Autoantibodies Status in a Sample of Iraqi Celiac Disease Patients

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Abstract
Fifty celiac disease (CD) patients (21 males and 29 females) with an age range of 2-35 years and 25 apparently healthy controls were investigated for 10 autoantibodies (anti-tissue transglutaminase IgA antibody; ATA, anti-tissue transglutaminase IgG antibody; ATG, anti-gliadine IgA antibody; AGA, anti-gliadine IgG antibody; AGG, anti-nuclear antibody; ANA, anti-double strand DNA antibody; AdsDNA, anti-thyroid peroxidase antibody; ATP, anti-phospholipid antibody; APP, anti-myeloperoxidase antibody; AMP and anti-proteinase 3 antibody; AP3) in their sera. Six autoantibodies (ATA, ATG, AGA, AGG, AMP and AP3) showed significant variations between CD patients and controls. The first four antibodies were not detected in sera of controls, while in patients, their percentage frequencies were 44.0, 22.0, 56 and 72.0%, respectively. With respect to AMP autoantibody, 36.0% of patient's sera were positive for this autoantibody; while in controls, the corresponding frequency was much lower (7.70%). AP3 autoantibody behaved in a similar manner, and its frequency was higher in patients compared to controls (46.0 *vs*. 19.2%).Autoantibody AGG recorded the highest sensitivity and specificity (76.0)(100.0) respectively.
Keywords: Autoantibodies Status in a Sample of Iraqi Celiac Disease Patients

حالات الاضداد الذاتية في عينة دم المرضى العراقيين المصابين بحساسية الحنطة

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الخلاصة:
اجريت الدراسة على 50 عينة مصل دم مرضى مصابين بداء الزلاقي (21 ذكور و 29 اناث) باعمار تتراوح من 2–35 سنة و 25 فرد من الاصحاء ظاهريا كسيطرة للكشف عن انواع من الاضداد الذاتية في مصل دم المرضى (اضداد النسيج ترانس كلوتامينيز IgA (ATA) واضداد النسيج ترانس كلوتامينيز IgG(ATG) واضداد gliadin IgA (AGA) واضداد IgG gliadin (AGG) واضداد النوي للاضداد ANA واضداد DNA (Ads DNA) واضداد الدرقية البيروكسيديز(ATP) واضداد المكافحة الفوسفورية.
(APP) و اضداد الميلوبيروكسيديز (AMP) واضداد للبروتين 3 (AP3) . اظهرت النتائج بان ستة اضداد ذاتية (ATA–ATG –AGA– AGG–AMP and AP3) اختلافات معنوية بين مرضى الداء الزلاقي ومجموعة السيطرة لم تظهر الاضداد الاربعة الاولى في مصول السيطرة بينما في مصول دم المرضى كانت نسبتهم المئوية 72% و 56 , 22.0, 44.0 على التوالي . اما بالنسبة للضد الذاتي AMP فكانت النسبة 36% بينما كان تردده في مجموعة السيطرة 7.7% فضلا عن السلوك المتشابه للضد الذاتي AP3 بتردد عالي بالمرضى 46% قياسا مع مجموعة السيطرة 19%. الضد الذاتي AGG سجل اعلى حساسية، وخصوية بلغ (76.0) و (100.0) بالترتيب.

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Introduction

Celiac disease (CD) is one of the most common chronic inflammatory conditions affecting human beings. It has been identified throughout the world, and its prevalence is approximately 1% in the general population, but there are emerging data to suggest that its prevalence might be increased in recent years [1]. It is an immune-mediated enteropathy, in which several autoimmune features have reported; for instance, production of highly disease-specific IgA and IgG autoantibodies to tissue transglutaminase, and presence of small intestinal intraepithelial lymphocytes, which can mediate direct cytotoxicity to enterocytes [2].

