Combination of HLA DR and DQ molecules determine the susceptibility to Insulin Dependent Diabetes Mellitus in Iraqi Patients

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Summary:

Background: Type 1 diabetes mellitus is an autoimmune disorder characterized by destruction of insulin producing β-cells of the pancreatic islets of Langerhance and lack of endogenous insulin. Susceptibility to type 1 diabetes mellitus is influenced by both genetic and environmental factors.

Patients and methods: Polymerase chain reaction-Sequence Specific Primers PCR-SSP is the method used to asses HLA-typing of 90 blood samples of 50 insulin dependent diabetes mellitus (IDDM) patients and 40 healthy normal controls.

Results: An increased frequency of HLA-DR3 and DR4 was observed for patients group versus control group with P-value (0.0001 and 0.05) respectively, while DR*0211 (DR2) may be formed the basis for protection against the disease. HLA-DQ8 and DQ3 on the other hand, yielded a significant association in Iraqi patients with IDDM with P value (.P values 0.01 and 0.05) respectively, conversely DQB1*0101, 02 (DQB1) is commonly expressed in non diabetic individuals which suggest that this allele helps prevent IDDM (P value < 0.005).

Conclusions: This finding demonstrated that HLA-DR3, DR4, DQ3 and DQ8 might play a role in IDDM susceptibility.

Key words: Insulin dependent diabetes mellitus, HLA, PCR -SSP assay.

Introduction

Insulin-dependent diabetes mellitus (IDDM), commonly referred to as type-I diabetes, is a condition caused when an autoimmune response induces the death of insulin-secreting β cells in the pancreas and as a result no way of producing insulin, so glucose levels in the blood are unable to be controlled, leading to hyperglycemia, or high blood glucose level. Longterm complications of hyperglycemia include cardiovascular, kidney, and eye diseases, as well as various nervous system disorders (1). IDDM tends to run in families, and there is substantial evidence that genetics plays a significant role in causing the disease. However, studies have shown that the concordance rate for IDDM among monozygotic twins is less than 50 percent, meaning that environmental factors must also play a significant role (2). The fact that both environment and genetics contribute to IDDM has led to the assumption that the autoimmune response causing insulitis and autoantibody release is generally triggered by an environmental stimulus, but occurs primarily in those who are genetically predisposed to the disease (3). Genetic susceptibility to IDDM, as with most autoimmune diseases, is believed to be primarily related to the MHC genotype. The MHC II genes that appear to play the most significant role in IDDM susceptibility are found at the HLA-DR locus, which is linked tightly to HLA-DQ.

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The two alleles HLA-DR3 and HLA-DR4 seem to be the strongest genetic causes of IDDM, since nearly all diabetics express at least one, or more often both of these alleles (4,5).

Patients and Methods:

Patients:

The present study included 50 Arab, Iraqi IDDM patients (32 females and 18 males), attending The Diabetic Center, Al-Mustansiriya University in a period between November 2006 and July 2007.Their age ranged between 4-62 years, compared with 40 healthy individuals (age and sex matched). Both groups were typed for HLA-class II (DRB & DQ) antigens.

Laboratory investigation: The basic material for typing with HISTO TYPE/DNA-SSP kit is purified DNA. The test procedure was done by using the Sequence Specific Primers (SSP). This method is based on the fact that primer extension and hence successful PCR relies on an exact match at the 3'-end of both primers.

Results:-

Table-1 below revealed the importance of DR-alleles through their frequencies in IDDM patients in comparison with healthy controls. As shown, DRB1*0301 (DR3) and DRB1*0401 (DR4) found in high frequencies in patients compared to healthy control groups with (P value <0.0001, and 0.05) respectively. While DRB1*0211 (DR2) may form the basis for protection against the disease; It's presence formed significant difference between

patients and healthy individuals (P value < 0.008). In spite of presence of DRB1*0401 (DR4) in high frequency (20.0% Vs.5.0%); its importance as a risk factor was less than DR*0301 (DR3).

Table-1: Observed numbers and percentage frequencies of HLA-DR alleles (serotypes and genotypes) in IDDM patients and controls.

