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Abstract

Serological test have an important role in disease diagnosis, anti-reticulin antibodies (ARA) and anti-gliadin antibody (AGA) have been evaluated as diagnostic and monitoring tests for celiac disease. The goal of this study was to compare between the diagnostic efficiency of two tests (anti-gliadin and anti-reticulin antibody) in different stages of celiac disease (CD) using ELISA technique, forty blood samples were collected from newly diagnosed patients and 20 samples of patients under gluten free diet (GFD) and 20 samples of control group was also included. All control samples were recorded negative results 20/20 for all tests included in this study therefore the specificity for all tests (IgA- anti reticulin, IgA anti gliadin, IgG- anti reticulin and IgG-anti gliadin) were 100%. While samples collected from patients were given 39/40, 35/40, 19/20 and 17/20 positive results for IgA- anti reticulin, IgA anti gliadin, IgG- anti reticulin and IgG anti gliadin, respectively, this result reflected the sensitivity 98.33%, 91.66%, 97.5% and 92.5%. Present study concluded that anti reticulin IgA and IgG more sufficient in celiac disease diagnosis.

Keywords

Celiac disease, reticulin, gliadin, sensitivity and specificity

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Evaluation of Diagnostic Efficiency of Anti-reticulin and Anti-gliadin antibody test in Celiac Disease

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ABSTRACT:

***S**erological test have an important role in disease diagnosis, anti-reticulin antibodies (ARA) and anti-gliadin antibody (AGA) have been evaluated as diagnostic and monitoring tests for celiac disease. The goal of this study was to compare between the diagnostic efficiency of two tests (anti-gliadin and anti-reticulin antibody) in different stages of celiac disease (CD) using ELISA technique, forty blood samples were collected from newly diagnosed patients and 20 samples of patients under gluten free diet (GFD) and 20 samples of control group was also included. All control samples were recorded negative results 20/20 for all tests included in this study therefore the specificity for all tests (IgA- anti reticulin, IgA anti gliadin, IgG- anti reticulin and IgG-anti gliadin) were 100%. While samples collected from patients were given 39/40, 35/40, 19/20 and 17/20 positive results for IgA- anti reticulin, IgA anti gliadin, IgG- anti reticulin and IgG anti gliadin, respectively, this result reflected the sensitivity 98.33%, 91.66%, 97.5% and 92.5%. Present study concluded that anti reticulin IgA and IgG more sufficient in celiac disease diagnosis.*

Keyword*: **Celiac disease, reticulin , gliadin, sensitivity and specificity***

INTRODUCTION

Celiac disease is an immune mediated enteropathy initiated by ingestion of gluten,in genetically susceptible individuals. It is characterized by lifelong intolerance to gluten which is a mixture of gliadin and related prolamines present in cereals such as wheat, barley and rye (Bhatnagar and Tandon, 2006). The correct diagnosis of celiac disease is essential as it requires lifelong adherence to gluten free diet (GFD). Early diagnosis and treatment has additional benefits because studies now suggest that delayed diagnosis is associated with increased prevalence of other autoimmune conditions, (Ventura *et al.*, 1999) mortality (Corrao *et al.*, 2001) and increased risk of osteoporosis and malignancies (Holmes *et al*., 1989). It is therefore imperative to have

standardized protocols using sensitive and specific tests that can confirm the diagnosis of celiac disease and identify individuals at risk (Bhatnagar and Tandon, 2006).

Gliadin from wheat is common food allergen that can induce baker's asthma wheat-dependent exercise- induce anaphylaxis, atopic dermatitis and celiac disease. The gliadin assay focuses on rapidly screen and check for gluten contamination in raw materials and in gluten free diet production process, not only for wheat sensitive patients but also for the industries producing gluten free foodstuff (Chu and Wen, 2013).

The antireticulin antibody (ARA) test was first introduced as a diagnostic test for CD in 1977 and is routinely detected by indirect immunofluorescence assay (IFA) on rat tissue (Eade *et al.*, 1977). These antibodies (IgG or IgA) are directed against the reticular fibers of endomysium, a layer of connective tissue which sheathes smooth muscle fibers. Five different patterns (R1 to R5) are associated withARAs; however, only the R1 type is associated with CD and dermatitis herpetiformis. To be considered positive, the characteristic R1 type staining pattern should be seen on three rodent tissues, namely, the liver, kidney, and stomach. There are several drawbacks to the ARA test, including multifaceted procedure, poor sensitivity due to the rodent substrate, and the inherent subjectivity associated with IFA-based testing (Lock *et al.*, 1999).

