IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 13, Issue 4 Ver. III. (Apr. 2014), PP 44-48
www.iosrjournals.org

Genotyping of HLA Class I and II Molecules in Type 2 Diabetic Iraqi Patients

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Abstract: *Whereas the genetic risk for type I diabetes mellitus (DM) is linked to human leukocyte antigen (HLA) class II genes, the HLA association in type 2 (non-insulin-dependent DM) diabetes is less clear. The association between HLA class I and II genotypes and type 2 diabetes was examined in adult Iraqis population with a high prevalence of type 2 diabetes .HLA-A*, HLA-B*, HLA-Cw*, HLA-DRB1* and DQB1* genotyping of 60 unrelated type 2 diabetes patients (age ≥35 years) who had a strong family history of diabetes (50 of 60 versus 0 of 40 for controls, P < 0.001) and 40 healthy subjects was done by PCR-Sequence-Specific Oligionucleotides (PCR-SSO). DRB1*1137(46.7 versus 0.0, P <0.001); DRB1*0401 (041.7 versus 2.5, P < 0.001) and DQB1*0201(83.3 versus 5.0 ,P <0.001) were positively associated, while DRB1*0701 (82.5 versus 23.3, P 0.001); DRB1*1601 (20 versus 0.0, P 0.029);A*0201(75 versus 1.7;P< 0.001);B*3559(75 versus 0.0 ;P< 0.001) and Cw*0410(77.5 versus 3 ;P <0.001) were negatively associated with type 2 diabetes. In Iraqis with type 2 diabetes, there is a significant association with select HLA class II genotypes, which were distinct from those in type 1 diabetes also significant association selected HLA class I with healthy individuals.*
Key words: *Type 2 diabetes mellitus, human leukocyte antigen.*

I. Introduction

Diabetes mellitus (DM) is a group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced. There are three main types of DM, type 1 DM results from the body's failure to produce insulin, and currently requires the person to inject insulin, this form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". Type2 DM results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. This form was previously referred to as non insulin-dependent diabetes mellitus (NIDDM) or "adult-onset diabetes". The third main form, gestational diabetes occurs when pregnant women without a previous diagnosis of diabetes develop a high blood glucose level (AL-Kayatt *et al*., 2011; AL-Mukhtar *et al*.,2012a ;AL-Mukhtar *et al*.,2012b).

Whereas type 2DM is the most common of diabetes , its specific etiology is not yet known .Its frequency varies in different racial and ethnic subgroups and is often associated with a strong familial , likely genetic, predisposition more than autoimmune type I DM.Human leukocyte antigen (HLA) system ,the most polymorphic and comprehensively studies gene locus , is extensively used to understand the evolution of genetic relatedness and migration of different world population. while the role for HLA in the pathogenesis of type 1DM was reported by several studies, its role in type 2DM is less clear, and weak links between HLA class (I and II) and type 2 diabetes were reported for some ethnic groups whoever some alleles of class II consider as markers of susceptibility for type 2 diabetes in another ethnic groups.HLA is a kind of genetic marker of human beings that presents a component of the immune system, encoded by highly polymorphic genes that vary across racial/ethnic groups, has been suggested to be a biologically based risk factor for type 2 diabetes and this may explain some of its variation by race/ethnicity (Prabhavathi *et al.*,2012 ; Ze-Jun *et al.*,2013).

II. Subjects, Materials And Methods

A total of 60 Iraqi patients with Type 2 diabetic were included in this study. They were from attendants seeking treatment in the specialized center for endocrinology and diabetes (Baghdad, Iraq) from June 2013 to November 2013. Diagnosis was made by specialized dentists in the College. Permission from the respective center and Baghdad University Institutional Ethical Committee for working on Human Subjects was received properly. Apparently healthy volunteers their ages and sexes were matched to patients consisted of 40 individuals who were considered as control. All of them didn't have medical history or clinic evidence of any chronic or acute diseases.Three ml of venous blood were withdrawn from each subject under aseptic technique, then transferred into two EDTA tube (1.5 mg/ ml), kept at -20ºC for the genotyping of HLA class I and II (A,

B,C, DR and DQ). The DNA was extracted by using the genome DNA extraction kit (Extra-Gene / BAG Company) according to (Miller *et al.*, 1988). All DNA was stored at -20°C until tested

HLA genotyping were performed by the PCR-SSO according to the manufac-turer's instruction, this method depends on reverse hybridization, using the PCR-SSO kit (Histo Type / DNA-SSO Kits-Innogenetics-Line Probe Assay, INNO-LiPA, Belgium). HLA- genotyping was carried out in the HLA-Laboratory of Forensic Medicine Institute/Baghdad.

Statistical Analysis: The results were presented in terms of percentage frequencies, and alleles showing variations between patients and controls were further presented in terms of odds ratio (OR). The significance of these differences was assessed by fisher's exact probability (P). P values of $p<0.05$ were considered statistically significant.

