HLA Class I and II Genotyping by PCR-SSO in Patients with Type-1 Diabetes Mellitus

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ABSTRACT:

BACKGROUND:

Type 1 diabetes mellitus is a chronic autoimmune disease that involves destruction of the pancreatic beta cells. It is well known that both genetic and environmental factors involved in pathogenesis of type-1diabetes mellitus.

OBJECTIVE:

This study seeks to determine whether there is any association between human leukocyte antigen class I and II alleles and type-1diabetes mellitus.

SUBJECTS AND METHODS:

Seventy type-1 diabetes mellitus patients compared to 30 apparently healthy individual were enrolled in this study. Human leukocyte antigens genotyping were analyzed by polymerase chain reaction sequence specific oligonucleotide technique.

RESULTS:

The present study revealed significantly high frequency of DQB1*0101 and DQB1*0201 alleles among patients in comparison with healthy control, while there was significantly low frequency of each HLA-A*3301, B*0826, DRB1*0701, *1101 and HLA-DQB1*0604 alleles in patients as compared to healthy controls.

CONCLUSION:

These findings indicate that higher frequency of HLA-DQB1*0101 and *0201 alleles may be a risk factor for type-1diabetes mellitus, meanwhile low frequency of HLA-A*3301, B*0826, DRB1*0701, *1101 and HLA-DQB1-*0604 alleles could be a protective factor.

KEY WORDS: Type 1 diabetes mellitus; HLA-alleles; PCR-SSO.

INTRODUCTION:

Diabetes mellitus (DM) is a common chronic metabolic syndrome of endocrine system characterized by hyperglycemia as a cardinal biochemical feature [(1)]. Type 1diabetes mellitus (T1DM) is an autoimmune disease characterized by a destruction of beta-cells of the Langerhans islets. The autoimmune process involves both the cellular and humoral arms of the immune system; however, an initiating event remains still unknown [(2,3)]. T1DM has been shown to involve a genetic component and an environmental component. This genetic component is the earliest predictor of disease and may eventually allow prediction in the prenatal phase leading to early prevention and/or treatment. The genes that are known to play a role in the genetic susceptibility include those in the human leukocyte antigen (HLA) complex on chromosome 6p21 [(4)].

The first associations between the HLA-complex and T1DM were related to the HLA-B8 and HLA-B15 molecules; these findings were overcome by more significant, to those of the HLA-DR locus: T1DM is associated with HLA-DR3 coded for by the allele DRB1*03 and HLA-DR4, determined by DRB1*04[(5,6)]. In (2012), Prabhavathi and colleagues studied the association between HLA and T1DM in Indian population, and observed that the HLA–DRB1*15 and HLA–DRB1*03 were found at higher frequencies than in many other alleles in patients. Whereas in controls, DRB1*07 was shown to be at a higher frequency [(7)]. On the other, the major susceptible alleles in Japanese patients have been reported to be DRB1*0405, DRB1*0901, DQA1*0301 and DQB1*0302, whereas DR3 and DR9 but not DR4 was associated with Chinese T1DM patients [(8)]. The current study seeks to determine whether there is any association

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between HLA class I and II alleles and T1DM in Iraqi patients.

SUBJECTS AND METHODS:

A total of 70 Iraqi Arab patients with TIDM (42 females and 28 males) with an age range of 1-30 years were included in this study. They were among patients attending to Al-Kadhumyia Teaching Hospital and Al-Eskan Hospital in Baghdad, during the period between September 2010 till April 2011. All patients were selected on the basis of absolute dependency on insulin according to the revised criteria for diagnosis of DM as defined by the American Diabetes Association [9]. Apparently healthy volunteers their ethnic and gender was matched with patient group, consisted of 30 individuals (16 females and 14 males), with age range was 1-28 years were included in this study. All of them had negative family history of DM.

Two ml of venous blood were withdrawn from each subject under aseptic technique, then

transferred into two EDTA tube (1.5 mg/ ml), kept at –20 ºC for the genotyping of HLA class I (A and B) and class II (DR and DQ).The DNA was extracted by using the Invisob® Spin Blood Mini Kit from (Invitek, Germany). All DNA was stored at -20°C until tested. HLA- genotyping were performed by the PCR-SSO according to the manufacturer'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-typing laboratory of Al-Karama teaching hospital, 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) and etiological fraction (EF). The significance of these differences was assessed by fisher`s exact probability (P). P values of $P<0.05$ were considered statistically significant [10].

RESULTS:

In this study the age of T1DM patients ranged between 1-30 years with a mean age of 11.66± 5. 95 years. There was female's predominance among patients, about 42 (60%) of patients were females and 28(40%) were males. Female/male ratio was 1:1.5.The mean age of onset of disease was 9.52 years. In addition 21% of patients had positive family history of RA as shown in Table-1.

Table 1: Demographical Picture of the Studied Groups.

