Original Article

Findings in Celiac Disease

Diagnostic Accuracy of IgA Anti-Tissue Transglutaminase Antibodies in Comparison with Histopathological Findings in Celiac Disease in Pakistan

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ABSTRACT

Objective: The objective of this study was to assess the diagnostic accuracy of most widely used serological test for diagnosis of celiac disease (CD) i.e. anti-tissue transglutaminase antibody (IgA) in comparison to histopathological lesions in CD.

Study Design: cross sectional study

Place and Duration of Study: This study was carried out at the Departments of Gastroenterology and Pathology of Fatima Memorial Hospital, Shadman, Lahore from March 2014 to October 2014.

Materials and Methods: 121 patients clinically suspected of celiac disease were included in this cross sectional study. The biopsy was taken from the second part of duodenum and was evaluated according to Marsh classification of CD. Blood sample of every patient was obtained to perform anti-tTG antibody test.

Results: The range of the patients included in the study came out to be 18-65 years with 30.24 years as mean age. Out of all the patients included in this study 34 (28.1%) were males and 87(71.9%) were females. The overall sensitivity and specificity of anti-tTG were 78.6% and 98.1%.The positive predictive value (PPV) and negative predictive value (NPV) came out to be 84.6% and 97.2% respectively.

Conclusion: We have come to the conclusion that currently there is no serological test which can be used as a sole tool for the diagnosis of celiac disease. Relying on serological test will lead to missed diagnosis of CD especially those patients which have Marsh lesions of lesser degrees.

Key Words: Celiac Disease, Anti-Tissue Transglutaminase Antibody, Sensitivity, Duodenal Biopsy.

INTRODUCTION

Celiac disease (CD) also called as gluten-sensitive enteropathy is an autoimmune disease triggered by gluten, affects small intestine in genetically susceptible children and adults. It is the only immune-mediated disease which is fully treatable only when a precise diagnosis is established. Gluten is a protein present in wheat, barley and rye etc. It is mainly composed of gliadin and glutenin (Catassi and Fasano, 2010).[1]

The prevalence of CD is becoming significantly higher than that recognized 20 years ago. The prevalence of celiac disease at global level is considered to be 1% (Mustalhati et al, 2010)[2]. According to a study the prevalence of CD varies from 2-13% (van der Windt, 2010)[3].

Scientists have found a strong linkage between presence of human leukocyte antigen (HLA) DQ2 or DQ8 and celiac disease. HLA-DQ typing can be used in ruling out the celiac disease. On the other hand presence of DQ2 or DQ8 does not exhibit the presence of disease as these genes are present in general population as well (Kapitani, 2006)[4].

The parameters to diagnose CD have significantly changed over the last 50 years. Diarrhea and malabsorption once thought to be major mode of presentation of celiac disease are becoming less common (Reily, 2012)[5]. Over time many specific and sensitive serological tests were introduced to make the diagnosis of CD less invasive process. Anti-gliadin antibody (AGA is among the first immunological assays used for screening CD (Fasano and Carlo, 2001)[6].

AGA is also found in diseases like rheumatoid arthritis and depression among elderly population which adds to its poor specificity (Anitta and Katri, 2012)[7]. Later these serological tests have been replaced by more sensitive and specific tests including antiendomysial (EMA), antireticulin (RA) and anti tissue transglutaminase (tTG) antibodies (Shinjini and Nitya, 2006)[8]. The major breakthrough in the shape of the discovery of anti-tTG as the autoantigen recognized by the EMA led to the development of ELISA based assays. These assays were projected at the detection of anti-tissue transglutaminase (anti-tTG) antibody (Dieterich et al, 1997)[9].

The discrepancy associated with anti-EMA and anti-tTG includes their unreliability in children of less than 2 years and IgA dependence (Wang et al, 2014)[10]. The sensitivity of AGA was better than that of anti-EMA and anti-tTGA in children aged less than 2 years (Mankai et al, 2005)[11].

Anti-tTG antibody test is routinely used as the first choice because of its high sensitivity, cost-effectiveness and easy interpretation. However discrepancy of tTG assays is their variable accuracy among manufacturers (Giersiepen et al., 2012[12], & Astrid and Juri, 2013)[13].

The role of pathologist in the diagnosis of celiac disease is of utmost significance. For the histopathological examination scientists have agreed that biopsy for the diagnosis of CD should be taken from 2nd part of duodenum. Marsh was the first scientist who explained the broad spectrum of inflammatory and structural changes which took place in CD. That is why Marsh classification became very popular. Later on Marsh classification was modified by Oberhuber (Oberhuber, 1999)[14].

Once a patient is diagnosed with CD, adherence to gluten free diet (GFD) for life is the only treatment available currently as it leads to complete recovery of the patient (Akobeng and Thomas, 2008)[15]. The patients of CD are suffering from the deficiency of many minerals and vitamins including Iron, copper, zinc, B12, B 6, folic acid and vitamin D which leads to increased risk of fractures. (Rubio et al, 2013)[16].

