# IMMUNOLOGICAL DIAGNOSIS OF CELIAC DISEASE IN SYMPTOMATIC CHILDREN IN KIRKUK GOVERNORATE [+]

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**Abstract:**

**The aim of the present study is to estimate the occurrence of the celiac disease among clinically suspected children using immunoglobulin antibodies against gliadin (IgA and IgG-AGA) and against human tissue transglutaminase (IgA and IgG-tTG) as screening method. The study included 60 children with signs and symptoms suggested to have celiac disease (average age 36 months) who attended the Children's Hospital/ Kirkuk during the period from November/ 2011 until March/ 2012 and 30 apparently healthy children (average age 45 months) were chosen as a control group. Negative results were obtained in 33 (55.0%) clinically suspected children and 30 (100%) of control children. Positive results included antigliadin antibodies (IgA-AGA and IgG-AGA or both) in 27 (45.0%) patients and tissue transglutaminase (IgA-tTG and IgG-tTG or both) in 10 (16.6%) patients. The biopsy of five children were positive for both AGA (IgA and IgG) and tTG (IgA and IgG) revealed that characteristic lesions of celiac disease. It can be concluded that among the 60 patients that were tested, positive results of AGA (IgA and IgG) and tTG (IgA and IgG) represented (13.3%) and (11.7%) respectively and (8.3%) were confirmed by biopsy.**

## التشخيص المناعي من حساسية الحنطة (الداء الزلاقي) في أطفال لديهم أعراض في محافظة كركوك

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**المستخلص:**

**الهدف من الدراسة الحالية هو تقدير حدوث مرض حساسية الحنطة بين اطفال مشتبه بإصابتهم سريرياً بالمرض باستخدام الأضداد المناعية ضد غلياديين (الكلوبيولينات المناعية A و G) وضد ترانسكلوتامنيز (الكلوبيولينات المناعية A و G) كطريقة مسحية. شملت الدراسة 60 طفلا لديهم علامات وأعراض توحي اصابتهم بالمرض (متوسط العمر 36 شهرا) الذين حضروا مستشفى الأطفال/ كركوك خلال الفترة من تشرين الثاني/2011 ولغاية اذار/2012 وتم اختيار 30 طفلا أصحاء (متوسط العمر 45 شهرا) كمجموعة سيطرة. تم الحصول على نتائج سلبية في 33 (55.0%) من اطفال مشتبه بإصابتهم سريريا و30 (100%) من الأطفال السيطرة. شملت النتائج الايجابية أضداد الغلياديين (الكلوبيولينات المناعية A و G او كليهما معا) في 27 (45.0%) مريضا وأضداد الترانسكلوتامنيز (الكلوبيولينات المناعية A و G او كليهما معا) في 10 (16.6%) مرضى. كشفت الخزعات لخمسة أطفال ايجابية لكلا أضداد الغلياديين (الكلوبيولينات المناعية A و G) وأضداد الترانسكلوتامنيز (الكلوبيولينات المناعية A و G) أن هناك تقرحات**

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مميزة لمرض حساسية الحنطة. يمكن الاستنتاج أن من بين 60 مريضا التي تم فحصهم، نتائج ايجابية من أضداد الغليادين (الكلوبيولينات المناعية A و G) وأضداد الترانسكلوتامنيز (الكلوبيولينات المناعية A و G) مثلت (13.3%) و(11.7%) على التوالي وتم تاكيد (8.3%) بواسطة الخزعة.

## Introduction:

Celiac disease (CD), an immune-mediated mucosal disorder primarily affecting the small intestine in genetically susceptible individuals, is triggered by the ingestion of dietary gluten [1]. Gluten is the alcohol-soluble protein component of the cereals wheat, rye and barley. It is composed of 2 major protein fractions: glutenin and gliadin; most of the toxic activity exerted by gluten in CD is due to gliadin [2].

Celiac disease, also known as celiac sprue, gluten-sensitive enteropathy, non-tropical sprue [3], characterized by inflammation, leading to injury to the mucosal lining of the small intestine [4], including villous atrophy with crypt hyperplasia and intraepithelial lymphocytosis, and subsequent nutrient malabsorption [5].

The disorder is a multifactorial condition, originating from the interplay of genetic and environmental factors. The necessary environmental trigger is gluten, while the genetic predisposition has been identified in the major histocompatibility complex region on chromosome 6p21, with over 90% of CD patients expressing HLA DQ2 and the remaining celiac patients express DQ8 [2].

