FREQUENCY OF HLA-DRB1/DQB1 ALLELES AMONG TYPE 1 DIABETES PATIENTS IN DUHOK, KURDISTAN REGION (IRAQ)

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***Submitted* 04/09/2018*; accepted* 03/03/2019**

ABSTRACT

Background: A large number of studies have demonstrated that specific alleles at the HLA-DRB1 andHLA- DQB1 loci are strongly associated with type 1 diabetes mellitus (T1DM). This preliminary study investigated the heterogeneity in HLA class 11 genotypes distribution among Kurd patients with type 1 diabetes.

Patients and Methods: The study was conducted at Duhok Diabetes Center, Duhok, Kurdistan Region (Iraq). The study participants comprised 96 unrelated T1DM patients and 48 healthy control subjects. Currently, HLA typing methods are relatively expensive and time consuming. We sought to determine the minimum number of HLA polymorphism among T1DM patients and healthy controls that could define the HLA-DR/DQ alleles relevant to T1DM patients .All participants were typed at a polymerase chain reaction-(PCR) for theDRB1 and DQB1 loci. The association analysis was performed by comparing the frequency of DR/DQ alleles among the diabetic patients with the frequency of alleles in the healthy controls.

Results: Number of specific DR/DQ alleles has been identified and a statistically significant association with diabetes has been established. Compared with the healthy controls, patients were more than two-third as likely to have HLA- DRB1*03and -DRB1*04. HLA-DQB1*02allele was also more frequent in T1DM patients. HLA-DRB1*01,-DQB1*05 and -DQB1*06 were less frequent in T1DM patients.

Conclusions: The data indicate that the HLA-DRB1*03, -DRB1*04 and -DQB1*02 were positively associated with TID and may be the most prone alleles, while the HLA-DRB1*01 followed by-DQB1*05 and -DQB1*06 were negatively associated with TID and may be the most protective alleles.

Duhok Med J 2019; 13 (1):66-73.

Keywords: HLA-DR/DQ Alleles, Type 1 Diabetes Mellitus, Kurd Population.

There is increasing evidence on the association between human leukocyte antigen (HLA) class 11 genes and type 1 diabetes mellitus [1]. Subsequent analysis on HLA region shows that HLA-DRB1/DQB1 genes have the strongest association with T1DM, and susceptible alleles and genotypes are implicated in the pathogenesis of the disease[2]. These genotypes are transmitted in more than 80% of affected siblings [3]. Despite evidence suggestive of possible widespread family aggregation in Kurd population[4,5], attempts to investigate the

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heterogeneity in HLA class 11 genotypes in T1DM patients have been few[6].Thus, this preliminary study was aimed to examine the frequency distributionof HLA-DR/DQ genotypes and alleles in a sample of T1DM patients in comparison with those of healthycontrols and to ascertain the relationship betweenHLA-DRB1/DQB1 genes and the risk of diabetes among Kurd population.

MATERIALS AND METHODS

The study was carried out between September 2014 and April 2015 at Duhok Diabetes Center, Duhok, Kurdistan Region (Iraq).The subjects enrolled in this study (April 2015 data freeze) comprise 96 T1DM patients (42 males and 54females, mean age 13.3+3.6 yr), diagnosis was according to clinical features and laboratory findings. The means of height and weight were used to calculate the body mass index (BMI).All T1DM patients were receiving insulin for controlling hyperglycemia, were not obese (body mass index 19.5+3.5 kg/m), were free of any concomitant complication, and were not receiving additional treatment at the time blood collection. Controls consisted of secondary school students and comprised 48 apparently healthy subjects (12 males and 36 females, mean age 12.6+3.5 yr). Control subjects had normal fasting/random glucose levels and no family history of T1DM or other autoimmunediseases'. Allparticipants (patients and controls) were Kurdish ethnicity, and an informed consent was obtained from all of them.

The genomic deoxyribonucleic acid (DNA) extraction was done for all subjects by using deoxyribonucleic acid isolation Kit (QIAampDNA Blood Mini Kit). The HLA-DRB1 and –DQB1 gene alleles were analyzed with the polymerase chain reaction-(PCR) sequence-specific priming technique with the SSP HLA DQB-DRB Combi tray genotyping Kit (Lot No. 78V) according to the manufacture specifications(Olerup SSP AB, Stockholm, Sweden).. PCR products were analyzed on 2% agarose gel.