This study aimed to evaluate the diagnostic efficiency values: Sensitivity (Se%) ,Specificity(Sp%), Diagnostic Efficiency (De%),Positive Predictive Value (PPV%) and Negative Predictive Value (NPV%) of Anti – reticulin antibody test IgG & IgA and anti – gliadin antibody IgG & IgA in gluten free diet and newly diagnosed of celiac disease patients.

MATERIALS AND METHODS

Serum samples collection:

A total of 80 blood samples were collected and classified as follows: 40 samples from newly diagnosed patients (ND), 20 samples from gluten free diet patients (GFD) and 20 from healthy donors. Sera for all collected blood samples were separated at 3000 rpm for 10 min. and kept at -20 C^{o} until use.

Anti-gliadin and anti-reticulin estimation:

Anti-gliadin and anti-reticulin IgA were measured for ND celiac disease patients while Anti-gliadin and anti-reticulin IgG were measured for GFD CD patients using ELISA Kits (Orgentec, Germany)

The following equations were used to calculate the corresponding diagnostic parameters:

- Sensitivity (se %) $tp\times100/(tp + fn)$
- Specificity (sp %) $tn\times100/(tn + fp)$
- Diagnostic efficiency (de %) $(tp +tn)\ 100/(tp + fp + tn + fn)$

- Positive predictive value (PPV%)= tp×100 / (tp + fp)
- Negative predictive value (NPV%)= tn ×100/ (fn + tn)

(Akobeng, 2006 & Parikh *et al*., 2008)

tp: true positive (sick people correctly diagnosed as sick); fp: false positive (healthy people wrongly diagnosed as sick); fn: false negative (sick people wrongly diagnosed as healthy); tn: true negative (healthy people correctly diagnosed as healthy)

Statistical analysis

The receiver operating characteristic (ROC) analysis was utilized to analyze the data using the SPSS. ROC curves were generated by plotting sensitivity versus1-specificity, and the area under the curve was used to carry out a pairwise comparison of the diagnostic performance of the antigens.

RESULTS

The OD of ELISA reading that equal or above cutoff value in patient's sera represented as true positive (tp) result. The number of tp sera which recorded in IgA anti-gliadin and anti-reticulin tests was 35 and 39 respectively. While OD under the cutoff value represented as a false negative (fn) result for IgA anti-gliadin and anti- reticulin tests were (5) and (1) respectively. Whereas, in control group the OD equal or higher than cutoff value recorded as false positive (fp) result which were (0) for IgA anti-gliadin and IgA anti-reticulin tests, while the OD under cutoff value recorded as a true negative (tn) result which were (20) for IgA anti-gliadin test and (20) for IgA anti-reticulin, table (1) .

Table (1): Numbers of tp, tn, fp and fn for ND patients in gliadin and reticulin test.

	Patients			Control		
Test	No.	tp	fn	No.	tn	fp
Gliadin	40	35	5	20	20	0
Reticulin	40	39	1	20	20	0

Concentrations values of anti – gliadin IgA and anti- reticulin IgA for patients and control group were shown in figure (1) and (2).

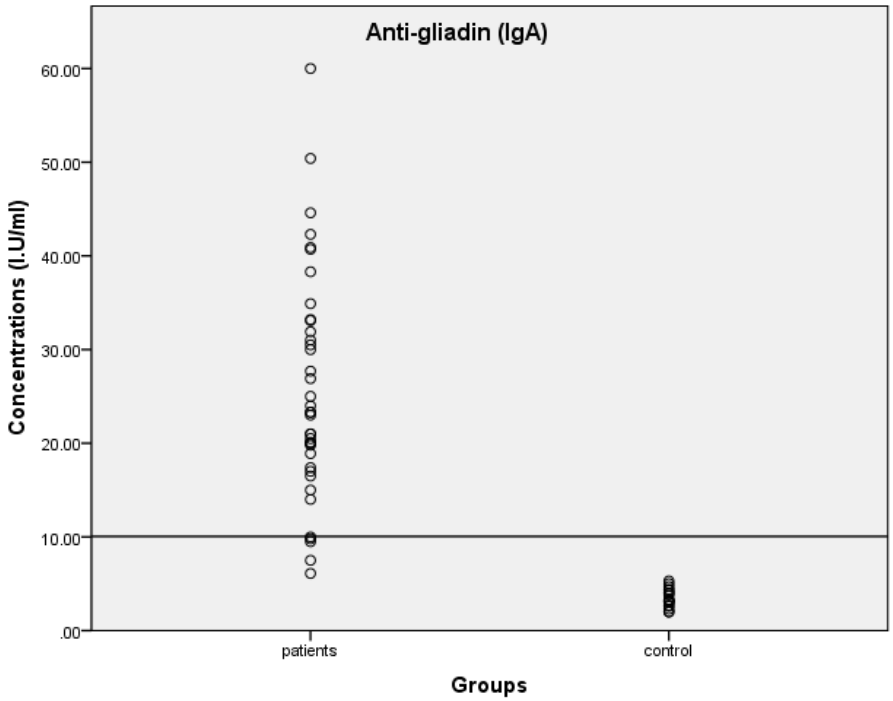

Figure (1): Values of IgA anti- gliadin test in patients and control group.