Celiac disease is one of the most common lifelong disorders on a worldwide basis affecting 0.5-1% of the general population in the USA and other developed countries [6]. In Iraq, the prevalence was found to be 1:400 among healthy blood donors by serological screening (7).

The symptoms vary considerably between individuals and also with age. In children younger than 2 years of age, gastrointestinal symptoms and failure to thrive are common. In older children and adults, the symptoms are often nonspecific, such as abdominal pain, anemia, osteoporosis, fatigue, and even depression. Consequently, the diagnosis is easily delayed or even missed [8].

Celiac disease prevalence is increased in at-risk conditions such as family history of celiac disease, autoimmune diseases, especially type 1 diabetes (T1D) and thyroiditis, IgA deficiency, and some genetic syndromes (Down, Turner, and William syndromes) [9].

The serological markers most commonly used in clinical routine are immunoglobulin A (IgA) antibodies against gliadin (AGA-IgA), tissue transglutaminase (tTG-IgA), and endomysium (EMA-IgA) [10]. However, some studies suggest that AGA-IgA should be discarded in view of its poor specificity and replaced with tTG-IgA [11].

In clinical setting, a patient with positive serological results requires duodenal biopsy to confirm CD diagnosis. However, a definitive diagnosis is only made when a response to gluten-free diet (GFD) is present [12]. Furthermore, duodenal biopsy has several pitfalls: (i) at least four forced biopsies are needed to achieve good readability; (ii) poorly oriented or inadequate biopsies may not be useful for diagnosis; and (iii) it is an invasive procedure, both in children and adults. In the last few years, a more prominent role for a definitive diagnosis based solely on serological assays has been proposed. In pediatric populations, strongly positive tTG antibody results (≥100 U) showed a high specificity for Marsh type IIIa or greater changes [13].

A strict life-long gluten-free diet is the only safe and efficient available treatment, yet it results in a social burden. Alternative treatment modalities focus on modification of dietary

components, enzymatic degradation of gluten, inhibition of intestinal permeability and modulation of the immune response [14].

The aim of the study was to **e**stimate the frequency of the celiac disease (CD) among clinically suspected children in Kirkuk governorate by using serological assays including immunoglobulin A and G for all antigliadin antibody (AGA) and anti-tissue transglutaminase antibody (tTG).

## Subjects and methods:

A total of 90 children were studied and classified into 2 main groups.

### 1. Patients group:

This group consisted of 60 children (28 male and 32 female) attending the Children's Hospital /Kirkuk. These children were referred from different hospitals in Kirkuk; because they were clinically suspected to have celiac disease.

### 2. Control group:

The control group consisted of 30 (16 male and 14 female) apparently healthy children who were not complaining of any gastro-intestinal problem.

Venous blood samples were also obtained from each subject included in this study and serum was stored at -20 C until testing. All patient and control groups ages ranged from 6 months - 8 years.

AGA-IgA and AGA-IgG were determined by enzyme-linked immunoassay (ELISA) (Immuchem, Belgium) in accordance with the manufacturer's instructions. Sera from patients and controls were tested at dilution 1:100, antibody levels were measured by (U/mL) and calculated from a 6-point calibrator curve. Values more than 12 U/mL were considered positive. tTG-IgA and tTG-IgG were measured by ELISA (Euroimmun, German) in accordance with the manufacturer's instructions. Sera from patients and controls were tested at dilution 1:201, antibody levels were expressed as relative units (RU/mL) and calculated from a 3-point calibrator curve. Values more than 20 RU/mL were considered positive.

SPSS version 18.00 was used to analyze the data. P value was calculated by using chi-square test. P value less than 0.05 was taken as significant.

## Results:

The study included 90 children (60 patients and 30 controls). The average age of patients in the study group was 36 months and male/female ratio was 28/32 (0.8/1). Negative results were obtained in all serological tests (IgA and IgG-AGA, IgA and IgG-tTG) in 33 (55.0%) patients and 30 (100%) control children. Nine (15.0%) and 26 (43.3%) children had positive titers of IgA-AGA and IgG-AGA respectively as compared with no positive result of IgA-AGA and IgG-AGA in control group (Table.1). Positive titers for IgA-tTG were 8 (13.3%) and IgG-tTG 9 (15.0%) as compared with no positive result of IgA-tTG and IgG-tTG in control group (Table.2).

