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The Predictive Value of Serological Markers in Diagnosis of Celiac Disease

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Abstract: ***Introduction:*** *Celiac disease is an autoimmune inflammatory disease of the small intestine that is precipitated by the ingestion of gluten, a component of wheat protein, in genetically susceptible persons. Serologic tests for antibodies against Endomysium, Reticulin, and Gliadin identify most patients with the disease. Early diagnosis and management are important to prevent serious sequelae of malabsorption, such as osteoporosis and anemia.* ***Aim of the study:*** *To compare the sensitivity, specificity, and predictive value of anti-reticulin and anti-gliadin antibodies according to anti-endomysium antibodies, in patients investigated for celiac disease antibodies.* ***Patients and methods:*** *Study population included 509 celiac disease patients (236 males & 273 females) who were referred to the clinical pathology department, from January till December 2015 for serological detection of auto-antibodies. Anti-Gliadin (IgG &IgA) measured by Enzyme Linked Immunosorbent Assay (ELISA), whereas Anti-Endomyseal IgA & Anti-Reticulin IgA were detected by indirect immunofluorescent technique.* ***Results:*** *Most the cases for celiac disease were reported in the age group (1-10) years as antibodies against Gliadin (IgG, IgA), Anti-Endomysial IgA antibodies and anti-Reticulin IgA were detected in 130 (66.7%),127 (65.8%), 39 (79.6%) and 49 (68.1%) respectively. Both anti-Gliadin IgA and anti Reticulin were detected significantly in females p values (0.047, 0.008) respectively. The study showed that anti-Gliadin IgA is the most sensitive according to anti Endomysial IgA and anti Reticulin was the most specific for screening of celiac disease. While Receiver Operating Characteristic (ROC) curve showed that anti-gliadin IgA had the best sensitivity and specificity according to anti Endomysium test results.* ***Conclusions:*** *The predictive values of the serological celiac disease tests showed that anti Gliadin IgA had the best sensitivity & specificity according to traditional anti Endomysium antibody test results.*

Keywords: Celiac disease, Endomysial Antibodies, Gliadin Antibodies, Reticulin antibodies

1. Introduction

Celiac disease (CD) is a multisystem autoimmune disease induced by gluten in wheat, barley and rye. It is characterized by polygenic predisposition, high prevalence (1%), widely heterogeneous expression and frequent association with other autoimmune diseases, selective deficit of IgA and Down, Turner and Williams syndrome. The basis of the disease and the key finding in its diagnostics is symptomatic or asymptomatic inflammation of the small intestinal mucosa which resolves by gluten-free diet. Therefore, the basis of the treatment involves elimination diet, so that the disorder, if timely recognized and adequately treated, it has an excellent prognosis (1).

Small bowel biopsy is always indicated when there is a high suspicion of celiac disease. It is reliable and technically straight forward by endoscopy, but relatively expensive, time consuming and unpleasant for patients. It is thus not appropriate for testing large numbers when the index of suspicion is low. There is therefore a need for a less invasive screening test to select patients for biopsy (2) Attempts to develop sensitive and specific serological tests to aid diagnosis started in 1958 when Berger described the anti Gliadin antibody AGA (Gliadin is the alcohol soluble fragment of gluten), which has been used clinically since the 1970s. Further antibodies have been discovered including anti Reticulin (ARA), and Endomysial antibody (EMA) (3).

CD is an inability of the body to process the protein gliadin properly which is a fraction of the gluten protein found in wheat and some other cereal grains. The body produces IgA and IgG antibodies to this protein. Both IgA and IgG anti-Gliadin antibodies (AGA) are detected in sera of patients with gluten enteropathy. A sensitive testing protocol includes testing for both IgA and IgG anti-Gliadin antibodies since a significant portion of celiac patients (approx. 2-5%) are IgA deficient. The Endomysium is the perivascular connective tissue which lines smooth muscle bundles, and which takes up silver stain. The commercially available tests for EMA detect IgA class autoantibody directed against the Endomysium in monkey esophagus by indirect immunofluorescence (IIF), as first described in1983. More recent work using human umbilical cord tissue as a substrate has shown improved sensitivity and correlation with villus atrophy. IgA class Reticulin antibodies were found only in 60% Celiac disease and 25% dermatitis herpetiformis (DH) patients (4).

2. Patients and methods

This study included 509 patients (236 males & 273 females) who were diagnosed at the internal medicine department and pediatric department to have celiac disease and were referred to the clinical pathology department for serological detection of the auto-antibodies from January till December 2015. Baseline data about patients were obtained from their history & clinical examination, a previously arranged questionnaire was used for this purpose. From each individual 5 ml of venous blood was collected and divided into several 0.5 ml aliquot and all frozen at -20 C° till used. AGA-IgG & IgA were measured by ELISA company (Eauskulisa-Germany),

whereas EMA IgA & ARA IgA were detected by IIF technique on monkey esophagus & kidney tissue respectively company (Euroimmun-Germany).

3. Results

The immunological celiac tests (AGA, AEA, and ARA) were mostly detected in the age group (1-10) years as shown in table (1).