Data were collected and analyzed using SPSS (Statistical Package for Social Science) software, version 17.0(SOSS, Chicago; Illinois, USA).

RESULTS

The descriptive characteristics of the study subjects are shown in **Table 1**.The patient group was age, sex and body mass index (BMI) matched with the healthy control group. The frequency distribution of HLA-DRB1/DQB1 alleles among the patients and the healthy controlsis shown in **Table2**.

Table 1- Descriptive Characteristics of the Study Subjects

Characteristic	Patients	Controls	*P*-value
N	**96**	**48**	
Age (years)	**13.3±3.6**	**12.6±3.5**	**0.12**
Male sex, n (%)	**42(43.7)**	**12(25.0)**	**0.07**
BMI (Kg/m^2)	**19.5±3.6**	**17.8±37**	**0.07**
Positive family history of T1DM,n(%)	**36(37.5)**	**-**	**-**
FBS (mg/dl)	**227.7±3.3**	**86.5±4.7**	**0.001**
HbA1c%	**10.2±2.4**	**4.8±0.4**	**0.001**

Table 2- Frequency of DRB1-DQB1Alleles in TID Patients and Healthy Controls

HLA Genotype	T1DM Patients		Controls				
	No. of alleles 192	F (%)	No. of alleles 96	F (%)	OR	95%Cl	*P*-value
HLA-DRBI							
*01	6	(3.1)	12	(12.5)	0.22	0.06-0.82	0.015
*03	64	(33.3)	16	(16.7)	2.5	1.26-4.95	0.007
* 04	72	(37.5)	14	(14.6)	3.5	1.74-7.08	0.002
*07	8	(4.2)	4	(4.2)	1.0	0.24-4.11	1
*08	2	(1.0)	5	(5.2)	0.19	0.02-1.67	0.211
*09	0	(0.0)	0	(0.0)	-	-	-
*10	4	(2.1)	7	(7.3)	0.27	0.05-133	0.169
*11	20	(10.4)	17	(17.7)	0.54	0.23-1.25	0.146
*12	0	(0.0)	0	(0.0)	-	-	-
*13	8	(4.2)	10	(10.4)	0.37	0.11-1.23	0.095
*14	0	(0.0)	4	(4.2)	-	-	-
* 15	8	(4.2)	7	(7.3)	0.55	0.15-1.95	0.351
*16	0	(0.0)	0	(0.0)	-	-	-
HLA-DQB1							
*02 80	(41.6)	13 (13.6)	4.56	2.23 -9.29	0.0004		
*03	84	(43.7)	34	(35.5)	1.4 1	0.79-2.53	0.238
*04	4	(2.0)	2	(2.0)	1.0	0.13-7.24	1.0
* 05	12	(6.2)	26	(27)	0.17	0.07-0.45	0.001
*06	12	(6.2)	21	(21.9)	0.23	0.09-0.62	0.003

Significant DRB1-DQB1 genotype differences were seen between T1DM patients and controls, 6 of 20 alleles being significantly different (P<0.05).Compared with controls, the most frequent allele of the HLA–DRB1 genotype among patients

Table 3: Frequency of DRB1-DQB1Alleles in TID Patients according to Parents History of Type 1 Diabetes.

	HLA Genotype Positive (n=36)		parents history of TID Negative (n=60)				
	No. of alleles 72	F (%)	No. of alleles 120	F(%)	OR	95%Cl	*P*-value
HLA-DRBI							
*01	0	(0.0)	6	(5.0)	-	-	-
*03	22	(30.5)	42	(35.0)	0.81	0.33-1.1.98	0.654
* 04	28	(38.8)	44	(36.6)	1.10	0.46-2.57	0.827
*07	2	(2.7)	6	(5.0)	0.54	0.05-5.42	1.000
*08	2	(2.7)	0	-	-	-	-
*09	0	(0.0)	0	(0.0)	-	-	-
*10	0	(0.0)	4	(3.3)	-	-	-
*11	8	(11.1)	12	(10.0)	1.10	0.29-4.29	1.000
*12	0	(0.0)	0	(0.0)	-	-	-
*13	6	(8.3)	2	(1.6)	5.36	0.53-53.7	0.293
*14	0	(0.0)	0	(0.0)	-	-	-
* 15	4	(5.5)	4	(3.3)	1.7	0.22-12.6	0.964
*16	0	(0.0)	0	(0.0)	-	-	-
HLA-DQB1							
*02	24	(33.3)	56	(46.6)	0.57	0.24 -1.34	0.199
*03	38	(52.7)	46	(38.3)	1.79	0.77-4.14	0.167
*04	4	(5.5)	0	(0.0)	-	-	-
* 05	0	(0.0)	0	(0.0)	-	-	-
*06	6	(8.3)	6	(5.0)	1.72	0.32-9.5	0.805