No.	Demographical Parameters	T1DM	Healthy Control
1	Age (years)[Mean ±SE]	11.66± 5. 95	13.93 ±1.20
2	Age of Disease Onset (years) (Mean)	9.52±5.38	-
3	Females [No (%)]	42 (60%)	16 (53.33%)
4	Males [No (%)]	28 (40%)	14 (46.67%)
5	Males: Females Ratio	1:1.5	1: 1.1
6	Positivity of Family History [No (%)]	15 (21%)	-
	Total number	70	30

Comparison between patients and control group showed several HLA-alleles deviations in their frequencies. Because of the large number of HLA-A, -B, -DR and -DDQ alleles that studied in the present study, so the tables [2,3,4,5] included only alleles that showing significant or high variations between T1DM patients and control group.

Regarding HLA-class I alleles this study noticed that the frequency of HLA-A*3301and B*0826 alleles were significantly higher in control as compared to patients ($P<0.05$), as demonstrated in Table-2 and Table-3. As well as HLA-class II genotyping evoked significant differences in some alleles between patients and control group. This result was showed significant decrease in the frequency of DRB1*0701, *1101 and HLA-DQB1-*0604 alleles among T1DM cases rather than in healthy control group with ($P=0.001$) as clearly shown in table-4 and table-5. In contrast the DQB1*0101 and 0201 alleles were found with highly significant frequencies among T1DM, in comparison with healthy control ($P<0.05$), Table-5.

Table 2: HLA-A genotypes in T1DM in comparison to healthy control group.

HLA-A Allele	Healthy control N=30		Diabetic patients N=70		OR	EF	*P* (Fishers Exact)
	No.	%	No.	%			
*0102	0	0	6	4.28	5.84	0.035	0.181
*0201	1	1.66	4	2.85	1.73	0.012	1.000
*0203	0	0	3	2.14	3.08	0.022	0.556
*0204	1	1.66	5	3.57	2.18	0.015	0.671
*0205	0	0	3	2.14	3.08	0.022	0.556
*0301	0	0	4	2.85	3.98	0.028	0.319
*0302	0	0	5	3.57	4.91	0.035	0.325
*1005	0	0	3	2.14	3.08	0.022	0.556
*1122	0	0	4	2.85	3.98	0.028	0.319
*3203	0	0	3	2.14	3.08	0.022	0.556
*3301	5	8.33	0	0	0.03		0.002

OR: odds ratio, EF: etiological factor.

Table 3: HLA-B genotypes in T1DM in comparison to healthy control group.

HLA-B Allele	Healthy control		Diabetic patients		OR	*EF*	*P*
	No.	%	No.	%			
*0503	0	0	2	1.42	2.18	0.015	1.000
*0701	0	0	2	1.42	2.18	0.015	1.000
*0809	0	0	4	2.85	3.98	0.028	0.319
*0826	4	6.64	0	0	0.04		0.008
*1529	0	0	4	2.85	3.98	0.028	0.319
*1561	2	3.33	0	0	0.08		0.089
*2609	0	0	2	1.42	2.18	0.015	1.000
*2718	0	0	2	1.42	2.18	0.015	1.000
*3501	0	0	4	2.85	3.98	0.028	0.319
*3503	2	3.33	0	0	0.08		0.089
*3527	2	3.33	0	0	0.08		0.089
*3801	2	3.33	0	0	0.08		0.089
*3802	0	0	4	2.85	3.98	0.028	0.319
*4051	2	3.33	0	0	0.08		0.089
*4101	0	0	2	1.42	2.18	0.015	1.000
*4105	0	0	2	1.42	2.18	0.015	1.000
*4411	0	0	2	1.42	2.18	0.015	1.000
*4501	0	0	2	1.42	2.18	0.015	1.000
*4901	1	1.66	4	2.85	1.73	0.012	1.000
*5102	0	0	3	2.14	3.08	0.022	0.556
*5131	0	0	2	1.42	2.18	0.015	1.000
*5204	0	0	3	2.14	3.08	0.022	0.556

Table 4: HLA-DR genotypes in T1DM in comparison to healthy control group.

HLA-DR Allele	Healthy control		Diabetic patients		OR	Ef	P
	No.	%	No.	%			
*0101	0	0	3	2.14	3.08	0.014	0.556
*0102	0	0	2	1.42	2.18	0.007	1.000
*0201	0	0	3	2.14	3.08	0.022	0.556
*0203	5	8.33	5	3.57	0.42		0.585
0303	0	0	3	2.14	3.08	0.014	0.556
*0304	0	0	4	2.85	3.98	0.028	0.319
*0404	0	0	4	2.85	3.98	0.028	0.319
0603	0	0	3	2.14	3.08	0.014	0.556
*0701	7	11.6	0	0	0.02		<0.001
*0703	0	0	5	3.57	4.91	0.035	0.325
*1101	9	15	2	1.42	0.08		<0.001
*1103	0	0	8	5.71	7.76	0.055	0.108
*1118	0	0	8	5.71	7.76	0.055	0.108
*1130	0	0	3	2.14	3.08	0.022	0.556
*1501	4	6.64	2	1.42	0.20		0.067
*5001	0	0	3	2.14	3.08	0.022	0.556

Table 5: HLA-DQ genotypes in T1DM in comparison to healthy control group.