Table 1: Distribution of celiac immunological tests according to Age groups (year)

Age groups (year)		Immunological tests (positive cases)			
		AGA IgA	AGA IgG	AEA IgA	ARA IgA
1 – 10	N	127	130	39	49
	%	65.8%	66.7%	79.6%	68.1%
11 – 20	N	49	45	6	22
	%	25.4%	23.1%	12.2%	30.6%
21 – 30	N	9	8	2	-
	%	4.7%	4.1%	4.1%	
31 – 40	N	8	10	1	1
	%	4.1%	5.1%	2.0%	1.4%
> 40	N	-	2	1	-
	%		1.0%	2.0%	
Total	N	193	195	49	72
	%	100.0%	100.0%	100.0%	100.0%

Table (2) shows that celiac disease tests were mostly detected in females than in males with significant p value for AGA & ARA (0.047 and 0.008) respectively.

Table 2: Distribution of celiac immunological tests according to gender

Gender		Immunological tests (positive cases)			
		AGA IgA	AGA IgG	AEA IgA	ARA IgA
Male	N	92	94	22	28
	%	47.7%	48.9%	44.9%	38.9%
Female	N	101	101	27	44
	%	52.3%	51.8%	55.1%	61.1%
Total	N	193	195	49	72
	%	100.0%	100.0%	100.0%	100.0%
P value		0.0475*	0.069	0.199	0.0088*

*Significant P value < 0.05.

To study validity of different tests (Gliadin & Reticulin) according to anti- Endomysial antibody test that is recognized as the best serological screening test for celiac disease, the specificity; the proportion of subjects without the disease who have a negative test that indicates how good a test is at identifying the non-diseased was measured as shown in table (3). The most specific test was anti Reticulin IgA Ab (92.45%) second anti Gliadin IgA (76.58%) last anti Gliadin Ab IgG (75.22%).

While sensitivity; the proportion of subjects with the disease who have a positive test that indicates how good a test is at identifying the disease. Both anti Gliadin antibodies IgA & IgG were the most sensitive for detection of CD with sensitivities (77.55%, 63.26%) respectively.

Table 3: The validity of anti Gliadin (IgG, IgA) & anti Reticulin Antibody according to Anti-Endomysial Antibody Test

Validity of the tests	AEA IgA		
	AGA IgA	AGA IgG	ARA IgA
Sensitivity %	77.55	63.26	44.89
Specificity %	76.58	75.22	92.45

To quantify the predictive performance of the tests according to anti Endomysial antibodies we employed receiver operating characteristic (ROC) curves (sensitivity versus 1 minus specificity), the area under the ROC curve (AUC) represent the false positive (healthy people with positive test for celiac disease), the best curve was that of anti Gliadin IgA as shown in figure (1) in comparison to Anti Gliadin IgG & anti Reticulin IgA Figures (2) & (3) respectively.

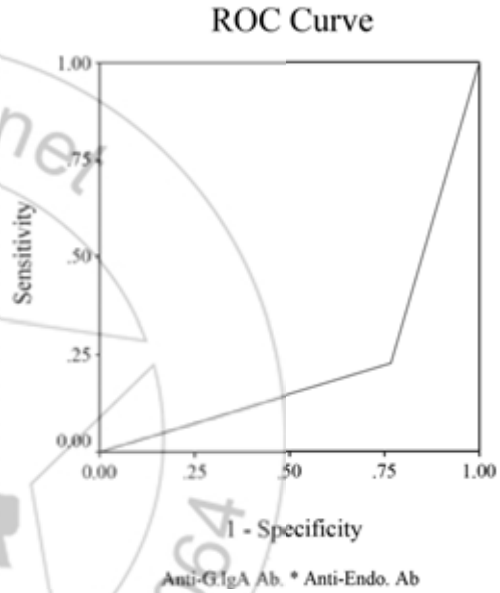

Figure 1: ROC curve for Anti-gliadin IgA.

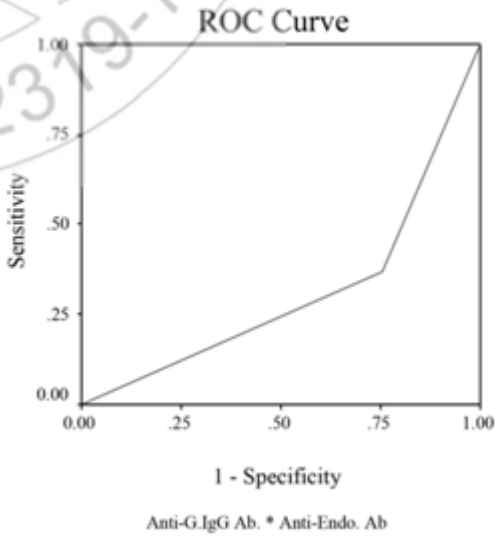

Figure 2: ROC curve for Anti-gliadin IgG.