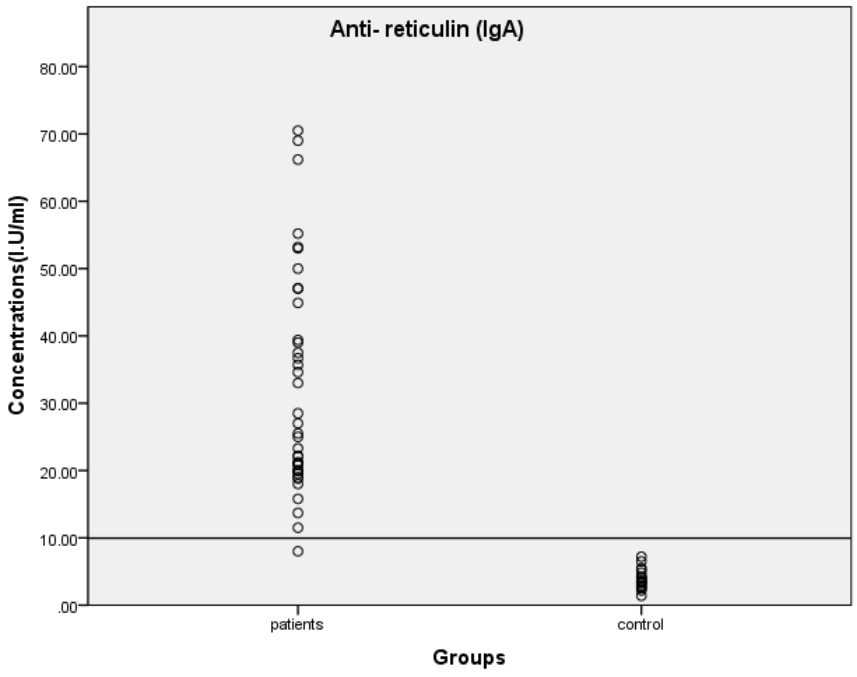

Figure (2): Values of IgA anti- reticulin test in patients and control group.

The values of tp sera which recorded in IgG anti-gliadin and anti-reticulin test were 17 and 19 respectively. Whereas the number of sera that recorded as fn were (3) and (1) and, respectively. (fp) result were (0) for IgG anti-gliadin test and IgG anti-reticulin, while (tn) result were (20) for IgG anti-gliadin test and (20) for IgG anti-reticulin, table (2).

Table (2): Numbers of tp, tn, fp and fn for GFD patients in gliadin and reticulin test.

	Patients			Control		
Test	No.	tp	fn	No.	tn	fp
Gliadin	20	17	3	20	20	0
Reticulin	20	19	1	20	20	0

Patients and control group values of anti-gliadin and anti-reticulin IgG were shown in figure (3) and (4).

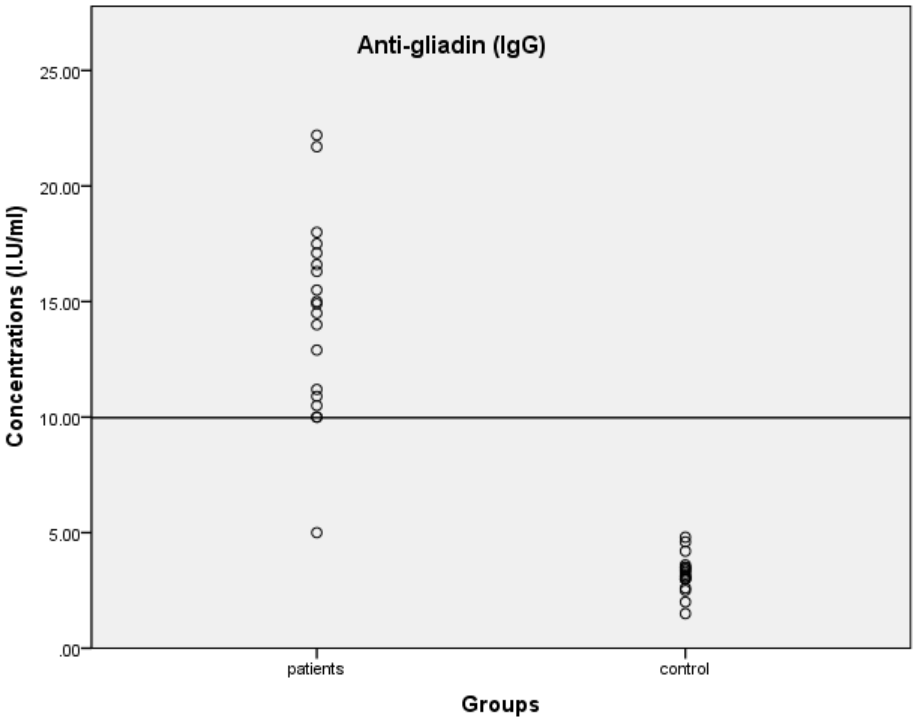

Figure (3): Values of IgG anti- gliadin test in patients and control group.

