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SHORT COMMUNICATION

Diagnostic Accuracy of Anti-Endomysial Antibody in Celiac Disease

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Abstract

The objective of the study was to determine the diagnostic accuracy of anti-EMA antibody in comparison to histopathological findings in patients suspected of CD. This cross-sectional study was conducted at Gastroenterology Department, Fatima Memorial Hospital, Lahore, from March to October 2014. One hundred and twenty-one patients aged between 5 - 60 years of either gender were recruited in the study. Every patient went through serological testing and biopsy specimens were obtained from second part of the duodenum. Histopathological evaluation was done according to the Modified Marsh classification. The overall sensitivity of anti-EMA came out to be 85.7% which varied with the histological lesions being 75.0%, 83.3%, and 100% for Marsh IIIA, IIIB and IIIC, respectively. Although anti-EMA has high sensitivity but serological tests as a sole mean of diagnosis are currently unable to replace the biopsy.

Key Words: *Celiac disease. Anti-endomysial antibody. Duodenal biopsy. Marsh classification.*

Celiac disease (CD) is precipitated by consuming food which contains gluten in genetically susceptible individuals. Its diagnosis still remains challenging. Its prevalence varies from 2 to 13%.[1] The combination of environmental, genetic factors, and immunological mechanisms is involved in activation and progression of celiac disease.[2] Serological tests for diagnosing CD include antigliadin antibody (AGA), anti-tissue transglutaminase (anti-tTG) antibody, and anti-endomysial antibody (anti-EMA).[3,4] However, in children less than 2 years of age the performance of AGA is far better than anti-EMA and anti-tTG antibodies.[5,6] Anti- EMA and anti-tTG are considered as the serological tests of choice these days in adults as they are more sensitive. Tissue biopsy is regarded as gold standard of diagnosing CD.[7] In Pakistan, scanty data is available on celiac disease and its criteria for diagnosis. This study was aimed to determine the diagnostic accuracy of anti-EMA antibody test in comparison to histopathological findings graded according to Modified Marsh classification in patients suspected of celiac disease.

It was a cross-sectional study carried out from March to October 2014 in the Department of Gastroenterology, Fatima Memorial Hospital, Shadman, Lahore, Pakistan. The sample included 121 patients of either gender, ages ranging from 5 to 60 years coming to the outpatient department and clinically suspected of celiac disease. Extremes of ages, having any other comorbid conditions etc. and previously diagnosed celiac patients, were excluded from the study. Formal consent from each patient or the guardian, in case of children, was taken before inclusion into the study. The whole study was performed according to Ethical Principles for Medical Research outlined in the Helsinki Declaration (revised in 2000). The study was approved by the Ethical Review Board of Khyber Medical University, Peshawar and Fatima Memorial Hospital, College of Medicine and Dentistry, Lahore.

Small bowel biopsies and blood samples were taken from these patients at the endoscopic unit of Department of Gastroenterology. Histopathological examination was conducted at the Hospital's Pathology Unit and serological tests, i.e. anti-EMA test and total IgA etc., were carried out in a community based laboratory. Data including age, gender, complete clinical history, and mode of presentation were collected. The serological test for anti-EMA was performed on each blood sample through commercial kit in accordance with guidelines provided by the manufacturer (D-Tek, Blue Well, Mons, Belgium).

For every case, duodenal biopsy samples were placed in clearly labelled, separate specimen collection jars. Duodenal biopsy specimens were fixed in buffered formalin and embedded in paraffin wax. Standard 3 - 5 µm thick sections were stained with hematoxylin and eosin and the slides were examined by two independent pathologists blinded to the serology reports of those patients. No inter-observer variation was reported. Villous atrophy and crypt hyperplasia were documented according to the modified Marsh classification. CD3 marker was used for visualization of intra-epithelial lymphocytes (Table I).

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Table I: Modified Marsh classification.

Stages	IEL* per 100 enterocytes	Crypts	Villi
0	Less than 30	Normal	Normal
1	More than 30	Normal	Normal
2	More than 30	Increased	Normal
3 a	More than 30	Increased	Mild atrophy
b	More than 30	Increased	Marked atrophy
c	More than 30	Increased	Absent

**Intra-epithelial lymphocytes*

Table II: Celiac disease on anti-EMA versus histopathology-cross tabulation.

Celiac disease on anti-EMA	Celiac disease on histopathology		Total
	Positive	Negative	
Count	12	1	13
% of celiac disease on anti-EMA (sensitivity)	85.7%	14.3%	100.0%
Positive predictive value	92.3%		
Count	2	106	108
% of celiac disease on anti-EMA (specificity)	0.9%	99.1%	100.0%
Negative predictive value		98.1%	
Count	14	107	121

Sensitivity =85.7%, Specificity=99.1%, PPV=92.3, NPV=98.1%, Accuracy= 97.5%

Sample size was calculated as 121 patients, using WHO formula, with a prevalence rate of 13%, margin of error 6% and a confidence interval of 95%. Study variables were the age, gender, serology and histopathology for celiac disease. Mean ± standard deviation for the age of patients, frequency and percentage were calculated. Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) were determined by taking histopathology as gold standard. Data was analyzed by using SPSS software version 16.

The mean age of patients was 30.24 ±9.00 years. Eighty-seven (71.9%) patients were females and 34 (28.1%) were males. No patient was found to have IgA deficiency. The frequency of CD in this study was 11.6% (14/121) on histopathology. The total patients positive for celiac disease on anti-EMA test were 13 (true positives 12, false positive 1) and 108 patients were negative for anti-EMA test (true negatives 106, false negatives 2, Table II). Among the CD patients 85.7% (12/14) tested positive for anti-EMA and 14% (2/14) yielded negative results for anti-EMA. On the other hand 99.1% (106/107) of non-CD were negative and 0.9% (1/107) came out to be positive for anti-EMA. The overall sensitivity and specificity of anti-EMA were 85.7% and 99.1%. The PPV and NPV came out to be 92.3% and 98.1%, respectively (Table II). Out of 14 patients diagnosed for CD on intestinal biopsy, 3 patients showed Marsh IIIA, 5 patients exhibited Marsh IIIB, and 6 patients had Marsh IIIC lesions. The sensitivity of anti-EMA antibody for Marsh IIIA, IIIB and IIIC was 75.0%, 83.3% and 100%, respectively.

As compared to the other studies, the sensitivity of anti-EMA test for total villous atrophy (VA) in this study was 100% as reported by Rostami *et al*. but the sensitivity of anti-EMA test for partial VA in this study was 75.0% (Marsh IIIA) to 80 % (Marsh IIIB), which is considerably higher than the disappointing 31% (Marsh IIIA) as documented by them.[8] A similar study by Tarmure *et al*. concluded that anti-EMA had a lower sensitivity in patients with Marsh-I and Marsh II lesions.[9]

This is the first study conducted in Pakistan which determined the accuracy of anti-EMA test against histopathology in diagnosing CD. Studies done previously were mostly on clinical presentation of celiac disease and diagnosis through anti-tTG.[10] In Pakistan, even large established laboratories in cosmopolitan cities such as Lahore and Karachi are not providing the facilities of highly specific and sensitive IgA-EMA testing. Taking the diagnostic accuracy in consideration, it is highly recommended that it should be used along with anti-tTG antibody test.

It is concluded that the serological test as a sole mean of diagnosis is currently unable to replace the intestinal biopsy as its sensitivity varies with the grading of histological lesions. Therefore, when the symptoms of celiac disease persist and patient is reported seronegative for the antibody, still an intestinal biopsy is necessary to avoid missing the disease.

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