Eight (13.3%) children had positive titers of both IgA-AGA and IgG-AGA. While, 7 (11.7%) children were positive for both IgA-tTG and IgG-tTG (Table.3). Six (10.0%) children were positive for all AGA (IgA and IgG ) and tTG (IgA and IgG), while only 5 (8.3%) children underwent biopsy and consistent with celiac disease.

Only one child in the study sample had a positive titer of IgA-AGA, but normal IgG-AGA. tTG (IgA and IgG) was negative for this child and refused endoscopy. Also only one child in the study sample had a positive titer for IgA-tTG without IgG-tTG.

Eighteen (30.0%) children had positive IgG-AGA but, negative IgA-AGA test. IgG-tTG had positive titer for two of these children and all refused endoscopy. Serological tests and biopsy results of five celiac children can be seen in (Table.4).

**Table (1): Frequency distribution of AGA (IgA & IgG) among the study groups**

| AGA | | Patients No=60 | Control No=30 | P value |
|---|---|---|---|---|
| | | No (%) | No (%) | |
| IgA | Positive | 9 (15.0) | 0 (0.0) | 0.025* |
| | Negative | 51 (85.0) | 30 (100.0) | |
| IgG | Positive | 26 (43.3) | 0 (0.0) | 0.004* |
| | Negative | 34 (56.7) | 30 (100.0) | |

*Significant using Pearson Chi-square test at 0.05 level of significance. AGA; antigliadin antibody

**Table(2): Frequency distribution of tTG antibodies (IgA & IgG) among the study groups.**

| tTG | | Patients No=60 | Control No=30 | P value |
|---|---|---|---|---|
| | | No (%) | No (%) | |
| IgA | Positive | 8 (13.3) | 0 (0.0) | 0.036* |
| | Negative | 52 (86..7) | 30 (100) | |
| IgG | Positive | 9 (15.0) | 0 (0.0) | 0.025* |
| | Negative | 51(85.0) | 30 (100) | |

*Significant using Pearson Chi-square test at 0.05 level of significance. tTG; tissue transglutaminase

**Table(3): Frequency distribution of AGA antibodies (IgA, IgG, IgAIgG) and tTG antibodies (IgA, IgG, IgAIgG) positive titers among patients group.**

| Test | | +ve IgA | +ve IgG | +ve IgAIgG | -ve IgAIgG | Total |
|---|---|---|---|---|---|---|
| AGA | No (%) | 1 (1.7) | 18 (30.0) | 8 (13.3) | 33(55.0) | 60 |
| tTG | No(%) | 1 (1.7) | 2 (3.3) | 7 (11.7) | 50(83.3) | 60 |

AGA; antigliadin antibody, tTG; tissue transglutaminase

**Table(4): Serological tests and biopsy results of five celiac children.**

| No | IgA-AGA U/mL | IgG-AGA U/mL | IgA-tTG RU/mL | IgG-tTG RU/mL | Biopsy |
|---|---|---|---|---|---|
| 1 | 74.1 | 61.3 | 75.1 | 74.3 | + |
| 2 | 119.3 | 107.8 | 245.8 | 250 | + |
| 3 | 28.9 | 42.7 | 113.7 | 95.7 | + |
| 4 | 110.2 | 118.5 | 247.2 | 240.8 | + |
| 5 | 127.7 | 60.3 | 266.3 | 214.1 | + |

**AGA; antigliadin antibody, tTG; tissue transglutaminase**

## Discussion:

The study was carried out in Kirkuk governorate/Iraq in order to diagnose celiac disease, serological markers including immunoglobulin A and G for all antigliadin antibody (AGA) and anti-tissue transglutaminase antibody (tTG) were used. Gliadin antibodies were less specific and sensitive than anti-tTG antibodies, except in children younger than 2 years of age, in whom anti-gliadin antibodies measure was more sensitive test [15].

The present study revealed that the seropositive of IgA-AGA was (15.0%) and IgG-AGA (43.3%) as compared with no positive result of IgA-AGA and IgG-AGA in control group. This finding is disagree with a study in India which showed that the seropositive of IgA-AGA was (10%) and IgG-AGA (74%) among clinically suspected children compared with only seropositive of IgG-AGA (4%) in healthy control [16].