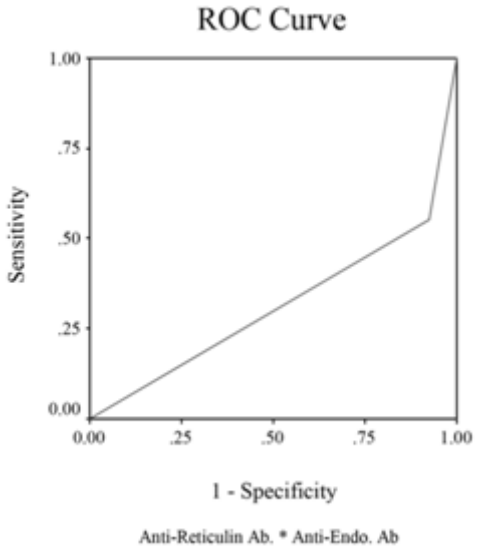

Figure 3: ROC curve for Anti-Reticulin antibody

4. Discussion

Celiac disease (CD) is a permanent intolerance to gluten that results in damage to the mucosa of the small intestine. This damage consists of mucosal inflammation and loss of absorptive surface area and is manifested by a broad spectrum of symptoms and nutritional deficiencies (4). For almost 30 years, intestinal biopsy has been the standard for the diagnosis of this disease. Although the mucosal damage is primarily cellular, untreated celiac disease is also associated with a humoral immune response that consists of both secreted intestinal and circulating serologic antibodies directed against the Reticulin and Endomysium of connective tissue, "Endomysial antibodies" (EMA)), is an indirect immunofluorescence assay that uses monkey smooth muscle esophagus as a substrate. Many variables may affect the test, including the light source, level of ambient light, training and experience of the operator, substrate used, and the initial screening dilution. Published results suggest that the Endomysial immunoglobulin A (IgA) indirect immunofluorescence assay is the most accurate test available, marker traditionally used for celiac disease and also the best serological marker of Gluten Sensitive Enteropathy in patients with dermatitis herpetiformis DH with a reported sensitivity of 95 to 100% and a specificity of 99 to 100% (5). Antibodies against various peptides derived predominantly from wheat, "anti-Gliadin antibodies" (AGAs) were variable for IgA and IgG antibodies, particularly for sensitivity; Whilst IgA class Anti Reticulin antibodies are found only in celiac disease & dermatitis herpetiformis (6).

The prevalence of celiac disease in childhood was higher than in adults in current study in agreement with a study done M marine et al (7) that showed celiac disease is significantly higher in children. Whether this difference is due to environmental factors influencing infancy, or latency of celiac disease in adulthood, remains to be demonstrated in prospective longitudinal studies (7). Nevertheless, differences in CD prevalence between studies may also be due to bias in the age and gender of individuals included. In fact, the predominance of CD in female patients is clearly established (8) and some studies have shown higher frequency of CD in infancy and adolescence than in adulthood.

This latter finding was unexpected in a disease considered long lasting and it remained unnoticed and not adequately demonstrated. If confirmed, important questions could be raised such as environmental factors (lifestyle, infections) affecting the youngest groups or the possibility of frequent evolution towards latency in CD detected by mass screening. The only way to demonstrate unequivocally the existence of gender – and age – related differences in CD prevalence is by performing a cross-sectional study in which the sample represents the structure of a reference population according to gender and age (9,10). The aim of this study was to collect and compare available information on the performance of diagnostic tests for detecting celiac disease.

On diagnosis, monitoring, and risk assessment of celiac disease, Setty et al (2008) (11) stated that currently, serological screening tests are utilized primarily to identify those individuals in need of a diagnostic endoscopic biopsy. The serum levels of immunoglobulin IgA Anti-Endomysium antibodies (EMA) are the first choice in screening for celiac disease, have close to 100 % specificity and a sensitivity of greater than 90 % therefore the validity of anti Gliadin & anti Reticulin antibodies were valid accordingly in diagnosis of celiac disease.

The sensitivity and specificity of AGA tests are known to vary particularly for sensitivity. Current study confirms the results of previous studies (12, 13) that indicated that the specificity of AGA-IgA and AGA-IgG tests does not approach that of the EMA test. AGA-IgA tests were more sensitive and more specific than AGA-IgG tests according to Anti-Endomysial Ab reported sensitivity for AGA IgA was (77.55%).

The ROC analyses for ARA & AGA were based on the supposition that IgA-EMA is a diagnostically significant reference marker of CD. The results for patients with positive IgA-EMA results were designated true positive, and those for patients with negative IgA-EMA results were designated true negative. By using different threshold values, the fraction of positive test results for the true-positive group was plotted against the fraction of positive test results for the true-negative group (1 − number of sample with true negative results). Thereafter, the area under the curve was calculated, and a suitable cutoff value was selected. The study showed the best (ROC) curve was that of Anti Gliadin IgA so it's the best reliable marker for diagnosis of celiac disease according to anti Endomysial antibodies in absence of total IgA deficiency in patients. This result agrees with a study done by Euroimmune laboratory organization in 2009 (14).

5. Conclusion

The basic criteria of standardization and quality assessment must be fulfilled by any given test protocol proposed for serologic investigation of celiac disease as serologic methods have been used widely to test for the disease and have gained importance in diagnostic definition and in new epidemiologic findings.

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