The risk of premature and low weight infant births, abortions and infertility rises in women suffering from CD. The treatment of CD decreases these risks (Jamot, 2011)[17]. Researchers have shown that those patients who did not get treatment for CD have revealed considerably lower bone mineral density (Mora, 2001)[18].

The celiac disease patients are rarely diagnosed in Pakistan because of two main reasons. Firstly physicians are unaware if its existence and its clinical presentation. Secondly no proper protocol is present to successfully diagnose this disease. Mostly anti-tTG antibody test is used to diagnose or exclude diagnosis of this disease. In addition to this there is no data available about prevalence rate of CD in Pakistan and sensitivity of anti-tTG antibody test. In this study we evaluated the diagnostic accuracy of anti-tTG antibody test in comparison to histopathological lesions according to Marsh classification of CD in order to draft a proper approach to diagnose this disease.

MATERIALS AND METHODS

This cross sectional study was conducted at the departments of gastroenterology and pathology of Fatima Memorial Hospital, Shadman, Lahore, Pakistan from March 2014 to October 2014. In this research, 121 patients were recruited according to our inclusion and exclusion criteria. We included patients from both gender from age 5 to 60 years. These patients were clinically diagnosed for CD. The clinical diagnosis included typical clinical presentation including diarrhea, weight loss, fatigue, iron deficiency anemia and also atypical clinical presentation including non specific GI symptoms for a long duration like abdominal pain, abdominal bloating, short stature and constipation etc. We excluded patients with any other known disease (comorbidity).

All the patients after receiving the oral and written explanation of the whole research study signed the informed consent form. This study protocol was approved by ethics committee.

Four to five biopsies were taken with sterilized forceps from the second part of the duodenum in all patients through endoscopy for histopathological examination. At the same time 5ml blood sample was taken from every patient for serological evaluation. The results of intestinal biopsy were considered as gold standard of our research. The age, gender and complete clinical history of every patient were documented.

RESULTS

Biopsy specimens were kept in labeled and separate collection jars. After fixation in buffered formalin the biopsy specimens were embedded in paraffin wax. The thickness of the sections was kept at standard 3µm.These were stained with hematoxylin and eosin and slides were prepared. The slides were evaluated by expert pathologists who were blinded to the serology results. The number of intraepithelial lymphocytes, crypt hyperplasia and villous atrophy were documented according to modified Marsh classification (Table 1).

Table No.1 Modified Marsh Classification

Marsh Type	*IEL/100 Enterocytes (Duodenum)	Crypt Hyperplasia	Atrophy of Villi
0	<30	Normal	Normal
1	>30	Normal	Normal
2	>30	Increased	Normal
3A	>30	Increased	Mild
3B	>30	Increased	Moderate
3C	>30	Increased	Total

(*Intraepithelial Lymphocytes (IELs) Per 100 Enetrocytes)

According to patient's history and mode of presentation other diseases which cause duodenal damage e.g. Giardia lambia infection, food protein hypersensitivity were also considered.

Serum Analysis: The serum analysis was performed in a laboratory with hundreds of routine samples. The laboratory staff neither knew biopsy results nor clinical presentation of the patient. The serology test was performed on each blood sample with following method through commercial kit in accordance with

guidelines provided by the manufacturer. For IgA tissue Transglutaminase, kit by IBL international, Hamburg, Germany was used. Those patients who were diagnosed as celiac patients on biopsy were also tested for total serum IgA level to rule out deficiency of IgA.

Transglutaminase IgA ELISA: Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The wells are coated with antigen. Specific antibodies of the sample binding to the antigen coated wells are detected by a secondary enzyme conjugated antibody (E-Ab) specific for human IgA. After the substrate reaction the intensity of the color developed is proportional to the amount of IgA-specific antibodies detected. The values less than 8u/mL were interpreted as negative. The values more than 12u/mL were considered as positive whereas values from 8 to 12 were termed as equivocal as per the manufacturer's guidelines.

Data Analysis Procedure: The collected data was analyzed through SPSS version 16; study variable were the age, gender, celiac disease for serology and histopathology. Mean ± standard deviation such as age of the patient, frequency and percentage were calculated. Sensitivity, specificity, positive predictive value (PPV), negative predictive value(NPV) were determined by taking histopathology as gold standard from 2 x2 table (Table 2).

Table No.2: 2 x 2 table Anti-tTG (CD) Antibody test

	Histopathology (CD)		
		+	–
Anti-tTG (CD)	+	a	B
	–	c	D

Sensitivity of serology = (a/a+c)x 100, Specificity of serology = (d/b+d)x 100, Positive predictive value (PPV) for serology = (a/a=b) x100, Negative predictive value (NPV) for serology = (d/c+d)x100, Accuracy of serology = (d + a)/overall patients x 100

a=True positive, b=False positive, c=False negative, d=True negative

Among CD patients 78.6% (11/14) tested positive for anti-tTG and 21.4 % (3/14) were negative for anti-tTG antibody test. On the other hand 98.1% (105/121) of non-CD were negative and 1.9 % (1/121) came out to be positive for antibody. The overall sensitivity and specificity of anti-tTG antibody were 78.6% and 98.1%. The PPV and NPV came out to be 84.6% and 97.2% respectively.