HLA-DR Alleles		Insulin dependent diabetes mellitus patients (No. = 50)		Healthy control (No. = 40)	
Serotype	Genotype	No.	%	No.	%
DR 1,-	DR*010101,0102,0201-0204,04-13	3	5.0	N.D	N.D.
DR 1	DR*010103	1	1.6	N.D.	N.D.
DR2*	DR*0211	3	5.0	32	80.0
DR3	DR*0312-13,14	10	15.5	8	20.0
**DR3	0306	38	63.3	10	25.0
DR3	0307	10	15.5	8	20.0
DR4***	DR*0401	12	20.0	2	5.0
DR4	DR*0404	5	8.2	2	5.0
DR4	DR*04011-04012,31,32	7	12.1	2	5.0
DR 103	DR*0103	2	3.3	4	10.0
DR 8,-	DR*0820	N.D.	N.D.	1	2.5
DR 10	DR*100101,0102	2	3.3	3	7.5
DR 11(5)	DR*1107	1	1.6	3	7.5
DR 11(5)	DR*1123,25,45	2	3.3	1	2.5
DR11(5),-	DR*1153	2	3.3	2	5.0
DR 13(6),-	DR*1305,06,0701,11,12,1401,21,25,30,42, 49, 50, 56, 58, 62	1	1.6	1	2.5
DR 13(6),-	DR*1309	2	3.3	3	7.5
DR 13(6),-	DR*1315,19,53,57	1	1.6	2	5.0
DR 14(6)	DR*1346	1	1.6	N.D.	N.D.
DR 14(6),-	DR*140501,08,2302,34,43,44,45	1	1.6	N.D.	N.D.
DR 14(6),-	DR *1419,20,41	3	5.0	N.D.	N.D.
DR 14(6),-	DR*1433	1	1.6	N.D.	N.D.
DR 14(6),-	DR*1446	1	1.6	N.D.	N.D.
DR 14(6),-	DR*1449	N.D.	N.D.	1	2.5
DR 14(6),-	DRB3*0109 / *0204,19 / *0303*	2	3.3	1	2.5

N.D.: Not detected
* P=0.008
** P=0.0001

*** p=0.05

A survey of the distribution of HLA-DQ frequency yielded a strong association between DQ Ag and IDDM patients, since DQB1*0801 and DQB1*0301 present in high frequencies in IDDM patients compared to healthy control group (P values 0.01 and 0.05) respectively, conversely DQB1*0101,02 (DQB1) is commonly expressed is non diabetic individuals which suggest that this allele helps prevent IDDM (P value < 0.005), (table-2).

Table-2: Observed numbers and percentage frequencies of HLA-DQB alleles (serotypes and genotypes) in IDDM patients and controls.

HLA-DQB Alleles		Insulin dependent diabetes mellitus patients (No. = 50)		Healthy Controls (No. = 40)	
Serotype	Genotype	No.	%	No.	%
DQB5(1)	DQB1*050101-050302,0504	4	6.6	2	5.0
DQB 6(1)	DQB1*060101-0103	1	1.6	1	2.5
DQB 6(1) / 1 /	DQB1*0602-18,20-22,24-26N	4	6.6	2	5.0
DQB 2	DQB1*020101-0102,0202-04	4	6.6	2	5.0
DQB 8 /*	DQB1*080101,080102,09,16	15	32.0	1	2.5
DQB 3 /**	DQB1*030201,030202,030501-0503,07,08,11	12	26.0	1	2.5
DQB 9(3) / 3 /	DQB1*030302,030303,06,12,15	1	1.6	1	2.5
DQB 7(3) /	DQB1*0304,14	1	1.6	4	10.0
***DQB1	DQB1*0101,02	2	3.3	28	70.0
DQB 4	DQB1*0401,02	3	5.0	3	7.5

N.D.: Not detected
* P=0.01
** P=0.05
*** P=0.005

Estimated Frequencies of HLA Haplotypes: Observed numbers and percentage frequencies of possible allelic combination patterns of HLA region in IDDM patients and controls are outlined in table (3) . This table show that the most common allelic combination patterns was DRB1*0401-DQB1*0801 (36.6% Vs. 2.5%), this result revealed significant (P value=0.005) increase in frequency in patients compared to controls. The next haplotype was DRB1*0301-DQB1*0801 (30.0% Vs. 7.5%) with (P value = 0.005), fallowed by DRB1*0301-DQB1*0301 (P value 0.05). In contrast DRB1*0201-DQB1*0301 genotype decreased in patients compared to healthy control groups, with p value (0.05).

