Serological detection of celiac disease among children in Diyala province- Iraq

Assis. Prof. Dr. Abulrazak SH. Hasan, Collge of Veterinary Med., Diyala University
Prof. Dr. Nadhim Gh. Noaman, College of Medicine, Diyala University
Assis. Prof. Dr. Mehdi SH. Al-Zuheiry, College of Medicine, Diyala University
Shahad KH. Al-Qaisi, College of Education, Diyala University

Abstract:

Background: celiac disease (CD) is an autoimmune disorder that is common in the general population. Early serological diagnosis of CD access to treatment and improves the patient's quality of life.

Objectives: to explore the rate of CD among clinically suspected children in using serological tests and to assess the validity of these markers for the diagnosis of CD.

Materials and methods:

This case control study was conducted in Diyala province-Iraq during the period from September 2011 to April 2012 in Al-Batool Teaching Hospital for Maternity and Children. 156 children who were clinically suspected as having CD and 124 healthy children as control group were enrolled. The patient's age range was 1 month to 6 years and above. Information regarding age, sex, residence, family history, and clinical signs were collected in a special questionnaire. Commercially available serological kits for anti-gliadin IgA (AGA-IgA) and anti-tissue transglutaminase IgA (anti-tTG-IgA) antibodies (Aeskulisa, Germany) were used by ELISA technique. Data were statistically analyzed and P value less than 0.05 was considered significant.

Results:

Based on the seropositivity of both anti-AGA IgA and anti-tTG IgA, 15 (9.6%) were considered CD patients. whereas, patients who had either anti-AGA IgA (16.7%) or anti-tTG IgA (14.7%) positive were considered as symptomatic non-CD patients. The results showed that the anti-AGA IgA seropositivity was highly significant ($P< 0.001$) in CD patients compared to symptomatic non-CD patients and control groups. Likewise, the anti-tTG IgA positivity was highly significant ($P< 0.001$) in CD patients compared to symptomatic non-CD patients and control groups. Both tests had similar sensitivity, but the anti-tTG IgA has higher specificity, accuracy, and positive predictive value.

Conclusion:

Serum Anti-tTG IgA is a good marker for the diagnosis of CD among clinically suspected patients; however, for more accuracy both anti-tTG IgA and anti-AGA IgA can be used.

Keywords: celiac disease, anti-glutin, anti-transglutaminase

Introduction:

Celiac disease (CD) is regarded as an autoimmune disorder in genetically susceptible individuals, triggered by gluten and related prolamins and characterized by severe malabsorption and flat intestinal mucosa [1]. CD is one of the best-known autoimmune human leukocyte antigen-dependent disorders, that has a relatively increased prevalence in first-degree relatives [2,3].

The identification of CD is challenging because it can begin not only with diarrhea and weight loss but also with atypical gastrointestinal and extra-intestinal symptoms, or it could be completely symptomless [4,5]. The accuracy of serological diagnosis of CD has progressively increased with the development of highly reliable tests. Serum antibody assays may serve as a first-step diagnostic tool: immunoglobulin A tissue transglutaminase (IgA tTG), IgA endomysial antibody (IgA EMA), IgA antigliadin antibody (IgA AGA), and IgG antigliadin antibody (IgG AGA) offers the best diagnostic accuracy regardless the patient's age. [6-8]. These tests usually have high sensitivity and specificity exceeding 95% with no evidence that a combination of tests was better than a single test using either the EMA IgA or tTG IgA. [9,10]. However, the positivity of these markers does not always correlate with mucosal appearance in the small intestine [4,11].

Following the application of simple serological tests for the diagnosis of CD, the prevalence of CD in Middle East, North Africa and India countries among low risk populations was found to be similar to that of Western countries, but it was higher in high risk populations ranging between 3 and 20% [12-15]. Thus, it has been suggested that in the developing countries, both serological screening in the general population and serological testing in groups at risk are necessary for an early identification of celiac patients [16,17].

Material and methods:

This case control study was conducted in Diyala province-Iraq during the period from September 2011 to April 2012 in Al-Batool Teaching Hospital for Maternity and Children. 156 children who were clinically suspected as having CD and 124 apparently healthy children as control group were enrolled. The patient's age range was 1 month to 6 years and above. Sociodemographic data including age, sex, residence, family history, and clinical signs were collected in a special questionnaire. For human privacy, the patient parent's consensus was taken. Commercially available serological kits for anti-gliadin IgA (AGA-IgA) and anti- tissue transglutaminase IgA (anti-tTG-IgA) antibodies (Aeskulisa, Germany) were used by Enzyme-Linked Immunosorbant Assay (ELISA) technique following the manufacturer's instructions. Statistical analysis was done through the computerized software, Statistical Package Social

Sciences (SPSS) version 20 by using the Chi-square. P value less than 0.05 was considered significant.

Results:

For statistical comparison, children who had positive serum anti-AGA-IgA and anti-tTG-IgA were considered CD patients, and those who had one of these markers positive were considered as symptomatic non-CD patients. Table (1) showed the range, mean and standard deviation of the study groups. The statistical analyses showed that there were insignificant differences among the study groups.

Table (1) Range, mean and standard deviation among the study groups.

Age (ys)	Healthy control (n= 124)	Symptomatic Non-CD (n= 141)	Celiac disease (n= 15)	P value
Range	(0.1-7)	(0.1-14)	(0.1-12)	0.08 [NS]
Mean	1.6	3.2	4.0	
SD	1.99	3.81	3.78	
SE	0.41	0.32	0.98	

[NS]: insignificant

Results showed that the overall anti-AGA IgA seropositivity rate in suspected patients group was 16.7% , which was significantly higher compared to control group (P= 0.031). Furthermore, the anti-AGA IgA seropositivity was highly significant (P< 0.001) in CD patients compared to symptomatic non-CD patients and control groups, table (2).

