ASSOCIATION OF CELIAC DISEASE WITH HLA-DRB1 AND HLA-DQB1 ALLELES IN A SAMPLE OF IRAQI PATIENTS

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ABSTRACT

Celiac disease (CD) is a complex disorder triggered by gluten affecting genetically predisposed individuals. The CD is triggered by the binding of one or more gliadin peptides to CD associated HLA class II molecules. Fifty patients with CD and fifty control group were studied. The sera were qualitativelymeasured for anti-TTG-IgA,IgG antibodies and anti-gliadin- IgA,IgG antibodies by ELISA method. The HLA class II (DRB1, DQB1) were genotyped by using Polymerase Chain Reaction-Sequence Specific Primers (PCR-SSP). In the current studypositivity for Anti-TTG antibodies showed a frequency of 38% in CD patients as compared with the control group 0.0%, while high frequency of Anti-gliadin antibodies positivity in celiac disease patient's sera showed 22% as compared with the control group 0.0%with a highlysignificant difference were highly (P=0.001).Human leukocyte antigen genotyping revealed that the DR-alleles, DRB1*03(01,06,08,10), DRB1*0701 and DQB1*02 (01,02) showed highly significant increased frequency in CD as compared withthe controls, while the DRB1*1302 and DQB1*0601 alleles showed significant decreased frequency in CD when compared with the control groups.

Key words: Celiac disease(CD), Anti-gliadinantibodies (AGA), anti-tissue transglutaminase antibodies (TTG), Human leukocyte antigen (HLA), PCR-SSP

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علاقة مرض حساسية الحنطة بالنمط الوراثي لمستضد كريات الدم البيضاء الصنف الثاني في عينة من المرضى العراقيين

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الخلاصة

إن مرض حساسية الحنطة يحفز بواسطة الكلوتين الذي يتأثر بالإستعداد الوراثي للبشر والتأثيرات البيئية، إذ أن مرض حساسية الحنطة يحفز عن طريق إرتباط واحد أو أكثر من ببتيدات الكلوتين بالمرض والمتعلق بمستضدات الكريات الدم البيضاء الصنف الثاني. تم دراسة خمسون عينة من مرضى حساسية الحنطة ومثلها من مجموعة السيطرة. تم قياس Anti-gliadin antibody , Anti-TGG antibodies في مصول المرضى ومجموعة السيطرة بطريقة اليزا. تم تحديد الأنماط الوراثية لمُستضد الكريات الدم البيضاء الصنف الثاني بتقنية البادئ المناوع لتعاقب سلسلة تفاعل البلمرة (PCR- SSP). إن إيجابية فحص (Anti-gliadin antibody) بلغت 22% لمرضى حساسية الحنطة مقارنة بفحص(Anti-TGG antibodies) والذي بلغت ايجابية الفحص 38% مقارنة بمجموعة الأصحاء مع فارق عالي المعنوية(P=0.001). أظهر التنميط الجيني بأن الأليلات DR *0701 ,(01,06,08,10)03، DQB1*02 (01,02) مرتبطان بمرض حساسية الحنطة وبفارق معنوي عالي مقارنة بمجموعة الأصحاء (Pc=0.0001) ، في حين أظهر الأليلان DRB1*1302، DQB1*0601 تكرار واطئ المعنوية في مرضى حساسية الحنطة مقارنة بمجموعة الأصحاء (Pc=0.0001).

INTRODUCTION

Celiac disease (CD) is an autoimmune disorder which affectsgenetically predisposed individuals upon the ingestionof gluten(1).The CD can present at any age after the introduction of gluten into the diet and can affect organ systems other than gastrointestinal tract (2,3).Its prevalence has been underestimated, but it is now considered one of the most common geneticdisorders in the West with a prevalence of 1%-2.67%. (4,5) .As in many other immune-mediated inflammatory diseases, there are environmental, genetics, and immune components in its pathogenesis(6). This disease is widely considered an autoimmune disease due to serologic reactivity to tissue matrix auto antigens(1,7). The diagnosis is made by biopsy which demonstrates acute inflammatory infiltrates below the dermis and granulated deposits of IgA and complement at the basement membrane by immunohistochemistry.Gliadin peptides have not been formally proven in these immune complexes, but are inferred by the loss of IgA deposits with a gluten-free diet(1,8). The availability of new, simple, very sensitive and specific serological tests has shown that CD is as common in Middle Eastern countries as in Europe, Australia and New Zealand where the major dietary staple is wheat (6). The most common serological tests for initial screening of CD are tissue transglutaminase (TTG), gliadin antibodies and deamidatedgliadin peptides (DGPs) (9,10).The use of TTG antibody may replace the use of the small bowel biopsy to diagnose celiac disease in children (11). Genetics play a role in the development of the disease: as much as 98% of the celiac patients are HLA-DQ2 (95%) or –DQ8 (3%) positive. However, the majority of people with these genetic factors do not develop celiac disease. This suggests that additional genetic and/or environmental factors play a role in disease development. Many genetic and immunological studies have been performed in an attempt to unravel the complexity of this multi-factorial disease (12). In addition, the possible role of environmental factors, such as early feeding, in the development or prevention of celiacdisease has been studied (13).The aim of study is toevaluate the interest of anti-gliadin-antibodies and of anti-TTG for diagnosing coeliac disease and to elucidate the HLA DRB1 and DQB1 polymorphism in some Iraqi patients with typical form of celiac disease in comparison withapparently healthy control group.