Immunoglobulin G (IgG)-AGA is very sensitive but less specific, and IgA-AGA is less sensitive but more specific. Their use in combination can give results of a high detection rate [17]. The current study revealed that the seropositive of IgA IgG-AGA was (13.3%). This finding is lower than that the study showed in Sudan which found that (31.3%) positive for IgA IgG-AGA [18].

Many individuals without CD express IgG-AGA antibody [19].This was borne out by this study as 18 children were IgG positive and IgA negative. However, AGA is not disease specific, and some patients with active CD lack these antibodies. In contrast, patients with diseases other than CD and healthy individuals occasionally have elevated levels of IgA-AGA and IgG-AGA [20].

Immunoglobulin A (IgA) against tissue transglutaminase (tTG) antigen is considered as the best serology screening tool performed by ELISA method (21). The current study showed that the frequency of IgA-tTG was (13.3%). This finding is higher than that in a study in Iran which found that the prevalence of CD was (6%) among 350 patients suspected to have CD depending on IgA-tTG and histopathological examination method [22]. This study also revealed that the seropositive of IgA IgG-tTG was (11.7%). This finding is higher than that in a study in Egypt which found the percentage was (4.7%) among 150 patients with suspected CD depending on tTG (IgA & IgG) and histopathological examination method [23]. This difference is attributed to the heterogeneity of the studied populations, subject selection, diagnostic strategies used, and whether confirmatory biopsies were performed or not [24].

Immunoglobulin G (IgG) tTG and IgA-tTG were used in combination as a screening test for celiac disease to assess IgA deficiency. The present study revealed that among 18 children positive for IgG-AGA and negative for IgA-AGA, only 2 children showed positive for IgG-tTG test while negative for IgA-tTG. This finding indicates that they may have IgA deficiency, and so it agrees with a study conducted that IgG-tTG is reliable serological assay for the diagnosis of CD in patients with selective IgA deficiency and perform better than IgG-AGA [25].

IgA deficiency is the most common human immunodeficiency and it is 10-15 times more common in CD patients. Approximately 3% of CD patients have this deficiency, which may produce false-negative for anti-tTG IgA and AGA IgA [26].

Only five children were introduced for biopsy due to difficulty in handling the histopathological examination, and all of them were seropositive for AGA (IgA & IgG) and tTG (IgA & IgG). The diagnosis of these five children consistent with characteristics CD. This finding is in agreement with a study which found that high levels of IgA-tTG and IgG-tTG antibodies were associated with the grade of mucosal villous atrophy and a more severe clinical presentation, and that the combined measurement of IgA-tTG and IgG-tTG enables a noninvasive prediction of small intestinal villous atrophy with high accuracy, and may reduce the need for a biopsy in patients with suspected CD [27].

## References:


1. Freeman HJ. Celiac disease (gluten-sensitive enteropathy). Minerva *Gastroenterol. Dietol.* (2010); 56: 245-249.
2. Silano M, Agostoni C & Guandalini S. Effect of the timing of gluten introduction on the development of celiac disease. *World. J. Gastroenterol.* (2010); 16(16): 1939–1942.
3. Papadopoulos GK, Wijmenga C & Koning F. Interplay between genetics and the environment in the development of celiac disease: perspectives for a healthy life. *J. Clin. Investig.* (2001); 108(9): 1261-6.
4. Westerberg DP, Gill JM, Dave B, DiPrinzio MJ, *et al*. New strategies for diagnosis and management of celiac disease. *J. Am. Osteopath. Assoc.* (2006); 106(3): 145-51.
5. Compilato D, Campisi G, Pastore L & Carroccio A. The production of the oral mucosa of antiendomysial and anti-tissue transglutaminase antibodies in patients with celiac disease: a review. ***Scientif.*** *W. J.* **(**2010); 10: 2385-94.
6. Catassi C. & Fasano A. Celiac Disease. *Curr Op In Gastroenterol.* (2008); 24(6): 687-691.
7. Mohamad T, Maki H. Fayadh & Sana A. Broad look on Celiac disease in Iraq. (2004); M.Sc. thesis submitted to College of Medicie/ Baghdad University.
8. Green PH & Jabri B. Celiac disease. *Lancet*. (2003); 362: 383–91.
9. Presutti RG, Cangemi JR & Cassidy HD. Celiac disease. *Am. Fam. Phys.* (2007); 76: 1795-1802.
10. . Farrell RJ & Kelly CP. Diagnosis of celiac sprue. *Am. J. Gastroenterol.* (2001); 96: 3237–46.
11. Reeves GE, Squance ML, Duggan AE, *et al.* Diagnostic accuracy of coeliac serological tests: a prospective study. *Eur. J. Gastroenterol. Hepatol*. (2006); 18: 493–501.
12. Hill ID, Dirks MH, Liptak GS, Colletti RB, *et al.* Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. *J. Pediatr. Gastroenterol. Nutr.* (2005); 40: 1-19.