Etiologically, genetic and environmental components have been suggested to have role. Evidence for a genetic component has been presented by the strong association with the presence of HLA-DQ2 (encoded by the alleles DQA1*05 and DQB1*02) and HLA-DQ8 (DQA1*03 and DQB1-*0302) alleles, and more than 95% of CD patients express HLADQ2 while the remainder express HLA-DQ8 [3-4]. With respect to environmental factors, the major environmental trigger is ingestion of gluten, which is a protein fraction of wheat, barley, and rye. Such protein is rich in two amino acids (glutamine and praline), which render it to be not completely digested. These residual partially digested peptides can initiate innate and adaptive immune responses in those genetically predisposed individuals to develop CD [5].

However, it has also been demonstrated that multiple autoimmune diseases can occur in CD patients, but only some hypotheses are exist to explain the concordance of separate autoimmune diseases in CD patients, and elucidation of the mechanisms of multiple autoimmune conditions in the same individual will provide deeper understanding of the specific conditions that lead to co-occurrence of CD and other autoimmune diseases [6]. In this regards, it has also observed that different types of autoantibodies can occur in CD patients of different ethnicities [7]; therefore the present investigation aimed to shed light on the autoantibody status in sera of Iraqi patients with CD.

Subjects, Materials and Methods

Subjects: The study was approved by the Medical Ethics Committee of the Iraqi Ministry of Health, in which 50 CD patients (21 males and 29 females) with an age range 2-35 years were enrolled. The patients attended the Gastrointestinal and Liver Diseases Hospital in Baghdad during the period May – July 2013 for diagnosis and treatment. The diagnosis was made by the consultant medical staff at the hospital, which was based on a clinical examination and laboratory tests that included assessment of anti-gliadin and anti-endomysium (EMA) antibodies in their sera, and only positive cases were involved. For the purpose of comparison, 25 apparently healthy controls (blood donors) were also enrolled, and matched patients for gender and age.

Methods: From each participant, 5 ml blood was collected in a plain tube and allowed to clot at room temperature. The clotted blood was centrifuged (2000 rpm for 10 minutes) and serum was collected, distributed into aliquots and frozen at -20°C until assessment for autoantibody status. The assessments included 10 autoantibodies (anti-tissue transglutaminase IgA antibody; ATA, anti-tissue transglutaminase IgG antibody; ATG, anti-gliadine IgA antibody; AGA, anti-gliadine IgG antibody; AGG, anti-nuclear antibody; ANA, anti-double strand DNA antibody; AdsDNA, anti-thyroid peroxidase antibody; ATP, anti-phospholipid antibody; APP, anti-myeloperoxidase antibody; AMP and anti-proteinase 3 antibody; AP3) by ELISA methods using commercially available kits (AESKU.Diagnostics GmbH) and the manufacture's instructions were followed.

Statistical analysis: The data were presented as percentage frequencies, and significant difference between these frequencies were assessed by two tailed Fisher's exact probability, which was estimated by using the computer package PEPI version 4. Estimations of sensitivity and specificity, positive predicative value (PPV) and negative predicative value (NPV) were made according to [8].

Results and Discussion

Six autoantibodies (ATA, ATG, AGA, AGG, AMP and AP3) showed significant variations between CD patients and controls. The first four antibodies were not detected in sera of controls, while in patients, their percentage frequencies were 44.0, 22.0, 56.0 and 72.0%, respectively. The estimated Fisher's exact probabilities of these differences were 2.4×10^{-5}, 0.013, 3.7×10^{-7} and 3.5×10^{-11}, respectively, which were highly significant, especially in the case of AGG. With respect to AMP autoantibody, 36.0% of patient's sera were positive for this autoantibody; while in controls, the corresponding frequency was much lower (7.70%), and the difference was significant (P = 0.01). AP3 antibody behaved in a similar manner, and its frequency was significantly (P = 0.04) higher in patients

compared to controls (46.0 *vs*. 19.2%). For ANA, AdsDNA, ATP and APP antibodies, neither patients nor controls were positive for them (Table 1).