MATERIALS AND METHODS

Samples collection

Fifty blood samples were collected from CD patients and Fifty samples from apparently healthy control .The diagnosis was made by the consultant medical staff at Alyarmok Teaching Hospitalfrom February 2011 to July 2011.The study population consisted mainly of adult patients (90% older than 18 years of age). Celiac patients and controls were similar in age and gender. The median of age was 47 years for coeliac group and 45 years for controls. Fiveml sample of venous blood was collected from each participating subject, and it was divided into two aliquots, 3 ml in plain tubes to collect serum and 2ml in EDTA tube.All samples were stored at -20°C until testing.

Serological tests

The sera were qualitativelyscreened for anti-gliadin- IgA and IgGantibodies (INOVA Diagnostics Inc.) and anti-TTG-IgA and IgG antibodies (BINDAZYME human IgA and IgG anti-tissue transglutaminase EIA kit) by ELISA methods.

HLA genotyping

Samples of EDTA blood (2ml) were used for DNA based HLA typing. The DNA-based HLA typing was performed using the polymerase chain reaction sequence-specific primer (PCR-SSP) method described previously byOlerup and Zetterquist(14). Briefly, DNA was extracted from peripheral blood by using EXTRA-GENE kit (Bag ,Germany), and the isolation was based on a selective erythrocyte lysis which was followed by a detergence break down step with subsequent salting out of the proteins and purification of DNA by precipitation.

Statistical Methods

The strength of an association between disease and genetic marker is generally expressed in terms of a relative risk value (RR). The level of significance (probability) is calculated by Fisher's exact probability (P) through constructing 2X2 contingency tables from the previous four entries (a, b, c and d) and to avoid a chance occurrence of an association (due to many comparisons), the P was multiplied by the number of alleles tested at each HLA locus; therefore the corrected probability (Pc) was given(15).

RESULTS AND DISCUSSION

High frequency of anti-TTG antibodies positivity was observed inceliac disease patient's sera 38% as compared with control group (0.0%). Positivity for anti-gliadin antibody showed a frequency of22% in CD patients while it was 0.0%in controls. Such difference were highly significant (P=0.001) table (1).

Table(1):Percentage of positivity of anti-gliadin antibody and anti-TTG antibody among celiac disease and apparently healthy control groups.

Studied groups		Anti-gliadin antibody(IgG,IgA)		anti-TTG antibody(IgG,IgA)		P value
		Negative	Positive	Negative	Positive	
CD patients	No.	39	11	31	19	P=0.001
	%	78.0*	22.0	62.0*	38.0	
Controls	N0.	50	0	50	0	
	%	100.0	0.0	100.0	0.0	

The frequency distribution was constructed to give an insight on which of the HLA- DRB1 and DQB1 alleles was deviated in fifty in CD patients. Regarding DRB1-locus, the statistical analysis revealed a highly significant increased frequency of: DRB1*03(01,06,08,10) and DRB1 *0701 alleles as compared with controls, (Pc =0.0001). The associated RR (4.94 and 13.50, respectively) and EF (0.414 and 0.48, respectively) demonstrated positive associations. On the other hand, DR*1302 allele showed significant decreased frequency(Pc=0.001) in CD patients when compared with controls(table 2).Among HLA-DQB1 alleles, it was observed that HLA-DQB1*02(01,02) might be considered as a risk factor due to its presence in high frequency (46%) among CD patients in comparison with healthy control (6%) with RR of 13.35, EF= 0.425 (Pc =0.0001),while the HLA-DQB1*0601 allele showed a significant decreased (Pc=0.0001) frequency in patients table(3).

Table(2):Observed and percentage frequencies of HLA-DRB1 alleles in celiac disease patients and controls.

