

Study the Correlation of Anti-gliadin IgA & IgG with Iron & Total Iron Binding Capacity & Albumin in sera of Patients with Celiac Disease

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Summery

Celiac disease (CD) is characterized by malabsorption of nutrients , chronic inflammation and damage of small intestinal mucosa caused by the ingestion of gliadin fraction of wheat gluten and barley . Since the anemia its diagnosis the CD, therefore were measured iron, total iron binding capacity(T.I.B.C.) , and its relationship with antigliadin IgG,IgA and serum albumin. Thirty patients with CD were studied and compared with twenty healthy individuals. Significant elevation of iron and albumin and anti-gliadin IgG,IgA were observed in the sera of patients with CD compared with the control group. Also a significant negative correlation between anti-gliadin IgG,IgA and iron were found in the sera of patients with CD while a non significant correlation were found between albumin and anti-gliadin IgG,IgA in the sera of patients with CD.

الخلاصة

يوصف داء الاحتشاء بأنه سوء امتصاص للمواد الغذائية , وهو التهاب مزمن بسبب ضرر في غشاء الامعاء الدقيقة وذلك عند تناول مواد غذائية حاوية على الكلايدين والموجودة في الحنطة والشعير. ونظرا لان الانيميا هي احدى اعراض داء الاحتشاء , فقد تم قياس الحديد والحديد الكلي في هذه الدراسة الحالية وايجاد علاقة مع مضادات الكلايدين IgA , IgG والالبومين , حيث تضمنت الدراسة الحالية جمع 30 عينة من المرضى المصابين بداء الاحتشاء و مقارنتها مع 20 عينة من الاصحاء . اظهرت النتائج وجود زيادة معنوية لمستوى الحديد والالبومين ومضادات الكلايدين IgA,IgG في مصول دم المصابين بداء الاحتشاء ومقارنتها مع مستوياتها في مصول دم الاصحاء وقد تبين ايضا وجود علاقة معنوية سلبية بين مضادات الكلايدين IgA , IgG مع الحديد في مصول دم المصابين بداء الاحتشاء . في حين تبين عدم وجود علاقة معنوية بين الالبومين ومضادات الكلايدين IgA,IgG في مصول دم المصابين بداء الاحتشاء.

Keywords:-iron,total iron binding capacity , anti-glaidin IgG, IgA,albumin,celiac disease.

Introduction:-

Celiac disease (CD) is a digestive disease that damages the small intestine and interferes with absorption of nutrients from food^(1,2) . People who have CD cannot tolerate gluten, a protein in wheat , rye and barley⁽³⁾.Gluten is found mainly in foods but may also be found in every day products such as medicines, vitamins. , and lipbalms^(4,5).When people with CD eat foods or use products containing gluten, their immune system responds by damaging or destroying villi-the tiny , finger like protrusion lining the small intestine^(6,7) .

Villi normally allow nutrients from food to be absorbed through the walls of the small intestine into the blood stream^(8,9).Without healthy villi , a person becomes malnourished , matter how much food one eats^(10,11).CD is both a disease of malabsorption , meaning nutrients are not absorption properly and an abnormal immune reaction to gluten⁽¹²⁾ . Furthermore , there is genetic meaning is runs in families , sometime the disease is triggered or becomes active for the first time after surgery , pregnancy , childbirth , viral in faction , or severe emotional stress⁽¹³⁾ . Testing sera for IgG,IgA immunoreactivity to gliadin is usually one of the first steps in the complex process of diagnosing gluten into lerance⁽¹⁴⁾ .

In addition, patients with CD sometimes present with iron deficiency anemia (IDA)^(15,16).However , IDA is a commonly observed sign in CD^(17,18) . Only aminority of CD patients present with classical malabsorption symptoms whereas most patients have subclinical or silent

forms in which IDA can be the sole presentation⁽¹⁹⁾. Among laboratory tests , measurement of albumin testing is used in a variety of setting to help diagnose disease , to monitor changes in health status with treatment or with disease progression , and as a screen that may indicate the need for other kinds of testing^(20,21,22).

