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Profile of HLA-DQ allele in a sample of Iraqi Celiac disease Patients.

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ABSTRACT

Celiac Disease (CD) is an intestinal inflammatory disorder that occurs in genetically predisposed individuals as a result of loss immunological intolerance to the wheat protein (gluten) and related proteins (prolamines) This study was carried out to investigate the possible association of HLA-DQBI alleles with celiac disease and severity of disease in some Iraqi patients with CD.. The results showed that the frequencies of human white blood cell antigens of DQBI * 02 alleles were significantly higher in CD patients compared to control group (75%, 35% respectively). This allele gave the highest value of relative risk (OR) (7.21) and the value of the causative factor (EF) (0.24), this is visualizing, it may be a causal factor for the occurrence of CD.

KEY WORDS: Celiac Disease, HLA genotype, DQBI allele polymorphism.

1. INTRODUCTION

Celiac disease (CD), is chronic inflammatory enteropathy induced in genetically predisposing individuals by ingestion of wheat gluten and related prolamines, leading to inappropriate reaction of immune system characterize by increased number of immune cells in lamina propria (LP) and signs of malabsorption [1]. Genetic predisposition is an important aspect of CD, it is major associated with the HLA-II system, which participates in the recognition of self and non self antigen, by the immune system. The variants of HLA-DQ2 and DQ8 are commonly presented in CD patients [2,3]. These genetic variants produce receptors that bind to gliadin peptides more tightly than other forms of the antigen presenting receptors. This may increase probability for immune cells activation and autoimmunity [4]. CD is multifactorial disorder, where HLA-DQ2 and/or HLA-DQ8 haplotypes are necessary but not sufficient for the development of disease. So, the presence of other genetic variations in several non-HLA genes, as well as environmental factors such as gluten introduction at childhood, infectious agents and socioeconomic features contribute to the development of celiac disease [5]. In Iraq, it has emerged as a major health-care problem in people of all ages, where the most of daily diet containing gluten as major component in our

country [6]. however, the current study aims at about survey of CD in specific samples of Iraqi patients by assessing the genotyping aspects in patients and apparently healthy subjects as controls.

2. MATERIAL AND METHOD

2.1 Patients and controls

A total 40 Iraqi Arab CD patients are included for this study, their age ranged from 2-55 years, were included for this study. They are attending the consultant clinic of Pediatric department in Pediatrician Welfare Teaching Hospital/ Medical city and Al-Kadhimiah Teaching Hospital, from October 2015 to March 2016. All, studied cases are diagnosed by clinicians and confirmed by serological and histological tests. 40 apparently healthy persons are included in this study as a control group, were selected randomly from Kidney transplant donor unit at Al-Karamah hospital. They had a negative history or clinical evidence for CD or any other chronic disease.

2.2 Collection of Blood

2 mls of venous blood are withdrawn from each subject and added in EDTA tube (1.5 mg / ml) and kept at –20 C for the genotyping of HLA-DQB1 alleles.

2.3 HLA Genotyping

Genomic DNA was extracted from peripheral blood samples using (**QIAamp DNA mini Kit, Germany**). All individuals were typed for DQB1 genes by specific sequence Oligonucleotide Probe -polymerase chain reaction (SSOP-PCR) method. In less than 60 minutes, DNA is extracted without the need to prepare any reagents or solutions. The yield is approximately 5-30 µg of DNA from 500µl of whole blood (Table 3). Based on addition of the sample of DNA to a composition of reagent which contained the following components: biotinylated primers, deoxynucleoside 5`-triphosphates (dNTPs) and thermostable DNA polymerase. By PCR-SSOP, the DNA for a whole region (e.g the HLA-DQ gene region is amplified. Then amplified DNA is tested by adding label oligonucleotide probes, which are complementary for DNA sequences, characteristic for certain HLA antigens. These probes will then type for the presence of specific DNA sequences of HLA genes. As a result, the Auto-LiPA-48 is designed as walk-away system with features of full automation of heating, cooling, aspiration and pipetting, finally reading of results by using the LiRAS software.

2.4 Statistical Analysis

The association between disease and genetic factor 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.