HLA-DRB1 alleles	Controls(50)		CD patients (50)		RR	EF	PF	P-value	Pc
	No.	%	No.	%					
DR*01(01,02,04	8	16.0	5	10.0	058	-	0.06	0.375	NS
DR*03(01,06,08,10)	9	18.0	26	52.0	4.94	0.41	-	3.4×10^{-4}	0.0001
DR*04(01-22 not 0415)	11	22.0	7	14.0	0.58	-	0.09	0.300	NS
DR *1302	24	48.0	5	10.0	0.12	-	0.42	2.8×10^{-3}	0.0001
DR*08(01-19,not 0805,0818)	5	10.0	4	8.0	0.78	-	0.02	0.728	NS
DR *0415	9	18.0	5	10.0	0.50	-	0.08	0.475	NS
DR*1001	8	16.0	3	6.0	0.34	-	0.10	0.213	NS
DR*11(01-31 not 11(09,10,13,16,17,20, 22)	7	14.0	0	0	0	-	-	0.006	NS
DR*12(01-03,05)	5	10.0	5	10.0	1	0	0	1.0	NS
DR*13(01,05,06,09,10, 16,18,20,27,28,31)	5	10.0	5	10.0	1	0	0	1.0	NS
DR *14(02,06,19,20)	4	8.0	3	6.0	0.73	-	0.02	0.697	NS
DR*15(01-03,06)	8	16.0	4	8.0	0.46	-	0.08	0.221	NS
DR *16(01-08)	6	12.0	0	0.0	0	-	-	0.025	NS
DR *0819	6	12.0	1	2.0	0.15	-	0.10	0.132	NS
DR*0701	4	8.0	27	54.0	13.50	0.48	-	4.03×10^{-7}	0.0001

Table(3):Observed and percentage frequencies of HLA-DQB1 alleles in celiac disease patients and controls.

HLA-DQB1 alleles	Controls (50)		CD patients (50)		RR	EF	PF	P-value	Pc
	No.	%	No.	%					
DQB1*0501	10	20.0	11	22.0	1.13	0.03	-	0.807	NS
QB1* DQB1* 0303	5	10.0	6	12.0	1.23	0.02	-	0.75	NS
DQB1*06(02,10,11,13)	12	24.0	8	16.0	0.60	-	0.09	0.32	NS
DQB1*02(01,02)	3	6.0	23	46.0	13 .35	0.43	-	3.3×10^{-6}	0.0001
DQB1*0301	7	14.0	4	8.0	0.53	-0.07	0.06	0.34	NS
DQB1*03(02,07)	16	32.0	10	20.0	0.53	0	0.15	0.17	NS
DQB1*0601	22	44.0	4	8.0	0.11	-0.64	0.39	3.3×10^{-5}	0.0001
DQB1*0401	11	22.0	6	12.0	0.48	-0.13	0.11	0.19	NS

A number of antibodies have been described as having an association with CD. These include anti-gliadin and anti-TTG antibodies that have consistently been shown to have high sensitivity and specificity for CD.According to the current study, anti-gliadin positive results were 22%in CD patients.When comparing these results to other studies, the current study results ishigher than that reported by Rabab**,** in Saudi Arabia where the percentage was7.6%, among 145 patients with suspected CD depends on serological methods (16). In another study reported by van and west in America theprevalence of CD among suspected patients was 0.5% (17). Our result showed higher percentage of anti-TTG antibodyand when it was compared with that ofLiorente result (18). The difference between our study and previous studies could be due to study population (selection of high risk group, symptoms, number of subjectsenrolled in the study. Most studies which are concerned with the prevalence of CD amongsymptomatic patients showed lower percentage than our study.In the present work, there was highly significant association of DRB1*03(01,06,08,10), DRB1 *0701 and DQB1*02 (01,02) with CD patients , as compared with healthy control group while the frequency HLA-DRB1*1302 and HLA-DQB1*06 allele was highly significant increase in healthy control group when compared with CD patients.This result is similar with Brazilian study by Sliva*et al.* that revealed statistically significant increase of DRB1*03, DRB1*07and DQB1*02 alleles in Brazilian patients compared with healthy control group. The frequency of HLA-DQB1*06 alleles was significantly decreased in CD patients(19).On other hand, previous study explained that the DRB1*03, DQB1*02,DQA1*0501was associated with CD of Tunisia origin.As for protection alleles was detected ahigh frequency of DRB*13,DQA1*0102 and DQB1*06(20).All the studies indicate the association of particular alleles with CD varies among different races, over 95% of Celiac disease patients have an isoform of DQ2 and DQ8, which are inherited in families. The reason these alleles produce an increased risk of celiac disease is that the receptors formed by these genes bind to gliadin peptides more tightly than other forms of the antigen-presenting receptor.

Therefore, these forms of the receptor are more likely to activate T lymphocytes and initiate the autoimmune process (21). The DQA1*0501, DQB1*0201 and DQAl*0301,DQB1*0302 heterodimers occur at a much higher frequency in the human population than the overall frequency at which symptomatic cases of CD (22).

CONCLUSIONS

The results show that HLA-DRB1*03, HLA-DRB1*07, and HLA-DQB1*02 alleles may confer susceptibility to CD in Iraqi patients. In contrast, HLA-DRB1*1302 and HLADQB106 alleles may confer protection against development of the disease.

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