The aim of this study was to evaluate the serum concentration of iron and its correlation with albumin and anti-gliadin IgG,IgA antibodies in CD .

Subjects and Methods:-

Subjects:-This study included 30 (17 male and 13 female) with age (1-60) year between June 2011 to September 2011 were involved in this study .The patients were referred to Al-Kadhimiya Teaching Hospital . Baghded, Iraq. All patients with CD were dignosed depend on symptoms of diarrhea , weight loss , anemia , and serologic examination by anti-gliadin IgG,IgA . No other disease associated with CD in those patients . As a control , 20 healthy(8 male and 12 female) with age (1.5-40) individual were included in the present study.

Serum Sampling:-Five milliliters of venous blood were collected in test tubes. The serum was separated from the cells by centrifugation at 3000rpm for 10 minutes, stored frozen until used to estimate the different parameters. The sera which were obtained from blood samples should be unhemolyzed in order to avoid any interference with the obtained results.

Determiation of anti-gliadin screen IgG,IgA :- Serum concentration of anti-gliadin screen IgG,IgA were measured by double antibody technique using enzyme–linked immunosorbent assay (ELISA) , Immuchem , Belgium^(23,24).

Determiation of Albumin Concentration :- The serum concentration of albumin was determined by dye-bindin method using albumin kit (Biolabo Reagents,France)^(25,26).

Determiation of iron & total iron binding capacity(T.I.B.C):- Serum iron and T.I.B.C. were determined spectrophotometrically in duplicate and trans ferrin iron saturation was calculated ,Biolabo Reagents , France^(27,28).

Statistical Anglysis:-The findings were expressed as the mean ± standard deviation (SD). The data were analyzed and correlation with student's independent t-test. All statistical analyses were performed with the program statistical package for the social science (SPSS for windows , version 11.5) values of $p < 0.05$ were considered significant.

Results:-

The concentration of anti-gliadin IgG,IgA were measured in the present study in the sera of control and patients with celiac disease by double antibody technique. The mean values presented in table (1), reflect a highly significant increase in the serum levels of anti-gliadin IgG,IgA ($p < 0.001$) in patients with CD compared with these of the control group .

Table (1):Serum anti-gliadin screen IgG&IgA levels in patients with CD and control groups.

	Groups	Sample number (n)	Range AU	Mean ±SD AU	P value
IgG,IgA	Control	20	9-15	10.66±1.8	P<0.001
IgG,IgA	Patients with CD	30	17-200	62.23±44.39	

The serum concentration of albumin was measured throughout this study in the sera of control and patients groups using dye-bindin method.The result presented in table(2) shows a significant

decrease ($p < 0.0001$) in albumin levels in sera of patients with CD comparison with these of the control group.

Table(2): Albumin concentration in the sera of control and patients with celiac disease.

Groups	Sample number (n)	Range mg/dL	Mean \pm SD mg/dL	P value
Control	20	3.53-4.42	3.88 \pm 0.25	P< 0.0001
Patients with CD	30	1.97-3.14	2.6 \pm 0.35	

In order to calculate the iron concentration was measured in the sera of the studied groups, using spectrophotometricall method. A highly significant decrease ($p < 0.0001$) in iron levels was observed in the present study in sera of patients with CD in comparison with these of the control group, table (3) .

Table(3):Iron concentration in the sera of control and patients with celiac disease