3. RESULT

Comparison between frequency of distribution of HLA- alleles for the CD patients and control groups, tables (1,2 and 3), showed several alleles deviations in their frequencies. Regarding DQBI locus, by Hardy-Weinberg equilibrium (HWE) calculations, the statistical analysis revealed a significantly increased frequency for only the: DQBI*02:02(27.50%), with OR of (7.21), (p = 0.006). As well as the DQBI*02:06 (20%) alleles are presented with higher frequencies in CD patients than healthy control group with OR of (3.08), but it did not reveal any significance, tables (1 and 2). In general, the statically analysis revealed a significantly increased frequency for the DQB1*02(%75) CD patients as compared with healthy control (%35), while DQBI*03 (%55) alleles are showed increased frequency for the healthy control with OR's of 0.44, comparison with CD patients table (3).

Table 1 observed and expected genotype frequencies of HLA-DQB1 alleles and their Hardy-Weinberg equilibrium (HWE) in celiac disease patients and controls.

HLA-DQB1 Genotypes	Celiac Disease Patients (No.= 40)				Controls (No. = 40)			
	Observed		Expected		Observed		Expected	
	No.	%	No.	%	No.	%	No.	%
01:01	Not detected				Not detected		0.03	0.08
01:02	Not detected				Not detected		0.40	1.00
01:03	Not detected				Not detected		0.68	1.70
01:04	Not detected				1	2.50	0.15	0.38
01:05	Not detected				1	2.50	0.38	0.95
01:06	Not detected				Not detected		0.35	1.33
02:02	11	27.50	10.51	26.28	2	5.00	1.60	4.00
02:03	7	17.50	8.71	21.78	4	10.00	5.40	13.50
02:04	1	2.50	0.51	1.28	2	5.00	1.20	3.00

02:05	3	7.50	4.10	10.25	3	7.70	3.00	7.50
02:06	8	20.00	6.66	16.65	3	7.50	2.80	7.00
03:03	3	7.50	1.81	4.53	5	12.50	4.56	11.40
03:04	Not detected		0.21	0.53	3	7.50	2.03	5.08
03:05	4	10.00	1.70	4.25	6	15.00	5.06	12.65
03:06	Not detected		2.76	6.90	4	10.00	4.73	11.83
04:04	Not detected		0.01	0.03	Not detected		0.23	0.58
04:05	Not detected		0.10	0.25	Not detected		1.13	2.83
04:06	Not detected		0.16	1.50	Not detected		1.05	2.63
05:05	Not detected		0.40	1.00	2	5.00	1.41	3.53
05:06	1	2.50	1.30	3.25	1	2.50	2.63	6.58
06:06	2	5.00	1.06	2.65	3	7.50	1.23	3.08
HWE	Chi-square = 9.84; D.F. = 10; $p > 0.05$				Chi-square = 15.35; D.F. = 15; $p > 0.05$			

Table 2 Distribution of HLA-DQB1 genotypes in CD patients and controls.

HLA-DQB1 Genotypes	Celiac Disease Patients (No. = 40)		Controls (No. = 40)		Odd Ratio	EF or PF	*P*	95% Confidence Interval
	No.	%	No.	%				
02:02	11	27.50	2	5.00	7.21	0.24	0.006	1.51-34.38
02:06	8	20.00	3	7.50	3.08	0.14	N.S.	0.77-12.39
02:02+02:06	19	47.50	5	12.5	6.33	0.40	0.001	2.09-19.21

EF: Etiological fraction; PF: Preventive fraction; *p*: Probability; N.S.: Not significant.

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

HLA-DQB1 Alleles	Celiac Disease Patients (No. = 40)		Controls (No. = 40)		Odd Ratio	EF or PF	Fisher's Exact Probability	95% Confidence Interval
	No.	%	No.	%				
*01	0	0.0	2	5.0	0.19	0.05	N.S.	0.01 - 3.94
*02	30	75.0	14	35.0	5.57	0.62	6.5×10^{-4}	2.15 - 14.47

***03**	**14**	**35.0**	**22**	**55.0**	**0.44**	**0.31**	**N.S.**	**0.18 - 1.07**
***04**	**1**	**2.5**	**6**	**15.0**	**0.15**	**0.13**	**N.S.**	**0.02 - 1.23**
***05**	**8**	**20.0**	**12**	**30.0**	**0.58**	**0.13**	**N.S.**	**0.21 - 1.61**
***06**	**11**	**15.0**	**11**	**15.0**	**1.00**	**0.00**	**N.S.**	**0.38 - 2.64**

EF: Etiological fraction; PF: Preventive fraction; N.S.: Not significant (p > 0.05).