Table 1: Observed numbers and percentage frequencies of autoantibody positive cases in celiac disease patients and controls.

Autoantibody	Autoantibody Positive Cases				Fisher's Exact P (two-sided)
	Patients (No.= 50)		Controls (No.= 26)		
	No.	%	No.	%	
Anti-tissue transglutaminase IgA antibody	22	44.0	0	0.0	2.4×10^{-5}
Anti-tissue transglutaminase IgG antibody	11	22.0	0	0.0	0.013
Anti-gliadine IgA antibody	28	56.0	0	0.0	3.7×10^{-7}
Anti-gliadine IgG antibody	38	72.0	0	0.0	3.5×10^{-11}
Antinuclear antibody	0	0.0	0	0.0	N.S.
Anti-double strand DNA antibody	0	0.0	0	0.0	N.S.
Anti-thyroid peroxidase antibody	0	0.0	0	0.0	N.S.
Anti-phospholipid antibody	0	0.0	0	0.0	N.S.
Anti-myeloperoxidase antibody	18	36.0	2	7.7	0.01
Anti-proteinase 3 antibody	23	46.0	5	19.2	0.04

N.S.: Not significant ($P > 0.05$)

It is worth to mention that there was no significant variation in the distribution of these autoantibodies in CD patients distributed by gender (Table 2).

Table 2: Observed numbers and percentage frequencies of autoantibody positive cases in celiac disease patients distributed by gender.

Autoantibody	Autoantibody Positive Celiac Disease Patients				Fisher's Exact P (two-sided)
	Males (No.= 21)		Females (No.= 29)		
	No.	%	No.	%	
Anti-tissue transglutaminase IgA antibody	10	47.6	12	41.4	N.S.
Anti-tissue transglutaminase IgG antibody	4	19.0	7	24.1	N.S.
Anti-gliadine IgA antibody	14	66.7	14	48.3	N.S.
Anti-gliadine IgG antibody	16	76.2	22	75.9	N.S.
Anti-myeloperoxidase antibody	6	28.6	12	41.4	N.S.
Anti-proteinase 3 antibody	9	42.9	14	48.3	N.S.

N.S.: Not significant ($P > 0.05$)

The deviated autoantibodies were further evaluated for sensitivity, specificity, PPV and NPV. It was found that AGG autoantibody scored the highest sensitivity and specificity (76.0 and 100.0%, respectively), followed by AGA (56.0 and 100.0%, respectively), ATA (44.0 and 100.0 %, respectively), AP3 (46.0 and 80.8%, respectively), AMP (36.0 and 92.3%, respectively), and finally ATG (22.0 and 100.0%, respectively). The highest NPV was observed in AGG, which was 68.4% (Table 3).

Table 3: Estimation of sensitivity and specificity, positive predicative value and negative predicative value for autoantibodies in celiac disease patients.

Parameter	Percentage					
	ATA	ATG	AGA	AGG	AMP	AP3
Sensitivity	44.0	22.0	56.0	76.0	36.0	46.0
Specificity	100.0	100.0	100.0	100.0	92.3	80.8
Positive predictive value	100.0	100.0	100.0	100.0	90.0	82.1
Negative predictive value	48.1	40.0	54.2	68.4	42.9	43.8
Probability	2.4×10^{-5}	0.013	3.7×10^{-7}	3.5×10^{-11}	0.01	0.04

ATA: Anti-tissue transglutaminase IgA antibody.
ATG: Anti-tissue transglutaminase IgG antibody.
AGA: Anti-gliadine IgA antibody.
AGG: Anti-gliadine IgG antibody.
AMP: Anti-myloperoxidase antibody.
AP3: Anti-proteinase 3 antibody.