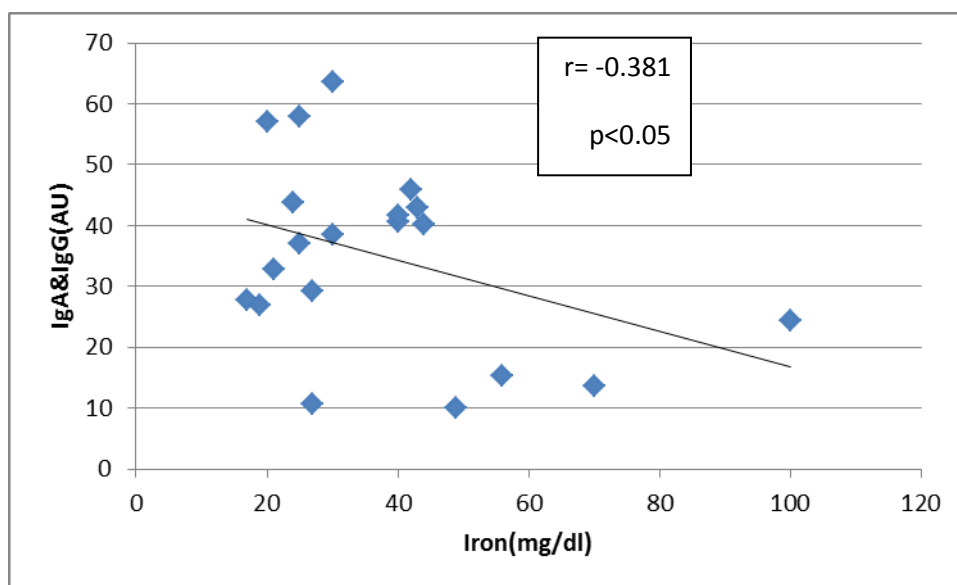
Groups	Sample number (n)	Range mg/dL	Mean \pm SD mg/dL	P value
Control	20	56.60-95.46	72.66 \pm 14.35	P<0.0001
Patients with CD	30	10-63.40	37.23 \pm 14.52	

When the mean values of both T.I.B.C. and transferring iron saturation in sera of patients with CD was compared with that of the control group , the results (Table 4) reflected non significant in T.I.B.C. and highly significant decrease trans ferrin iron saturation ($p < 0.0001$) .

Table (4):Mean values of T.I.B.C.& transferring iron saturation in sera of control and patients with CD.

Group	T.I.B.C.			transferring iron saturation		
	Range	Mean \pm SD	P value	Range	Mean \pm SD	P value
Control 20	295.00- 493.40	375.80 \pm 72.38	P>0.05	12.80- 38.25	22.65 \pm 7.9	P<0.0001
Patients with CD 30	156.80- 527.47	375.76 \pm 96.32		2.02- 27.90	11.55 \pm 5.57	

Upon analysis of the overall results of the present study , it was found a significant negative correlation ($r = -0.382$, $p < 0.05$) between anti-gliadin IgG,IgA & iron in the sera of patients with celiac disease (95 %) who have decrease in there iron Figure (1) . While a non significant correlation ($p > 0.05$) between anti-gliadin IgG,IgA and albumin were found in the sera of the same patients with CD.



Figure(1): The correlation between anti-gliadin IgG&IgA and iron in sera of patients with CD.

Discussion :-

Gluten sensitive enteropathy has a wide clinical spectrum including GI and extra-GI findings , which can be diagnosed at any age from childhood to the elderly ⁽²⁹⁾. Classical or typical form of CD is associated with features of malabsorption , however, a substantial number of CD patients have atypical manifestations , including hematologic , endocrinologic , renal , neurologic , psychiatric , dermatologic and cardiovascular symptoms⁽³⁰⁾ . Testing sera for anti-gliadin IgG,IgA immunoreactivity to gliadin is usually one of the first steps in the complex process of diagnosing gluten intolerance , because it is well known that antibodies to native gliadin sequences are present in patients with CD^(31,32). In the present study anti-gliadin IgG,IgA measurements (table 1) shows a presence of a highly significant increase of anti-gliadin IgG,IgA ($p<0.001$) in sera of patients with CD .