was DRB1*03and DRB1*04, (OR 2.5, 95%Cl 1.26-4.95, *p*=0.007) and (OR 3.5, 95%CL 1.74-7.08, *p*=0.002) respectively. HLA- DQB1*02 allele was also more frequent in T1DM patients (OR 4.56, 95%Cl 2.23-9.29, *p*=0.0004). HLA-DRB1*01,DQB1*05 and DQB1*06 were less frequent in T1DM patients than they were in healthy controls. In the case of alleles DRB1*9, DRB1*12 and DRB1*16 were unidentified in the study subjects. Comparing the family history risk for the most frequent DRB1*03, DRB1*04 ,DQB1*02 alleles with the less frequent alleles DRB1*01, DQB1*05, DQB1*06 alleles (OR 0.47),the results are shown in **Table 3**.

DISCUSSION

Subsequent analysis on HLA region shows that HLA-DR/DQ genes have the strongest association with type 1 diabetes[7].The association varies among various ethnic and racial groups[8].The association analyses presented here show a statistically significant risk hierarchy among the many associated DRB1-DQB1 alleles, ranging from highly positive to highly negative, consistent with results of previous studies[9,10].For example, the most frequent DRB1*03 and DRB1*04 has an odds ratio of 2.5 and 3.5 while theless frequent DQB1*05 and DQB1*06 has an odd ratio 0.17 and 0.23 respectively.However, comparing the type 1 diabetes risk of DRB1*04-DQB1*05 with the DRB1*04-DQB1*06 (OR 1.0) reveals the risk conferred by DRB1 alleles. The comparison also illustrate the importance of both DRB1 and DQB1 alleles inthe family history risk for the most frequent DRB1*03, DRB1*04,DQB1*02 alleles with the less frequent alleles DRB1*01, DQB1*05, DQB1*06 alleles (OR 0.47), and reveals the risk is an epistaticinteraction. It was noteworthy that DRB1*04-DQB1*0302 haplotypes in particular conferring strong disease susceptibility among variousethnicities[11], similar to what was shown here for Kurdpatients. It remains to be seen whether the lack association of HLA-DR3/4 and DQ*02 with family history of parents in Kurdistan region is directly linked with the generalized lower incidence of T1DM,as was suggested[12].Type 1 diabetes incidence rates are extremely low in Asian population[13].For example, in Japanese patients with classic T1DM, DRB1*0505-DQB1*0401 and DRB1*0901-DQB1*0303 are major susceptible HLA-DR-DQ haplotypes, whereas DRB1*1502-DQB1*0601 and DRB1*1501-DQB1*0602 are protective [14].While in Iraqi Arab population, DQB1*0101 and *0201 alleles were found with high frequencies among T1DM patients in comparison with healthy controls[15]. In contrast, the present study suggested that the DRB1*03, DRB1*04 and DQB1*02 alleles were statistically significant frequencies among Kurd T1DM patients. A finding also reported in Iranian[16], Israeli Jewish[17], Arabs[11] and others[18]. However, this is the first study to demonstrate an association between HLA-DR/DQ alleles and an increased risk for diabetes, further research with a larger cohort will be necessary.

In summary, the present study indicated that the specific HLA-DR/DQ alleles, DRB1*03-DRB1*04-DQB1*02 showed the strongest association, and negative

association ofDRB1*01-DQB1*05-DQB1*06 alleles with T1DM in Kurd population. This finding may have clinical implications due to increased risk of future diabetes.

ACKNOWLEDGEMENT

We acknowledge the support of the staff of Duhok Diabetes Center and secondary schools that provided the facilities for the interviews and the records.