The present findings demonstrate that some autoantibodies were specifically associated with CD (ATA, ATG, AGA and AGG), while others were shared between patients and controls (AMP and AP3), although their frequencies were significantly higher in the patients, and accordingly the autoimmune nature of CD is confirmed and its association with other disease-non-specific autoantibodies is also strengthen. The latter subject (one of the most controversial issues concerning possible complications of untreated CD) involves the association between CD and other autoimmune disorders [6], and studies highlighted the tendency for multiple autoimmune disorders to occur over lifetime of a patient with CD. The most frequent reported associations are with type 1 diabetes mellitus (3 to 8%), autoimmune thyroiditis and, more rarely, Addison's disease [9];[10]. Furthermore, several screening programs and case reports have revealed associations between CD and chronic autoimmune hepatitis, primary biliary cirrhosis and systemic lupus erythematosus [11]. A further studied the frequency of autoimmune antibodies in 56 CD patients and their 118 first-degree relatives [9]. All serum samples were tested for EMA, anti-neutrophil cytoplasmic (ANCA), anti-smooth-muscle (SMA), anti-mitochondrial (AMA), ANA, anti-liver-kidney microsomal (LKM), anti-gastric parietal cells (PCA), and anti-thyroidmicrosome (TMA) antibodies. EMA autoantibodies were detected in 100% of CD patients ingesting gluten and in 16.1% of the first-degree relatives. Twenty five percent of CD patients were positive for at least one of the autoantibodies, with significant prevalence of TMA, ANA and PCA, while the relatives showed 17.8% of positivity. A further studied 35 children with CD disease and 35 healthy controls for serum autoantibodies [12]. CD patients had increased prevalence of serum anti-single-stranded DNA (14%), AdsDNA (23%), anticardiolipin (14%) and EMA (63%). Further studies have observed that a significantly increased prevalence of other autoimmune diseases has been reported in individuals with CD and their first-degree relatives as compared to controls, with an estimated burden of autoimmune diseases in CD cases up to 15% [13]. In CD patients, an early diagnosis in life and having a family history of autoimmunity has been considered as risk factors for developing other autoimmune diseases, while the gluten-free diet has a protective effect. By contrast, in relatives of CD cases, the prevalence of autoimmune diseases rises with the age. Conversely, a significantly increased prevalence of CD has been documented in individuals with other AD [14];[15]. It has been suggested that these associations among CD and other autoimmune diseases may be explained by the sharing of a common pathogenic basis involving genetic susceptibility, similar environmental triggers, and the loss of intestinal barrier secondary to dysfunction of intercellular tight junctions with increased intestinal permeability, and possibly by other undiscovered mechanisms [12]; [16].

Regarding the diagnostic value of the investigated autoantibodies, ATA, ATG, AGA and AGG antibodies are of importance because the estimated specificity and PPV of them were 100%, although different sensitivities were observed. Small intestine biopsy is the widely accepted gold standard for the diagnosis of CD disease. However, given the invasiveness of endoscopy, the current guidelines recommend serological testing for CD screening [17], in which testing for human anti-tissue transglutaminase antibodies appears to give more consistent results and is likely to be the best test for CD, and initial reports have shown a sensitivity of 95 – 98% and a specificity of 94%. An even higher sensitivity (99.5%) and specificity (99.6%) have been achieved using recombinant human anti-tissue transglutaminase in a radioligand assay, confirming the reliability of such antibody testing [18]. It has also been suggested that ATA antibodies can replace endomysial antibodies as the preferred serological marker due to the ease of their use, their high sensitivity and specificity, and the high correlation between high titers and intestinal mucosal lesions [19]. The present study is in a good agreement with such suggestion and ATA antibodies were observed in 44% of CD patients, while none of the controls was positive for ATA antibodies.

In conclusion, the present results highlighted two aspects of CD in Iraqi patients. In the first, AMP and AP3 antibodies were observed with a significant increased frequency in patients, although they are

not specific for CD, while in the second aspect, the diagnostic value of ATA antibodies was observed. However, the study may have some limitations, which are related to the sample size and the number of autoantibodies investigated. Therefore, future work should include larger number of patients and controls and autoantibodies that specify a wide range of autoimmune diseases.

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