4. DISCUSION

Numerous studies demonstrated that HLA region plays a critical role in the genetic predisposition to many systemic autoimmune diseases, like CD. Specifically, HLA class II-DQ region containing genes encoding two alleles (HLA-DQ2 and HLA-DQ8). Although, not enough familial data in the Iraqi population are available to quantify the relative contribution of HLA and other genes in the pathogenesis of this disease. The study aims at finding out the HLA class II alleles that may play an important protective role for the development of CD. In this work, HLA-DQB1 genes is investigated in a sample of Iraqi Arab patients with CD who lived in Baghdad province, by molecular method, PCR-SSOP. HWE is used to test the deviations of the observed frequency from expected which was constructed on presumption of normal separation of alleles in a population assuming no linkage, no selection, no mutation and surely no migration. The association between the HLA alleles carried out by making a comparison between the observed and expected values, Table (1). Interestingly, the observed alleles are higher than the expected for some genotyping groups, in this case either the non- significant differences might be attributed to the small number of observations or the contribution of such alleles was too little to be associated with the incidence of the disease. The observed probability for specific alleles is more than the expected, which means abnormal separation of alleles and the most probable cause would be linkage disequilibrium. Another possibility is that, disease –driving genes are closely related to a special HLA haplotype by linkage disequilibrium and inherited together.

In Iraq, involvement of specific DQ variants in CD susceptibility has been studied by serologic techniques. It is demonstrated that the DQ2 is 39.6% and DQ8 is 35.8%. [7].

However, serological HLA typing method considered less sensitivity. Meanwhile, Abdullah and Al-Thawani [8], are reported that the most frequent HLA-DQ genotype variants in Iraqi patients with CD, is DQB1*02 :01 and DQB1*02 :02, which is agreement with this study. The most frequent genotype is DQB1*02:02 that significantly increased frequency for 11 CD patients (p = 0.006) as compared with healthy control (No. =2). The OR for this allele is (7.21), this mean that the individuals with DQB1*02:02 allele have 7.21times greater chance of acquiring CD than those of the same population who lack it. As well as, DQB1*02:06 is presented in this study with higher frequencies in CD patients than healthy control group with OR and EF (3.08; 0.14), respectively. As shown in Table (2). A result similar to those in Greek pediatric population that estimates the frequencies of HLA-DQB1* 02:02 in patients was 20.34 vs. 5.42% in controls, P < 0.001[9]. Notably, in the current study, significant proportion of the patients carried the HLA-DQB1*02 genotype (%47.50). So is proposed to be considered as A CD-related genotype in Iraqi population. Finally, Table (3), demonstrated that HLA-DQBI*02 are expressed in 30/40 (75%) of CD patients either heterozygote or homozygote. This result strongly coincides with the frequencies obtained from the groups ethnically closely related to our group. In Egyptian CD patients, frequency of alleles that contribute to DQBI genotype were 77.42% [10]. Similar to our group and slightly lower than in the Jordanian population 80% [11]. In addition, resemble with the frequencies presented in Iranian population, for which have been estimated that 83.03% of cases and 35.09% of controls were carriers of an HLA-DQ2 heterodimer, either in homozygous or heterozygous state [12]. Also, it has been revealed that the most common alleles in Turkish study are DQA1*0501 and DQB1*0201 [13]. Actually, our results seem in agreement with other reports on effect of HLA-DQ2 genes on CD in different areas such as the miscegenation Brazilian population with (68.5%) had the genotype DQ2, and (6.8%) have DQ2 and DQ8 [14]. While, HLA-DQ genotypes in Croatian CD group, were 93.7% [15]. In India, was founded significant geographic variations within India in HLA-DQB1 genotype prevalence among CD patients where ranged of HLA-DQ2 and/or DQ8 determinants between (28.5% - 75.8%) [16]. In fact, the discordant results obtained in the studies of association HLADQB1*2 with CD, might be due to distinct linkage disequilibrium in different cohort studies or to other interacting, genetic, environmental factors and the heterogeneity of methods.

5. RECOMMENDATION

Based on the current results, this present study suggests the following recommendations: Further studies are required to clarify the role of non-classical HLA in susceptibility and development of disease, as well as Familial study of HLA – genotyping for patients. More investigation is required to find the relationship between CD and other autoimmune diseases as a trigger factor especially Diabetes Mellitus. The highly polymorphism of HLA which may necessarily be taken into account during the HLA-genotyping include various ethnics in Iraq.

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