13. Donaldson MR, Firth SD, Wimpee H, Leiferman KM, *et al.* Correlation of duodenal histology with tissue transglutaminase and endomysial antibody levels in pediatric celiac disease. *Clin. Gastroenterol. Hepatol.* (2007); 5: 567-573.
14. Greetje J. Tack, Wieke H. M. Verbeek*, et al*. The spectrum of celiac disease: epidemiology, clinical aspects and treatment. *Gastrolo.& Hepatol*. (2010); 7: 204-213.
15. Rostom A, Dubé C, Cranney A, Saloojee N. *et al.* The diagnostic accuracy of serologic tests for celiac disease: a systematic review. *Gastroenterol.* (2005); 128 (Suppl. 1): S38–S46 .
16. Yachha SK, Aggarwal R, Srinivas S, Srivastava A, *et al.* Antibody testing in Indian children with celiac disease. *Indian J. Gastroenterol.* (2006); 25(3): 132-5.
17. Misra S & Ament ME. Diagnosis of coeliac sprue in 1994. *Gastroenterol. Clinics. of North America*. (1995), 24(1): 133–43.
18. Mohammed I.M., Karrar Z.E.A. & El-Safi S.H. Celiac disease in Sudanese children with clinical features suggestive of the disease. *Eastern Mediterranean Health Journal.* ( 2006); 12(5): 582-589.
19. Carroccio A, Iacono G, Montalto G, *et al.* Immunologic and absorptive tests in celiac disease: can they replace intestinal biopsies? *Scand. J. Gastroenterol*. (1993); 28: 673–6.
20. Bürgin-Wolff A, Gaze H, Hadziselimovic F, Huber H, *et al.* Antigliadin and antiendomysium antibody determination for celiac disease. *Arch. Dis. Child* . (1991); 66: 941-7.
21. Fasano, A., M. Araya, S. Bhatnagar, D. Cameron, *et al.* Federation of international societies of pediatric gastroenterology, hepatology and nutrition consensus report on celiac disease. *J. Pediatr. Gastroenterol. Nutr*. (2008); 47: 214–219.
22. Emami MH, Karimi S, Kouhestani S, Hashemi M, *et al*. Diagnostic Accuracy of IgA anti-Tissue Transglutaminase in Patients Suspected of Having Celiac Disease in Iran. *J. Gastrointestine Liver Dis.* (2008); 17(2): 141-6.
23. Abu-Zekry M, Kryszak yD, Diab M, *et al*. Prevalence of Celiac Disease in Egyptian Children Disputes the East–West Agriculture-dependent Spread of the Disease. *J. Pediatr. Gastroenterol. Nutr. (*2008); 47: 136–140.
24. Barada K, Bitar A, Abdul-Razak M. Celiac disease in Middle Eastern and North African countries: A new burden?. *World. J. Gastroenterol*. (2010); 16(12): 1449–1457.
25. Villalta D, Alessio MG, Tampoia M, Tonutti E, *et al*. Testing for IgG class antibodies in celiac disease patients with selective IgA deficiency. A comparison of the diagnostic accuracy of 9 IgG anti-tissue transglutaminase, 1 IgG anti-gliadin and 1 IgG anti-deaminated gliadin peptide antibody assays. Clin. Chim. Acta. (2007); 382(1-2): 95-9.
26. Silva TSG & Furlanetto TW. Diagnosis of celiac disease in adults. *Rev. Assoc. Med. Bras.* (2010); 56(1): 122-6.
27. Dahlbom I, Korponay-Szabo IR, Kovacs JB *et al.* Prediction of clinical and mucosal severity of coeliac disease and dermatitis herpetiformis by quantification of IgA/IgG serum antibodies to tissue transglutaminase. *J. Pediatr. Gastroenterol. Nutr.* (2010) ;50: 140-146.