HLA-DQ Allele	Healthy control		Diabetic patients		OR	*EF*	*P*
	No.	%	No.	%			
*0101	0	0	13	9.28	12.81	0.085	0.011
*0201	0	0	13	9.28	12.81	0.085	0.011
*0202	2	3.33	2	1.42	0.42		0.58
*0203	0	0	8	5.71	7.76	0.055	0.108
*0204	5	8.33	9	6.42	0.75		0.763
*0205	2	3.33	2	1.42	0.42		0.585
*0301	2	3.33	10	7.14	2.23	0.015	0.516
*0302	0	0	8	5.71	7.76	0.055	0.108
*0304	0	0	3	2.14	3.08	0.022	0.556
*0309	0	0	5	3.57	4.91	0.035	0.325
*0415	0	0	3	2.14	3.08	0.022	0.556
*0501	0	0	8	5.71	7.76	0.055	0.108
*0601	0	0	3	2.14	3.08	0.022	0.556
*0604	6	10	3	2.14	0.19		0.023
*0609	0	0	4	2.85	3.98	0.028	0.319
*0804	0	0	3	2.14	3.08	0.022	0.556

Note: because of the large number of HLA-(A, B, DR and DDQ) genotypes include in this study so, the tables (2,3,4 and 5) contain only genotypes that showing significant or high variations between patients and control group.

DISCUSSION:

Autoimmune DM is clearly a genetic disease. Susceptibility is inherited in a polygenic fashion, 18 IDDM–genes were shown to be involved in its genetic determination until now. Among the genes involved, those of the human major histocompatibility complex (HLA), play a paramount importance[11]. In the current study molecular typing techniques have been employed to identify HLA alleles at DNA level. This is the first Iraqi study concerning the genotyping of HLA

class I and II by PCR-SSO and its proposed association with T1DM.

The present work revealed that the frequency of HLA-A*3301 and B*0826 alleles were higher in healthy control as compared with patients. These results is at variance with other results conducted by Noble *et al.*, [12] who pointed out to that the most significantly T1DM –associated alleles were B*5701, B*3906, A*2402 and A*0201 (as predisposing and risk factors), while they observed that A*1101, A*3201, A*6601, B*0702, B*4403, B*3502, C*1601, and C*0401 were significantly decrease in patients (as protective factors).

Among the DRB1and DQB1 alleles, the present study observed that the DRB1*0304, DRB1*0404, DQB1*0101 and 0201 alleles were frequent in T1DM patients, but the frequency of DRB1*0304 and DRB1*0404 alleles was statistically not significant. This result is in line with similar findings in Iraq [13] and in other ethnic groups [14,15]. These alleles are reported to be the risk genes among T1DM Iraqi patients, similar to those from Asian populations[16]. This is unlike findings in Japanese and Caucasian populations[17,18], in whom DRB1*0401-DQB1*0302 was the most significant susceptibility haplotype. In Slovakian study conducted by Shawkatova etal., [19] reported that the most significant associated alleles with the disease development were DQB1*0302 and DQB1*0201, whereas the DQB1*0602 and DQB1*0301 alleles were observed as marker for protection. Correspondingly Saruhan-Direskeneli and colleageues [20] also noticed that DQB1*0302 and DQB1*0201 alleles frequency was higher in Turkish patients with T1DM, while DQB1*0503 and DQB1*0601 alleles were found negatively associated with disease. In 2010, Manan *et al.*, [21] studied HLA-typing by PCR/SSP technique in Saudi patients, observed that there was association of heterozygote DQ*0203 with T1DM.

In addition the frequencies of DRB1*0701, DRB1*1101 and DQB1*0604 in our study were significantly decrease in patients than healthy control, which may confer protective role in healthy individuals. This finding was comparable to other findings reported by [22].

The inheritance of both a risk and protective alleles will result in disease protection or will become a neutral gene. An example is the inheritance of DRB1*0401-DQB1*0301, which confers protection despite the risk role of DRB1*0401. This phenomenon could be attributed to the intricate interplay between different DRB1 and DQB1 alleles in determining susceptibility to T1DM. Moreover, the association of two protective alleles results in additional protection [23].

Different results regarding this association was reported, results differ in different population. This could be explained by the variable ethnic backgrounds of studied patients, or more likely the multiple etiologic bases for this disease, or may be related to the sample size.

CONCLUSION:

The current study indicate that higher frequency of HLA-DQB1*0101 and *0201 alleles may be a risk factor for T1DM, meanwhile low frequency of HLA-A*3301, B*0826, DRB1*0701, *1101 and HLA-DQB1-*0604 alleles could be a protective factor. So these findings give additional support to a genetic basis for T1DM pathogenesis.

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