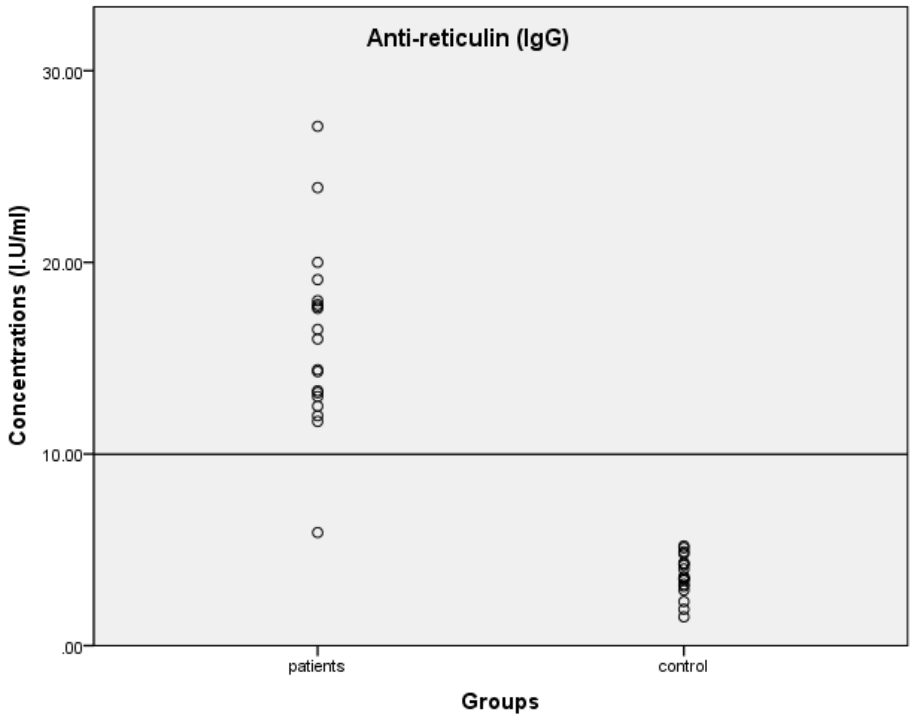

Figure (4): Values of IgG anti- reticulin test in patients and control group.

By using the tp, fn, tn and fp values, the Se%, Sp%, De%, PPV% and NPV% were calculated for (IgA and IgG) anti- reticulin test as in table (3). The Se% was (97.5 and 95) % for IgA and IgG,respectively while the Sp% was (100)% for the two immunoglobulins . The De%, PPV% and NPV% were (98.33, 100 and 95.23) % and (97.5, 100 and 95.23) % for IgA and IgG,respectively.

Table (3): Diagnostic accuracy of anti- reticulin test (IgA and IgG).

	Se%	Sp%	De%	PPV%	NPV%
IgA (ND)	97.5	100	98.33	100	95.23
IgG (GFD)	95	100	97.5	100	95.23

The Se%, Sp% and De% for (IgA and IgG) anti- gliadin test were (87.5 and 85)% ; (100)% ; (91.66 and 92.5)% respectively. Also PPV% and NPV% were (100)% ; (80 and 86.95)% , table (4).

Table (4): Diagnostic accuracy of anti- gliadin test (IgA and IgG).

	Se%	Sp%	De%	PPV%	NPV%
IgA (ND)	87.5	100	91.66	100	80
IgG (GFD)	85	100	92.5	100	86.95

According to the ROC curve result , AUC value for all tests examined in this study were 99.96 for anti – gliadin IgA and 1 for the other tests, figure (5), (6),(7) and (8).