Six patients exhibited Marsh 3C lesions (total villous atrophy), five patients showed Marsh 3B lesions (moderate villous atrophy) and three patients had Marsh 3A(mild villous atrophy) in small intestine out of fourteen patients diagnosed for celiac disease on intestinal biopsy.

Significantly the sensitivity of anti-tTG antibody test for Marsh IIIA, IIIB and IIIC was 66.6%, 60% and 100% respectively.

Table No.3: Celiac Disease on anti-tTG antibody Test

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Positive	13	10.7	10.7	10.7
	Negative	108	89.3	89.3	100.0
	Total	121	100.0	100.0	

Table No.4: Celiac Disease on Histopathology

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Positive	14	11.6	11.6	11.6
	Negative	107	88.4	88.4	100.0
	Total	121	100.0	100.0	

Table No.5: Celiac Disease on anti-tTG antibody * Celiac Disease on Histopathology Crosstabulation Count

		Celiac Disease on Histopathology		Total
		Positive	Negative	
Celiac Disease on anti-tTG	Positive	11	2	13
	Negative	3	105	108
Total		14	107	121

Table No.6: Celiac Disease on Histopathology * Celiac Disease on anti-tTG antibody Crosstabulation

			Celiac Disease on anti-tTG antibody		Total
			Positive	Negative	
Celiac Disease on Histopathology	Positive	Count	11	3	14
		% within Celiac Disease on Histopathology (Sensitivity)	78.6%	21.4%	100.0%
		% within Celiac Disease on tTG (PPV)	84.6%	2.8%	11.6%
	Negative	Count	2	105	107
		% within Celiac Disease on Histopathology (Specificity)	1.9%	98.1%	100.0%
		% within Celiac Disease on tTG (NPV)	15.4%	97.2%	88.4%
Total		Count	13	108	121
		% within Celiac Disease on Histopathology	10.7%	89.3%	100.0%
		% within Celiac Disease on TTG	100.0%	100.0%	100.0%

DISCUSSION

Our study revealed that the sensitivity of the serum IgA anti-tTG was 78.6%%. The specificity was reported to be 98.1% while PPV and NPV were 84.6% and 97.3% respectively. The diagnostic accuracy came out to be 96.7%. The sensitivity of IgA anti-tTG antibody in our study is substantially higher than the value of 38% documented by Emami M et al in their study conducted in Iran (Emami et al., 2008)1[9?]. A research conducted in USA revealed overall sensitivity of anti-tTG antibody

test at 70.6%, whereas over specificity was found to be 65.0 % (Abrams et al., 2006)[20]. In our study sensitivity of anti-tTG antibody test was 60% for partial villous atrophy which is higher than the 42.3% sensitivity reported by Abram et al. (2006) and 36.8% documented by Emami et al (2008)[19]. According to our research sensitivity of anti-tTG antibody test for total villous atrophy (Mrash III C lesion) was 100% which is again higher than 90.0% sensitivity reported by Abram J et al. for patients with total villous atrophy (Abrams et al., 2006)[20].

Celiac disease can be diagnosed correctly even if the physicians are aware of the several ways in which it is presented. Despite the fact that prevalence of CD is increasing all over the world, still physicians in Pakistan have not made their minds to include this new endemic disease even in their differential diagnosis. It would not be wrong to state that in reality CD is already wide-spread because of wheat consumption but is either misdiagnosed or undiagnosed. On one hand it is very important that physicians learn to recognize signs and symptoms of CD while on the other hand it is equally necessary that pathologist must recognize mild categories of CD including 1, 2 and 3a.

CONCLUSION

We conclude that there is no single serological test with 100% sensitivity and specificity therefore biopsy remains the gold standard for the diagnosis of this disease. On this antibody test many patients were reported as false negatives and also false positives. The sensitivity of anti-tTG antibody for lesser degree of Marsh lesion is considerably low at 60% as compared to 100% for Marsh 3C (total villous atrophy). It reveals that a significant proportion of patients suffering from celiac disease having mild to moderate degree of lesions will remain undiagnosed if only anti-tTG antibody test is used for diagnosis. Therefore it is recommended that when there is persistence of signs and symptoms of celiac disease and patient is reported seronegative for antibody test, still an intestinal biopsy is necessary for making or ruling out the diagnosis. The challenge of diagnosis of celiac disease can be fulfilled through good communication between the pathologist and the clinician. The pathologist's report should concisely highlight the histopathological findings which must be correlated with clinical presentation and serological results.

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