This was in agreement with the result obtained by Cindy Huany, *et. al.* who report for woman found to have positive anti-gliadin antibodies and positive tissue transglutaminase IgG antibodies that are consistent with celiac disease⁽³³⁾ . Celiac disease was diagnosed by characteristic histological abnormalities on small bowel biopsy , positive antibodies . Through this study serum iron and transferrin saturation was measured highly significant increase ($p<0.0001$) , T.I.B.C. non significant of its was observed in the patients with CD in comparison with that of the control group. This was in agreement with the result obtained by Kosnai *et. al.* who reported that was evidence of mild iron deficiency in the children with CD. Hb, transferrin saturation , MCV of red blood cells , and serum ferritin were below the lower limits of patients with CD⁽³⁴⁾ . Derya Vcardg *et. al.* show the prevalence of CD was significantly higher IDA of obscure origin patients than the control subjects ($p<0.05$)⁽³⁵⁾ .

Throughout this study highly significant increase ($p< 0.0001$) in sera albumin of patients with CD. This was in agreement with the result obtained by Itvatum M. *et. al.* who reported bovine serum albumin only few patients of both categories had raised IgG,IgA activity to BSA most of the IgG subclass activity resided in IgG1 and IgG4⁽³⁶⁾. Hilger C. *et. al.* individuals with medium and high levels of IgG and IgA antibodies to native bovine serum albumin were screened with recombinant BSA ⁽³⁷⁾ . A significant negative correlation ($p<0.05$, $r = -0.382$) was found in the present study

between anti-gliadin IgG ,IgA and serum iron in the sera of patients with CD while a non significant correlation ($p>0.05$) were found between anti-gliadin IgG,IgA and albumin the patients with CD.

Conclusions:-

From the results that been conclude a significant negative correlation between iron and anti-gliadin IgG & IgA in the sera of patients with CD who have decrease in total iron .This finding would appear to suggest a possibility to use the total iron as a tool with anti-gliadin IgG & IgA for diagnosis of celiac disease.

References:

- 1-Fasano A. , Catassi C. . Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum.Gastro enterology 2001;120:636-51.
- 2-Farrel RJ. , Kelly CP. Celiac sprue and refractory sprue . In:Feldman M. , Friedman LS, Sleisenger MH,eds.Sleisenger & Fordtran's gastro intestinal and liver disease pathophysiology diagnosis / management . Philadelphia : Elsevier Science , 2002;1818-41.
- 3-Farrell RJ,Kelly CP.Celiac sprue .NEJM2002;346:180-8.
- 4-Sollid , L.M.Coeliac disease :Dissecting a complex inflammatory disorder . Nature Rev.,2002;2:647-655.
- 5-Dieterich W. et al.Serum antibodies in celiac disease . Clin. Lab. 2000;46:361-364.
- 6-Loftus C. & Murray J. Celiac disease diagnosis and management . J.Clin. Outcomes manage 2002;2(9):341-350.
- 7-Ch'ngC.L., et al.Celiac disease and antommune thyroid disease. Clin. Med. Res. 2007;5(3):184-192.
- 8-Man Kai A. et al . Screening for celian disease in Tunisian patients with Grave's disease using anti-endomysium and anti- tissue transglutaminase anti-bodies . Gastroenterol.Clin. Biol. 2006;30(8-9):961-964.
- 9-Wahnschaffe U. ,et al. Celiace disease like abnormalities in a subgroup of patients with IBS. Gastroenterology 2001;121:1329-1338.
- 10-Brandimarte G. et al. Changing trends in clinical forms of celiac disease : which is now the main form of celiac disease in clinical practice ? minera gastroenterol.Dietol 2002;48(2):121-130.
- 11-Koop I. Ilchmann R., Izzi L. ,et al .Detection of autoantibodies against tissue transglutaminase in patients withceliace disease and dermatitis herpetiformis. Am J. Gastroenterol 2000;95:2009-14.
- 12-Howard MR , Turnbull AJ , Morley P, et al. Aprospective study of the prevalence of undiagnosed celiac disease in laboratory defined iron and folate deficiency . J Clin Pathol 2002;55:754-7.
- 13-Tatar G. , Elsurer R. , Simsek H. , et al .Screening of tissue transglutaminase antibody in healthy blood donors for celiac disease screeing in the Turkish population. Dig Dis Sci 2004;49:1479-84.
- 14-Konic Ristic A., Dodig D. Krstic R. , et al .Different levels of humoral immuneoreactivity to different wheat cultivars gliadin are present in patients with celiac disease and in patients with multiple myeloma BMC Immunol2009;10(32):1186-1203.
- 15-Stevens L. H. .Jama patient page : celiac disease.J.A.M.A. 2002;287:1484.
- 16-Annibale B. , Capurso G. , Chisstolini A., et al. Gastrointestinal cases of refractory iron deficiency anemia in patients without gastrointestinal syptoms. Am J Med 2001; 111:439-45.
- 17-Zamani F.,Mohamadnejad M.,Shaker R., et al. Gluten sensitive enteropathy in patients with iron deficiency anemia of obscure origin . World J Gastroenterol 2008;14:7381-5.