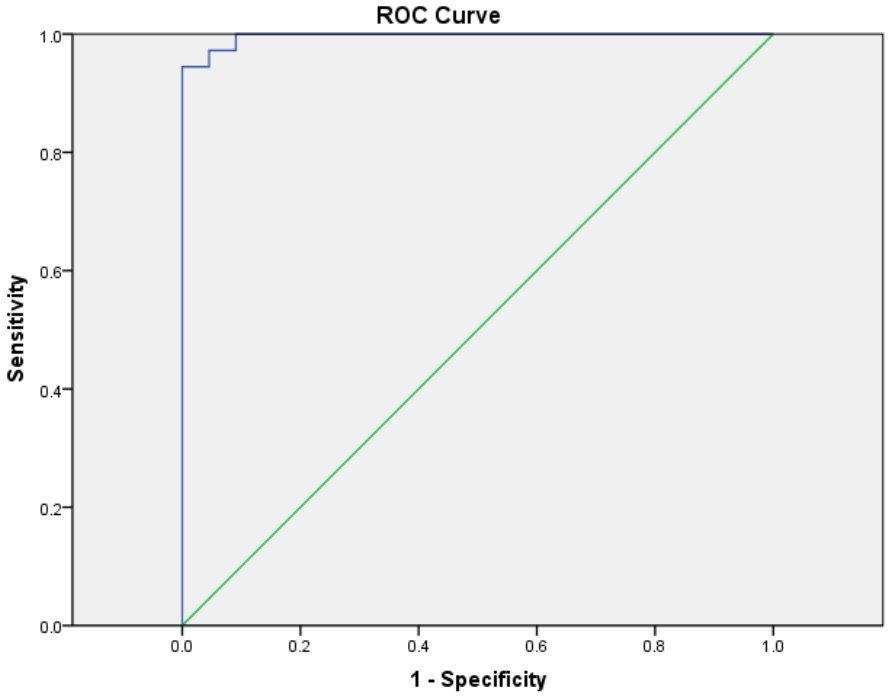

Figure (5): ROC curve for anti- gliadin IgA.

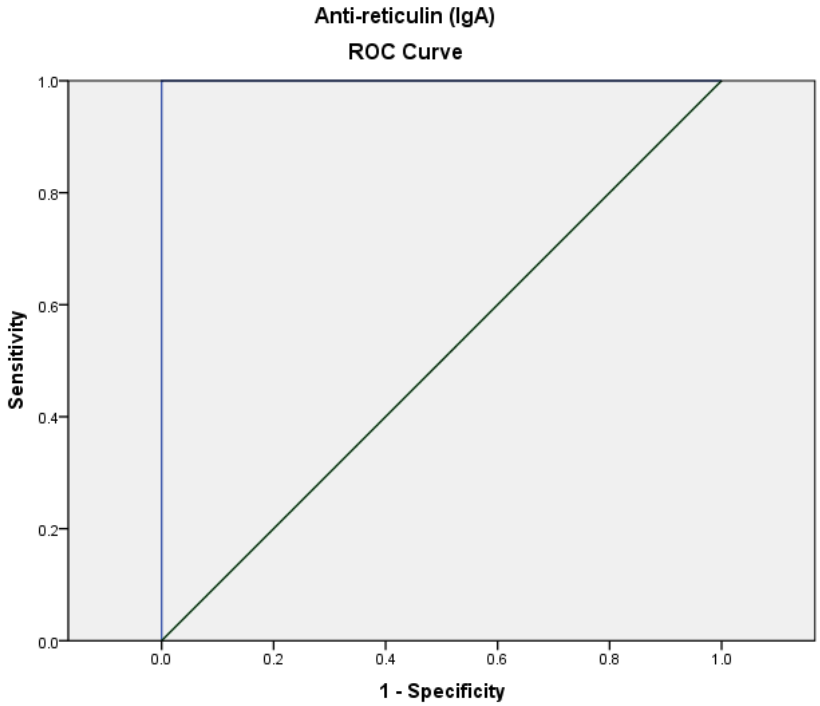

Figure (6): ROC curve for anti- reticulin IgA.