Table-3: Observed numbers and percentage frequencies of possible allelic combination patterns of HLA region in IDDM-patients and controls.

HLA allelic combination pattern	Patients (Numbers= 50)		Control (Numbers= 40)		P
	No.	%	No.	%	
DRB1*0301-DQB1*0801	15	30.0	2	7.5	0.005
DRB1*0401-DQB1*0801	17	36.0	1	2.5	0.005
DRB1*0301-DQB1*0301	9	18.0	3	7.5	0.05
DRB1*0301-DRB1*0401-DQB1*0801	5	8.3	1	2.5	N.S.
DRB1*0201-DQB1*0301	2	4.0	9	22.5	0.05

Discussion:

The association of HLA-DR3 or other HLA-DRB1 alleles with IDDM had now been studied in nearly every population. (1,5,6).Regarding to the fact says that DRB1 locus is the principle susceptibility region of MHC in patients with IDDM, several different alleles of DR*3 and DR*4 has been identified in patients with this disease from different populations Like other studies (5,6,7,8), our results showed that

there is a strong association between certain HLA-DRB1 alleles and IDDM. This evidence brought in hands when we estimated the frequency distribution of HLA-DR*3 (0301)and DR*4 (0401) alleles groups that formed an etiologic risk factor with (P value 0.0001 and 0.05) respectively. The same antigens but different alleles (0301 and 0407) were seen to be associated with IDDM in patients in other nearby and far country, as reported in Bahraini and on Koreans patients (5,9).The reason for such allelic variation is still mysterious; it may be due to gene drift, when these genes get associated together by chance or by gene flow which is the result of admixture between different populations (10).On the other hand, our results has shown that there was a significant loss in HLA-DR*2 alleles when compared with the healthy control group. These alleles could be considered as protective markers. HLA-DQ antigen, were encoded by genes within the HLA-class II region. A recent study revealed that the HLA-DQ locus may hold a real promise to define class II susceptibility to IDDM, since some researchers noted an increased freqsuency of some HLA-DQ Ags in IDDM and they suggested a possible causal role of this antigen in this disease. For instance Park and co-workers reported the association of DQB1*0201 and DQB1*0801 in patients from Koreans (9),while DQB1*0302 allele was reported in Bahraini patients with IDDM (5). Interestingly, our study showed that DQB1*0301 (DQ3) and DQB1*0801 (DQ8) was the risk alleles associated with IDDM in Iraqi patients. Furthermore, our results showed that most of DQB1*0101 alleles in the IDDM patients seem to be lost; again this alleles may act as a rather protection factor.In order to reach a better understanding of the role of chromosome 6 markers in the pathogenesis of IDDM, the data were interpreted in terms of combination between certain alleles or phenotypes. An significantly increased the phenotype, DRB1*0401-DQB1*0801 was observed in IDDM patients (36.0% Vs. 2.5%), compared to healthy control group with (p value 0.005). The other phenotype which showed a remarkable increased frequency among the IDDM patients was DRB1*0301-DQB1*0801. This phenotype was present in (30.0%) while its (7.5%) of healthy control group, this difference was also highly significant (P=0.005). This result suggests that the stretch of chromosome 6 containing DR and DQ antigens has an important role in the susceptibility to IDDM. Whether some alleles of DR and DQ are directly involved in the pathogenesis of the disease or not, require more studies.

A question that remains unsolved is weather the linkage and association observed is due to an effect of normal immunological function of the HLA molecules themselves or to a disease-causing gene encoded in linkage disequilibrium within the HLA gene region. A recent study reported that, the disease-causing genes are closely linked to a special HLA haplotype and that they therefore are inherited together, in addition this study revealed the association between haplotypes that increase the possibility that unlucky immunological event leading to disease will take place (11), also it has to be remembered that the HLA loci represent a tiny fraction of MHC genes most of which have important function in the immune system, inflammatory response, cell metaboloism. However this study provided important information on which further work can be based.So, generally the development of IDDM would depend upon the expression of the susceptible HLA alleles and absence of the protective alleles, along with the environmental factors that trigger the autoimmune process. This fact was confirmed recently by Dolekos (12).

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