genetic techniques [11].

This study aims at assessing the validity of the screening tests in estimating the occurrence of CD among Palestinian children living at Gaza strip who suffer chronic non-infectious diarrhea and propose sensitive and specific non-invasive immunologic tests to be introduced in health laboratories of Gaza Strip.

2. Materials and Methods

The present study is a descriptive one and performed to assess the validity of the ASMA, EMA and tTG screening tests in identifying CD among Gaza children. It comprised all children (age 6-96 months) suffering non-infectious chronic diarrhea more than three times daily. Representative samples (n=123) were collected from two clinics in Gaza city; Al-Rimal clinic (32%) and Ard El-Insan clinic (68%). The present study was approved by the Helsinki ethical committee at the Palestinian ministry of health, and it was performed in accordance with the ethical standards laid down in the 1964 and 1975 Declarations of Helsinki, and the modifications of 1996.

Blood samples were collected when children attend the

clinic. From each individual 5 ml of venous blood sample were withdrawn and serum was separated by centrifugation for 20 minutes at 3500 rpm at room temperature. The separated serum was frozen at -70 °C until assaying. The following assays were performed: (1) Anti-endomysial (EMAs) IgA antibodies qualitative test using indirect immunofluorescent assay (Biosystems, Spain), (2) tissue transglutaminase enzyme (tTG IgG, IgA) antibodies quantitative test using ELISA technique (Orgentic Company, Germany) with normal value < 15 IU/mL and positive: >15 IU/mL, (3) Antismooth Muscle Antibodies (ASMA) qualitative test using IFA (Biosystems, Spain), and (4) total IgA quantitative test using radial immunodiffusion (RID) technique (Liofilchem company, Italy). For quality control purposes, positive and negative controls, supplemented by the manufacturer, were run along with each test. Data were analyzed using SPSS (version 15.0).

3. Results

Initially we tested a portion of serum samples (n=123) for the presence of EMAs, and we found four positive samples only and the rest (n=119) were negative, which indicates that 3.25% of all patients suffer from CD. The next step was testing another portion of serum samples for the presence of ASMA where 88 samples (including the four EMAs positive samples) were negative, while 35 samples showed positive results for ASMA.

The third step was testing of serum samples for (tTG IgG and IgA) antibodies. Again the four the EMAs positive samples which were negative for ASMA showed positive results for (tTG IgG and IgA) antibodies. In addition another 11 samples out of the 35 ASMA positive samples showed positive results for (tTG IgG and IgA) antibodies (Table 1) with a total of 15 samples. This means that the percentage of CD among chronic diarrheic children jumped to 15 of 123 (12.2%).

We tested all positive (tTG IgG and IgA) antibodies samples (n=15) for total IgA. Ten samples showed normal results while five samples were deficient in total IgA. When these samples (n=5) were tested for (tTG IgA), only 2 samples were positive while the other three were negative. These were positive for (tTG IgG) only (Table 2).

Table 1. Results of three Immunological Tests

Test (n=123)	Results			
	No. of positive cases	Percent	No. of negative cases	percent
EMAs	4	3.25	119	96.75
ASMAs	35	28.5	88	71.5
(tTG IgG IgA)	15	12.2	108	87.8

EMAs: Anti-endomysial antibodies; ASMAs: Anti-smooth muscle antibodies
(tTG IgG and IgA): Tissue-transglutaminase antibodies

Table 2. Results of Total IgA

Test (n=15)	Normal IgA N (%)	Deficient IgA N (%)
TTG, IgA, n=5	10 (66.7)	5 (33.3)
TTG, IgG n=3	2 (40)	3 (60)
		3 (100)

4. Discussion

The aim of this study is to screen and estimate the occurrence of CD among children suffering from chronic diarrhea in Gaza Strip. As children should not be exposed to invasive techniques such as biopsy, this study focuses on adoption of highly sensitive and specific immunological test for diagnosis of CD. In our work we were looking for two different types of antibodies: anti-gluten and anti-self antibodies. The anti-gluten antibodies are the anti-gliadin IgG and IgA in addition to anti-partially deaminated gliadin peptides [11,12]. The anti-self antibodies include EMAs IgA and tTG IgA [13,14].

In the present study, only four serum samples showed positive results for EMAs i.e the occurrence was 3.25%. However, the clinical presentation and challenge tests suggested that more children suffer from CD. This was confirmed subsequently when 35 (28.5%) serum samples were positive for ASMA. It was evident that high titer of ASMA results in false negative EMAs, other researchers reported this finding [15].

The masking effect of ASMA might be due to fluorescence masking as the high intensity of ASMA test prevents EMAs color to be interpreted, another explanation is related to the high titer of ASMA antibodies which may prevent the EMAs antibodies from reaching the binding sites of endomysial tissue. However, when diluting the ASMA antibodies, the EMAs will bind endomysial epitopes and yields positive results.

Eleven serum samples were positive for (tTG IgG and IgA) in addition to the four EMAs positive samples. So the occurrence of CD increased to 12.2%. This result is lower than another reported finding which revealed an occurrence of 15% [14]. This difference might be explained on the basis of the characteristics of each population and how the environment affects the expression of specific genes. Anyhow, the percentage of CD in our study (12.2%) is not low, an important factor may be due to consanguinity in which the DQ2 and DQ8 genes pass to the siblings and increase the risk of CD. All samples which were positive for (tTG IgG and IgA) were subsequently examined for total IgA. Ten samples showed normal results, while the remaining five samples were deficient in total IgA (33.3%). It seems that total IgA deficiency is found more in patients suffering from CD when compared to healthy individuals [16]. In those patients, however, it is recommended to test for tTG IgG [16,17].

Five positive (tTG IgG and IgA) CD patients were randomly selected and sent to histopathology laboratory at Al Shifa Hospital for biopsy to confirm the immunological results. All of them were consistent with CD. The result is similar to other findings [18]. However, emphasis of this study was on non-invasive techniques. One of the limitations of this study is the limited service of histopathology laboratory in our area.

In light of this study, for diagnosis of CD, it is

recommended to start with non-invasive test; (tTG IgG and IgA) as this test is specific and sensitive. However, if histopathology service is available, it is recommended to confirm diagnosis of CD using histopathological examinations as this is the golden standard for diagnosis of CD. Introduction of genetic analysis for CD is recommended for persons who are at increased risk.

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