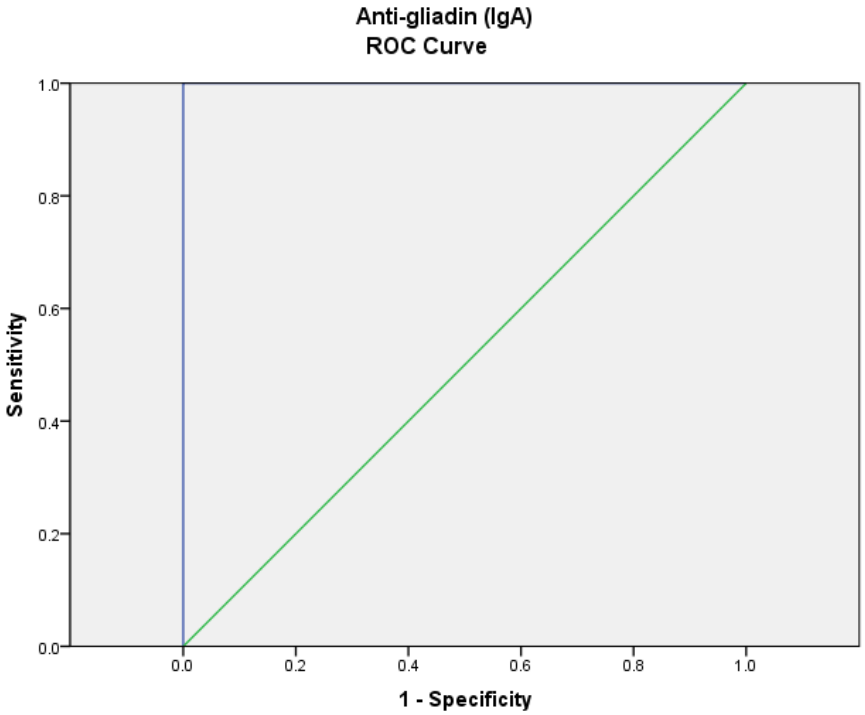

Figure (7): ROC curve for anti- gliadin IgG.

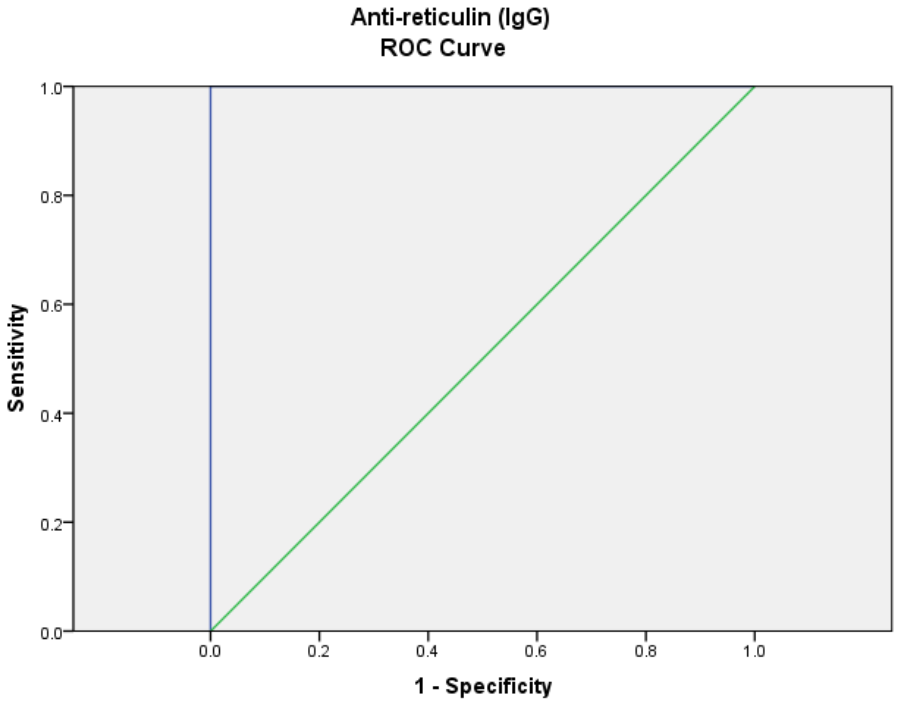

Figure (8): ROC curve for anti- reticulin IgG.

DISCUSSION

The diagnostic tools of CD include serologic testing for specific antibodies, histological analysis of biopsy specimens and HLA typing (DQ2 and DQ8) for suspected patients. The detection and quantitation of specific antibodies in appropriate clinical specimens are usually the first steps in the evaluation of CD and it is useful tool for diagnosis and follow-up of patients on a gluten-free diet (Bahia *et al.* ,2001).

This study was aimed to support the serological diagnosed tests for specific antibody in CD patients, for this purpose anti-gliadin and anti-reticulin were used to diagnose ND and GFD of CD patients.

In the present study , data collected according to the disease stage where sera were organized to two groups : ND and GFD, this division based on the fact that improvement of symptoms is generally seen within days to weeks after the initiation of GFD, while full mucosal recovery usually takes longer (Lee *et al*., 2003). Anti-tTG and anti -gliadin antibody titers will go down with the elimination of gluten from diet but may require many months or even years to completely disappear (Briani *et al*., 2008) . This fact lead to the immunoglobulins levels differ

when patient under GFD , when Elia (2013) examined anti-tTG and anti- EMA (IgA and IgG),he found that none of newly diagnosed patients give positive result when tested with IgG-tTG and IgG- EMA and found significant decrease in the mean of IgA-tTG and IgA-EMA concentrations. Also, Villata *et al*. (2007) refered to the facts that the IgA autoantibody has higher avidity to the tTG antigen and therefore that subsequent anti - tTG IgG binding is reduced . Moreover, IgA -tTG seems to be directed mainly against conformational tTG epitopes (Sblattero *et al*.,2002 & Seissler *et al*., 2001) and it is possible that IgG -tTG is directed against the same epitope (Dahlbom *et al*., 2005). Hence, a competition between IgA -tTG and IgG -tTG might take place and this competition would favor antibodies with a high avidity for tTG (Dahlbom *et al*., 2005).