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پوختە

جورێن ئێکانەHLA – DR / DQ لنك نەخوشێن شەکرێ جورێ ئێکێ–هەرێما کوردستانا عیراقێ–ظەکولینەکا دەستپێکی

پێشەکی: بەلطەییَت زێدة هەنة ل سەر ثەیوەندیێ بهێز لناظبەنیا طوطیَت خوینا سثی و بتایبەت HLA – DRB1 و ALA – DQB1دطەل بەتا یاشەکرێ ذ جورێ ئێکێ.

ئارمانج: ل طەریان ل جوازیا جینی ل ALA ذجورێ دووێ لناف کوردیَن توش بووی ب شەکرێ ذجورێ ئێکێ.

ڕێکێن ڤەکۆلینێ: ذ ظەکولین دیارة ك وثەیوەندیەکا طرنط هەیة لنافبەترا نەخوشیَن بەتایاشەکرێ ذ جورێ ئێکێ دطەل ئەلیلیَن DRB1O3وئەلیلیَنDRB_1*O_4 . زێدەباری ذ ئەنجاما دیاربوی کو ثەیوەندیەکا طرنط دطەل ئەلیلیَنDQB_1*O_2 دطەل هندێ ئەنجاما دیارکر کو ثەیوەندي یا نەباش دطەل ئەلیلیَن ذ جورێ$HLA – DRB_1*O_1$و DQB_1OS و DRB_1O_6.

ئەنجام: ئەظێ ظەکولینا نوکة دیارکر کو ئەلیلیَن ذجورێ$HLA – DRB_1*O_3$ و DRB_1*O_1 و DQB_1*O_2د ثەیوەندیەکا باش و طرنط دانة بوەبتایاشەکرێ ذ جورێ ئێکێ و دبیت ببنة فاکتەریَن مەترسیدار بو دیاربونا بەتایاشەکرێ ذ جورێ ئێکێ. ذبەرکو ئەلیلیَن$HLA-DRB_1*O_1$–DQB_1*O_5ئەلیلیَننیطەتظن و دێ بنة ذ فاکتەریَن خوثاراستنێ.

الخلاصة

الانماط الفردانية HLA – DR / DQ لدى مرضى السكري النوع الاول في اقليم كوردستان العراق دراسة اولية

الخلفية والأهداف: هناك أدلة متزايدة على وجود علاقة قوية بين اليلات خاصة من HLA – DRB1 و ALA – DQB1 مع داء السكري النوع الاول. التحري عن الاختلاف الجيني في ALA النوع الثاني ما بين الاكراد الذين يعانون من داء السكري النوع الاول.

المواضيع و طرق البحث: أجريت هذه الدراسة في مركز السكري/ دهوك، المشاركون في الدراسة كانوا 96 مريض تم تشخيصهم سابقاً بداء السكري النوع الاول و 48 من الاشخاص الاصحاء. حالياً طرق التصنيف ذات كلفة عالية وتستغرق وقت طويل، لذا تم اختيار اعداد قليلة من المرضى والاشخاص الاصحاء ذو امكانية ان تظهر الاليات ذات العلاقة. ثم اجريت عملية التصنيف الاليلي لـ (DR/DQ و HLA class) باستخدام مسيار اوليلوثيوكليوتايد الاحادي لتفاعل متسلسلة البلمرة ذو التسلسل الخاص.

النتائج: أكدت الدراسة وجود ارتباط ايجابي بين داء السكري من النوع الاول مع الاليلDRB1O3والاليلDRB_1*O_4 اضافة لذلك بينت النتائج وجود علاقة ايجابية مع الاليلDQB_1*O_2في حين اظهرت النتائج علاقة سلبية مع الاليلات$HLA – DRB_1*O_1$و DQB_1OS و DRB_1O_6 .

الاستنتاجات: تؤكد الدراسة الحالية على ان الاليلات$HLA – DRB_1*O_3$ و DRB_1*O_1 و DQB_1*O_2 على ارتباط ايجابي بداء السكري النوع الاول ويمكن ان تكون عوامل الخطر لقابلية ظهور داء السكري في حين ان الاليلات-HLA $DRB_1*O_1–DQB_1*O_5–DQB_1*O_6$ في ارتباط سلبي وقد تكون من ضمن العوامل الوقائية.