- 20-Boza JJ, Dangin M., Moennoz D., et al. Free and patient bound glutamine have identical splanchnic extraction in healthy human volunteers. *Am J Physiol Gastrointest liver physiol.* 2001;281:267-274.
- 21-Clase CM. S., Pierre MW., Churchill DN. Conversion between bromocresol green and bromocresol purple-measured albumin in renal disease. *Nephrol Dial Transplant.* 2001;16:1925-1929.
- 22-Dieterich W., et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nature Med.*, 1997;3:797-801.
- 23-Dieterich W., et al. Autoantibodies to tissue transglutaminase as predictors of celiac disease. *Gastroenterol*, 1998;115:1317-1321.
- 24-Young D.S. "Effect of Drugs on clinical laboratory test", 1998; 4thed. P.3-16 to 3-22.
- 25-Hallbach J., Hoffmann G. E., Guder W. G., Overestimation of albumin in heparinized plasma. *Clin. Chem.*, 1991; 37(4), p.566-568.
- 26-Tietz N.W. "Text book of clinical chemistry", 1999; 3rd ed, p1701-1703.
- 27-Tietz N. "Clinical guide to laboratory test", 1995; 3rd ed, p374-375.
- 28-Ciclitira PJ., King AL., Fraser JS. A technical review on celiac sprue. *American gastroenterological association. gastroenterology.* 2001;120:1526-40
- 29-Van De Wal Y., Kooy Y., Van Veelen P., Vador W., Koning F., Pena S. Celiac disease: it takes three to tangle gut. 2000;46:734-7.
- 30-Mothes T., Vhlig HH., and Richter T. Recent aspects of antibody determination for the diagnosis of coeliac disease. *Dtsch Med Wochenschr.* 2009;134(3):1525-1528.
- 31-Caja S., Maki M., Kaukinen K., and et al. Antibodies in celiac disease: implications beyond diagnostics. *Cell Mol Immunol.* 2011;8(2):103-109.
- 32-Ortiz M., Fragoso A., and Osullivan CK. Detection of Antigliadin autoantibodies in celiac patients samples using a cyclodextrin – based supramolecular biosensor. *Anal Chem.* 2011;28.(Epub ahead of print).
- 33-Cindy H., Amy T. Xiangbing W. Hyperthyroidism iron – deficiency anemia and celiac disease. *Thyroid Science* 2009;4(3).
- 34-Gaurav K., Patwari A., Shashi N., Anand V. Iron supplementation in children with celiac disease. *Indian J. pediatrics* 2003;70(12), 955-958.
- 35-Derya U., Safa G., Ozcan C., Fahri Y., Pinar A., Osman C. Celiac disease prevalence in patients with iron deficiency anemia of obscure origin. *Turk J. Gastroenterol.* 2009;20(4):266-270.
- 36-Hvatum M., Scott H., Brandtzaeg P. Serum IgG subclass antibodies to a variety of food antigens in patients with celiac disease. *Gut* 1992;33:632-638.
- 37-Hilger C., Gregion F., De Beaufort C., Michel G., Freilinger J., Hentges F. Differential binding of IgG & IgA antibodies to antigenic determinants of bovine serum albumin. *Journal list, Clin expimmunol*, 2001;123(3):387-394.