The result of this study showed that IgA-gliadin was recorded lower sensitivity than IgA-reticulin. The sensitivity of IgA-gliadin and IgA-reticulin were (87.5 and 97.5)% respectively. While the specificity of IgA -gliadin and IgA –reticulin were (100)%, for the two tests in ND patients. Also, results obtained from ELISA test IgG anti- gliadin reveled lower sensitivity (85) % than IgG anti-reticulin (95) % and their specificity were (100) %.

The lower accuracy associated with the AGA tests may due to post infection malabsorption, Crohn's disease and cow's milk protein intolerance can give false positive tests for AGA. There is sufficient evidence to show that IgG- AGA tests generally are poor in sensitivity and specificity whereas the IgA- AGA tests are poorly sensitive but more specific for diagnosis of the disease (Bhatnagar and Tandon, 2006).

In contrast the results of Bahia *et al.* (2001) disagree with present study , they revealed that sensitivity of anti-gliadin IgA was 95.5% and specificity was 88.5% while the result obtained by Ghedira *et al* (2001) agree with present work, the sensitivity of IgA AGA was 86%, and disagree with specificity value (83%).
Mustafa Kamil (2015) study referred to the sensitivity of both AGA IgA &IgG were (77.55% ,63.26%)respectively and the specificity of anti Gliadin IgA was (76.58%) this result also agreed with recent work result. Bottaro and his colleagues (1995) concluded that AGA has great importance in suspect CD though their results were evaluated on the basis of age. The results contrast of different studies may be due to the difference of samples selection, some studies selected samples without a previous known of CD diagnosis and others included children only. This study recorded higher sensitivity than other studies, where the sensitivity of ARA was 35% in children below two years of age and 89% in children aged between two and 15 years (Ghedira *et al.*, 2001).

Our results indicated 100% specificity for ARA IgA test. This finding is consistent with the Ghedira *et al.* (2001) results. In contrast, Mustafa Kamil (2015) result revealed that the specificity of ARA IgA was (92.45%). The 100% specificity for our result may be due to the accuracy of samples collected from CD patients as all the samples were confirmed clinically and serologically.

In this study the De % of ARA tests was higher than that of AGA tests. Also other diagnostic parameters PPV and NPV revealed the values of sensitivity and specificity together and gave high value of ARA. Nandiwada and Tebo (2013) showed that ARA tests are requested by quite a number of clinicians in the routine evaluation for CD.

This study was included four tests (IgA- anti gliadin , IgA –anti reticulin , IgG anti gliadin and IgG anti reticulin) to choose which test has more accuracy statically , ROC curve test was used . This test revealed that area under the curve (AUC) for the tests were 99.96 for IgA AGA and 1 for the other tests , this result indicated that all tests have high efficiency especially (IgG AGA, IgA-ARA and IgG-ARA).

Conclusion:

1- Diagnostic efficiency of ARA (IgA and IgG) was higher than diagnostic efficiency of AGA (IgA and IgG).
2- The specificity of ARA (IgA and IgG) and AGA (IgA and IgG) were 100%.

Recommendation:

1- Tasting the efficiency of diaminated gliadin in CD diagnosis.
2- Study AGA and ARA tests related to age.
3- Do screening study for AGA and ARA to confirm the value of specificity .

REFRENCES

- **Akobeng,A**. (2006). Understanding diagnostic tests 1: sensitivity, specificity and predictive Values. *Found Acta Pædiatrica*., 96: 338–341.
- **Bahia,M.; Rabello, A. ; Brasileiro Filho, G. and Penn, F.** (2001). Serum antigliadin antibody levels as a screening criterion before jejunal biopsy indication for celiac disease in a developing country. Brazilian Journal of Medical and Biological Research 34: 1415-1420.
- **Bhatnagar, Sh. and Tandon,N.** (2006). Diagnosis of Celiac Disease . Indian Journal of Pediatrics, 73: 703 -710.
- **Bottaro ,G.; Rotolo, N.; Spina, M.; Sciuto, C.; Castiglione, S.; Sanfilippo, G. and Musumeci, S.** (1995). Evaluation of sensitivity and specificity of antigliadin antibodies for the diagnosis of celiac disease in childhood. Minerva Pediatr. , 47(12):505-510.
- **Briani, C. ; Samaroo, D. & Alaedidi, A.** (2008). Celiac disease: From gluten to autoimmunity. Autoimmunity Reviews 7:644 – 650.
- **Catassi, C.; Ratsch, I.; Gandolfi, L.; Pratesi, R.; Fabiani, E.; El Asmar, R.; Frijia, M.; Bearzi, I and Vizzoni, L.** (1999). Why is coeliac disease endemic in the people of the Sahara? 354(9179) : 647-648.