III. Results

The demographic and clinical characteristics of patients group and controls group included in this study are presented in Table (1). No statistically significant differences ($p>0.05$) in age or gender existed between two study groups. The mean age of patients was 40.15 ± 10.53 years, and there was male's predominance among patients. Furthermore, (26.6%) of patients had positive family history of CP, while (73.4%) showed negative family history. Regarding body mass index (BMI), the current study revealed that there was no significant differences in the mean of BMI between patients group and control group (P<0.05). Moreover, the mean levels of HbA1c and median of fasting plasma glucose were significantly elevated (P<0.001) among patients as clearly shown in table (1).

Table 3.1: Demographic and clinicalcharacteristics in study groups.

Characteristics		Study groups		P-value
		Control group n=40	Patients group n=60	
Age and Sex				0.017
Age (years)	Range	35 – 70	35 – 70	
	Mean ± SD	46.9±10.8	51.9±9.5	
Gender type	Female	21	26	0.38
	Male	19	34	
Family history	Positive	0.0%	26.6%	
	Negative	100%	73.4%)	
BMI (Kg/m^2)				0.28
BMI	Mean ± SD	29.7±6	31±6.4	
Fasting plasma glucose	Median	5.9±	11.6±	< 0.001
HbA_{1c}		5.5±0.6	9±1.5	< 0.001

Frequencies of HLA class I and II alleles

Sixty patients with type 2 DM and forty healthy individuals were typed for HLA-genotyping class I (A, B and C) and class II (DRB1 and DQB1).The frequency of distribution of HLA alleles for studied groups. Comparison between type 2 DM patients and control groups showed several alleles deviations in their frequencies. There are specific alleles of HLA-class I and II that associated as susceptible or protective factors for the development of the diabetes also (%, OR, EF, PF, P) were estimated.

Table (2) revealed significant increased (p <0.001) in frequencies of many alleles in patients (DRB1*1137; DRB1*0401; DRB1*1306; DQB1*0201 as compared with healthy control. However; the higher frequencies of (A*0201 with 0.001; B*3559; Cw*0410; DRB1*0701; DRB1*1601; DQB1*0501), (p <0.001) were found among control.

Table-2: The risk of being diabetic compared to healthy controls for selected HLA genes (only those with statistically significant associations are selected).

	Healthy controls (n=40)		Cases (type-II DM) (n=60)			Inverse OR			P (Fisher's exact)	Adjusted P
	N	%	N	%	OR		EF	PF		
HLA-A gene										
A-0201	30	75.0	1	1.7	0.01	177.0	**	0.749	<0.001	<0.001
HLA-B gene										
B-3559	30	75.0	0	0.0	0.003	351.5	**	**	<0.001	<0.001
HLA-Cw gene										
Cw-0410	31	77.5	3	5.0	0.02	65.4	**	0.763	<0.001	<0.001
HLA-DRB1										
DRB1-1137	0	0.0	28	46.7	71.03	**	0.460	**	<0.001	<0.001
DRB1-0401	1	2.5	25	41.7	27.86	**	0.402	**	<0.001	<0.001
DRB1-1306	0	0.0	9	15.0	14.94	**	0.140	**	0.008	NS
DRB1-0701	33	82.5	14	23.3	0.06	15.5	**	0.771	<0.001	<0.001
DRB1-1601	8	20.0	0	0.0	0.03	31.6	**	**	<0.001	0.029
HLA-DQB1										
DQB1-0201	2	5.0	50	83.3	95.00	**	0.824	**	<0.001	<0.001
DQB1-0501	33	82.5	14	23.3	0.06	15.5	**	0.771	<0.001	<0.001

Note: ** = cannot be calculated, equal to zero or not applicable**
Note: the complete data of these alleles seen in the appendix .
N: number
OR: odd ratio
EF: etiological fraction
PF: preventive fraction

IV. Discussion

Human leukocyte antigen plays a pivotal role in cellular immunity and may be an important genetically determined host trait. Certain HLA alleles that are predominantly associated with disease susceptibility or resistance in one population may or may not show any association in other population for the same disease. Despite of these limitations, HLA association are widely studied across the population worldwide and are found to be important in prediction of disease susceptibility, resistance and of evolutionary maintenance of genetic diversity (AI-Shammari, 2004; Yogita *et al*., 2005; Mahdi, 2013).

The principle aim of this study was to measure the association between certain HLA-A, B, C, DR and DQ alleles, and the incidence of type 2 DM. The frequency distribution was constructed to give an insight on which of the HLA-A, B, C, DR and DQ alleles was more frequent or infrequent in patients and controls for each of the five loci, which were used in this study.

The current study revealed that the HLA-DQB1*0201 was in highest frequency among type 2 DM patients (83.3%) vs. (5.0%) in healthy group, with OR (95), (P<0.001). The same significance probability continues after corrected P value, and this statistical application is important to exclude a chance of occurrence of an association due to many comparisons that were made (Al-Hakbany, 2009). This explain these alleles had

the strong effect provoked the development of this diseased in susceptible person, EF (0.824) greater chance of acquiring diseased than those of the same population who lack this type of alleles.