Table (2): Antigliadin IgA seropositivity rate among study groups.

Study groups	**Anti-gliadin IgA**			**P value**
	No.	**Positive**	**%**	
Control group	**124**	**0**	**0.0**	**0.031**
Suspected patients group	**156**	**26**	**16.7**	
Control group	**24**	**0**	**0.0**	**< 0.001**
symptomatic patients non-CD	**141**	**11**	**7.8**	
Celiac patients group	**15**	**15**	**100.0**	

Table (3) showed that the median serum concentration of anti-AGA IgA in CD patients was highly significant (P< 0.001) when compared to both symptomatic non-CD patients and control groups, while there was insignificant difference (P= 0.87) between the symptomatic non-CD patients and control group.

Table (3): concentration of anti-gliadin IgA among study groups.

◌Anti-gliadin Concentration	Healthy control (n= 124)	Symptomatic Non-CD (n= 141)	Celiac disease (n= 15)	P value
Range	(0.095-5)	(0.07- 88)	(12-300)	< 0.001
Median	2.03	2.10	32.0	
Interquartile range	(1.6-2.65)	(1-3)	(15-52)	
Mean rank	20.1	15.0	32.0	

The overall seropositivity rate of anti-tTG IgA in suspected patients group was 14.7%. The results revealed that there was insignificant difference (P=0.16) between the suspected and control groups, but it was highly significant (P< 0.001) in CD patients compared to symptomatic non-CD patients and control groups, table (4).

Table (4): Anti-tTG IgA positivity rate among study group.

Study groups	**Anti-transglutaminase IgA**			**P value**
	No.	**Positive**	**%**	
Control group	**124**	**5**	**4.8**	**0.16 [NS]**
Suspected patients group	**156**	**23**	**14.7**	
Control group	**24**	**1**	**4.2**	**< 0.001**
Symptomatic patients (not celiac)	**141**	**8**	**5.7**	
Celiac patients group	**15**	**15**	**100.0**	

The median concentration of anti-tTG IgA in CD patients was highly significant (P < 0.001) when compared to control and symptomatic non-CD patients group, whereas there was insignificant difference between the symptomatic non-CD patients and control group (P= 0.24), table (5).

Table (5): concentration of anti-transglutaminase IgA among study groups.

◌Anti-transglutaminase	Healthy control (n= 124)	Symptomatic Not celiac (n= 141)	Celiac disease (n= 15)	P value
Range	(1-21)	(0.1 -304)	(20-302)	< 0.001
Median	2.50	2.80	150.0	
Interquartile range	(2.25-3.4)	(2.3-4.8)	(26-300)	
Mean rank	72.5	85.2	169	

Table (6) revealed that the anti-AGA IgA and anti-tTG IgA were equal in their sensitivity, but the anti-tTG IgA test had higher specificity, accuracy, and positive PV.

Table (6): Validity of anti-AGA IgA and anti-tTA IgA.

Test	Sensitivity	Specificity	Accuracy	Positive PV		Negative PV
				50%	90%	100%
Anti-AGA IgA	100.0	92.2	92.9	92.8	99.1	100.0
Anti-tTGIgA	100.0	94.3	94.9	94.6	99.4	100.0

Discussion:

The present study is the first one in Diyala province, and one of the few studies on CD in Iraq. It was designed to explore the CD among clinically suspected children depending on serological markers, the Anti-AGA IgA and Anti-tTG IgA and to assess the validity of these tests for the diagnosis of CD. It had been documented that the correct diagnosis of CD in environmentally deprived children is frequently hindered by the common presence of other causes for the classical CD symptoms; malnutrition, failure to thrive and frequent diarrhea [4,18]. In this regard, malnutrition was well documented among Iraqi children as consequences of long term wars and deterioration of infrastructures beside the high prevalence of diarrhea causing diseases [19].

In the present study, 9.6% of the clinically suspected children had both anti-AGA IgA and anti-tTG IgA antibodies positive, while 16.7% had anti-AGA IgA positive and 14.7% had anti-tTG IgA positive. These results clearly revealed that CD in our region is within the range in the Middle East, India and North Africa [12,14,15, 18, 20] . These results can be explained on the fact that wheat has been the major staple food in these regions for a long time and it is possible that the continuous and high level of exposure to wheat proteins has induced some degree of immune tolerance, leading to milder symptoms, which are misdiagnosed as irritable bowel syndrome or unexplained gastrointestinal disorders. Of note, there was accumulating evidence of substantial underdiagnosis of CD in health care settings, where several studies had suggested that as few as a quarter of patients are recognized [21-22].

Our results also document that the combination of anti-AGA IgA and anti-tTG IgA tests represent a good diagnostic tool to confirm CD in suspected children, as these tests are manifested by high sensitivity, specificity, accuracy, and positive predictive values. Similar results were obtained by others [14,23,24]. Over the past decade, the diagnostic accuracy of serology for CD has markedly increased with the development of highly sensitive and specific tests, which were found to be an important screening tools for detection of latent CD, as these markers has the ability to discover a high number of silent CD, which can be classified as potential CD [10,11, 25]. Thus, a mass screening studies using serological markers is recommended, as the early detection of these patients would result in significant improvement of quality of their lives [2].

The study concluded that CD in our region is more prevalent than expected, and may well be under diagnosed, the combination of of serum anti-AGA IgA and anti-tTG IgA tests are a good diagnostic markers to confirm CD in suspected cases.

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