- **Chu,P and Wen, H.** (2013). Sensetive detection and quantification of glutin contamination in glutin free food with immunomagnatic beads based liposomal fluorescence immunoassay. Anal Chim Acta., 787:6438-6492.
- **Corrao, G. Corazza, G. Bagnardi, V. Brusco, G. Ciacci, C.; Cottone, M.; Sategna, Guidetti, C.; Usai, P. Cesari, P. Pelli, M.; Loperfido, S; Volta, U.; Calabro, A. and Certo, M.** (2001). 358(9279) : 356-361.
- **Dahlbom, I. ; Olsson, M. ; Forooz, N. ; Hasson, T. ; Truedsson, L. & Sjoholm, G.** (2005). Immunoglobuline G Anti-Tissue Transglutaminase Antibodies Used as Markers for IgA - Deficient Celiac Disease Patients. Clinical and Diagnostic laboratory Immunology. 12(2):254-258.
- **Eade, O.; Lloyd, R.; Lang, C. and Wright, R.** (1977). IgA and IgG reticulin antibodies in coeliac and non-coeliac patients. Gut., 18:991–993.
- **Elia, Z.** (2015). Assessment of immunological parameters in patients afflicted with celiac disease. M.Sc. thesis submitted to college of medicine / Hawler Medical University.
- **Ghedira, I.; Sghiri ,R.; Ayadi, A.;Sfar, M.; Harbi ,A.; Essoussi ,A.; Amri ,F.; Korbi, S. and Jeddi, M.**(2001). Anti-endomysium, anti-reticulin and anti-gliadin antibodies, value in the diagnosis of celiac disease in the child. Pathol Biol (Paris), 49(1):47-52.
- **Holmes, G.; Prior, P.; Lane, M.; Pope, D. and Allan, R.** (1989). Malignancyin coeliac disease—effect of a gluten free diet. Gut., 30(3): 333-338.
- **Lee, SK. ; Lo, W.; Memo, L.; Rotterdam, H. & Green, PH.** (2003). Duodenal histology in patients with celiac disease after treatment with a gluten –free diet. Gastrointest Endosc 57:187-91.
- **Lock. R.; Gilmour, J. and Unsworth, D.** (1999). Anti-tissue transglutaminase, anti-endomysium and anti-R1-reticulin autoantibodies-the antibody trinity of coeliac disease. Clin. Exp. Immunol. ,116:258–262.
- **Mustafa Kamil,L. (**2015). Evaluation of coeliac disease serological markers J Fac Med Baghdad , 57(2): 156-159.
- **Nandiwada, S, and Tebo, A.** (2013). Testing for Antireticulin Antibodies in Patients with Celiac Disease Is Obsolete: a Review of Recommendations for Serologic Screening and the Literature. Clinical and Vaccine Immunology,
- **Parikh,R.; Mathai,A. ; Parikh, S.; Sekhar,C. and Thomas,R.** (2008). Understanding and using sensitivity, specificity and predictive values. *Indian J Ophthalmol*., 56(1): 45–50.
- **Sblattero, D. ; Florian, F. ; Azzoni, E. ; Zyla, T. ; Park, M. ; Baldas, V.** (2002). The analysis of the fine specificity of celiac disease antibodies using tissue transglutaminase fragments. Eur. J. Biochem. 269:5175 – 5185.
- **Seissler, J. ; Wohlrab, C.; Wuensche, W.A. & Boehm, B.O.** (2001). Autoantibodies from patients with celiac disease recognize distinct functional domains of the auto antigen tissue transglutaminase. Clin. Exp. Immunol., 125:216 – 221.

- **Ventura, A.; Magazzu, G. and Greco, L.** (1999). Duration of exposure to gluten and risk for autoimmune disorders in patients with celiac disease. Gastroenterology, 117(2): 297-303.
- **Villata, D. ; Alessio, M.G. ; Tampoia, M. ; Tonutti, E. ; Brusca, I.** (2007). Testing for IgG class antibodies in celiac disease patients with selective IgA deficiency. A comparison of the diagnostic accuracy of 9IgG anti-tissue tranglutaminase, 1 IgG anti-Gliadin and 1IgG anti-deaminated gliadin peptide antibody assays. Clin. Chim. Acta., 382:95 – 99.