In comparison with healthy control, the second alleles which showed moderate frequency was DRB1*1137 (46.7% vs.0.0 %) with OR (71.03), (P<0.001) .Among the DRB1*0401 alleles, the highest frequent rate among patients in comparison with healthy control were (41.7% vs. 2.5%) with OR of 27.86 (P<0.001), these allele may act as risk alleles in susceptible person, and observed in high frequency among patients group.

Considering the protective effects of alleles and in comparison with control, the frequencies of A*0201,B*3559,Cw*0410, DRB1*0701, DQB1*0501 alleles were seem to be decline among patients that means, they negatively associated with type2 DM or may act as protective alleles in control subjects.

Linkage and association studies are the two major types of investigations to determine the contribution of genes to disease susceptibility. Association of a particular HLA allele with a disease implies that the frequency of the allele is different in the patient population as compared with that of normal population. While linkage studies can only use family data, association studies can be family or population based. Since HLA alleles of family members were not examined in the current study, the patient's haplotypes could not be determined directly.

Whole genome linkage scan have shown that the (MHC)/HLA region on chromosome 6p21 contain the major genetic component of the IDDM (Cox *et al*., 2010). The HLA-DR and DQ loci in the class II region have the strongest influence on type1 DM (Daneman, 2006). Variation at the HLA-DQ locus further defines susceptibility or resistance to type 1DM, this locus encodes for several variants of the HLA-DQ molecules (Dorman and Bunker, 2000); while β chain only for HLA-DR (Chapel *et al*., 1999).

Indeed, in this study,we can't compare our finding with Iraqi studies because no available studies for type 2 DM on molecular level while huge of studies on type 1 DM using PCR-SSP or PCR-SSO techniques even with serological methods in some thesis were revealed high significant association of the same allele of our finding DQB1*0201 as risky in patients group. These results are in conformity with the type I results of (AL-Ramahi, 2009; Jaleel, 2011 ; Lazim, 2011; Saleh,2006) . There were other Iraqi study which depend on using serotyping technique rather than genotyping showed agreement with above results but with low resolution alleles (Al-Khayali,1993; Al-Samarrai,2001). For this, it is considered as markers of susceptibility for type I and II diabetes in Iraqi ethnic groups. Similar results are arrived by Almawi *et al*,(2004) for type 1 DM in Bahraini patients as Arabic ethic /region and which is agreement with a broad study by Park *et al*.(2004) from Korean patients this may confer that DQB1*0201 is strongly associated with type 1 DM etiology and type 2 DM pathogenesis .

Regarding Arabic studies, in Peninsula Bahrainis type 2 DM patients the study done by Motala *et al*.(2005),using PCR-SSP technique revealed that the alleles which showed significant positively association with disease DRB1*0401(12.21%vs.5.62% in control); DRB1*0701(21.51%vs.8.43%) followed by DQB1*0302 (6.9%vs.0.7%), DQB1*201(17.8%vs.7.61%) .

While the DRB1*1101(6.98%vs.14.61%);DRB1*1601(6.4%vs.12.36%) ,followed by DQB1*0501(7.02 vs.3.49%) were negatively associated with type 2 DM. In Bahrainis with type 2 DM ,there is a significant association with select HLA class II genotypes , which were distinct from those in type 1 diabetes.

Mix study for type 2 diabetes patients from Bahrain and Lebanon done by Almawi *et al*,(2006) ,using PCR-SSP technique revealed that the allele which showed significant positively associated with diabetes was DRB1*0701 (Lebanese and Bahraini) while, DRB1*1101; DQB1*0201(Lebanese).

Hamid *et al*,(2011) study type 2 diabetes in Pakistan patients using PCR-SSP revealed that DRB1*15 being the commonest in both patients and control groups. The allele DRB1*13 had statistically significant higher frequency in patients group as compared to controls (p=0.005) .

Regarding abroad studies in Indian type 2 diabetes patients depending on PCR-SSP technique revealed that the DRB1*15 and DRB1*03 were at higher frequencies than in many other alleles .In controls, DRB1*07 was shown to be at a higher frequency. However, some of the common alleles in the population such as HLA-DRB1*1911 were not observed in the this study of diabetic patients.HLA-DRB1*16,08 and 04 were recorded with less frequency in diabetic patients in India type 2 diabetes (Prabhavathi *et al*.,2012).

Other abroad study was done in China, type 2 diabetes patients using PCR-SSO technique revealed that the DQA1*0301 alleles(15.5% versus 8.0%, p<0.01) and the HLA-DQA1*0501 alleles(16.6% versus 8.5% ,p<0.01),whereas the HLA-DQB1*0501 allele had a protective effect on type 2 DM (OR=0.53,P<0.05),these data suggest that DQA1*0301 and DQA1*0501 alleles are markers of susceptibility for type 2 DM (Ze-Jun *et al*.,2013).The variation between the above mentioned studies and the current findings may be related to genetically differences in population and the sample size.

Acknowledgement

The authors gratefully acknowledge the technical support provided by the Department of paternity and kinship in Iraqi medical legal institute /ministry of health.

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