Distribution of HLA class I and class II Antigens in T1DM Children and their Siblings

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Abstract

Background: Genomic studies have confirmed that the main locus defining the genetic susceptibility to T1DM is encoded within the Major Histocompatibility Complex- HLA (Human Leukocyte Antigen) region on human chromosome 6.

Objective: To investigate the role of HLA-class I and class II antigens in the etiology of type 1 diabetes mellitus (T1DM) and in prediction of this disease in siblings.

Patients & methods: Sixty T1DM children who were newly onset of the disease (diagnosed less than five months) were selected. Their age ranged from 3-17 years. Another 50 healthy siblings were available for investigation of HLA-typing, their ages range from 3-16 years. Eighty apparently healthy control subjects, matched for age (4-17) years, sex and ethnic backgrounds (Iraqi Arabs), underwent the HLA-typing. Serological typing of HLA antigens was done by microlymphocytotoxicity assay.

Results & recommendations: At HLA-class I region, T1DM patients showed a significant increased frequency of antigens A9 (40.0 vs. 18.75%) and B8 (28.33 vs. 8.75%) as compared to control subjects, while at HLA-class II region, DR3 and DR4 were significantly increased in patients (53.33 vs. 26.25% and 50.0 vs. 12.5% respectively) as compared to controls. In addition to that, T1DM was significantly associated with DQ2 (33.33 vs. 15%) and DQ3 (40.0 vs. 20%) antigens as compared to controls, suggesting that these antigens had a role in disease susceptibility, while the frequency of DR2 and DQ1 antigens were significantly lowered in patients compared to controls (6.66 vs. 25% and 6.66 vs. 22.5% respectively). These molecules might have protective effect. In siblings a significant increase frequency of DR4 antigen (34.0 vs. 12.5%) was observed in comparison to controls, suggesting that it might be much useful for predicting T1DM in affected families. It is potentially valuable to predict TIDM in siblings by screening for HLA risky alleles in correlation with autoantibodies.

Keywords: T1DM patients, Siblings, HLA.

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Introduction

Type1Diabetes Mellitus (T1DM) is one of the most common chronic childhood diseases.

Its incidence seems to be increasing in countries around the world and is predicted to be about 40% higher in 2010 than 1997 [(1)]. It is known to be polygenic disease that appears from interaction of mutation of multiple genes [(2)]. Genomic studies have confirmed that the main locus defining the genetic susceptibility to T1DM is encoded within the Major Histocompatibility Complex- HLA (Human Leukocyte Antigen) region on human chromosome

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6 [3]. The MHC consists of three major regions, A, B, and C that encode class I genes, and the D region which encodes class II genes. The class I molecules are highly polymorphic and present peptide fragments of foreign antigens to cytotoxic T- Lymphocytes. The class II molecules present foreign processed antigen to helper T- Lymphocytes and thus are involved in initiating the immune responses [4]. There are two major classes of class II genes, the DR and DQ genes. It has been estimated that 60% of the genetic susceptibility to T1DM is conferred by the HLA [5].

The role of HLA alleles in T1DM was first indicated by the association with HLA-B8, -B15, and -B18 [6], and then with HLA- DR3 and DR4 encoded by the DRB1 locus and susceptibility with the DQB1 and DQA1 genes, which are in linkage disequilibrium with DR3 and DR4 [7, 8, 9]. DQB1 *0201 and DQB1 *0302 present a high risk for disease occurrence [10]. A study conducted by Mezal, showed that A1, B8, and DR3 were the high risk antigens [11], while others found that A24, B8, B15, DR3, DR4, DQ2, and DQ3 were highly associated with T1DM among Iraqi patients [12]. Strong natural protection against T1DM is also conferred by the DR2.DQ6 haplotypes [13], which occurs in approximately 20% of the healthy white population but it is rarely found among patients with diabetes. DR2, DQ1, DQ4, and B35 were found among the protective alleles in Iraqi patients [11, 12].

In the present study, we investigate the occurrence of T1DM in relation to genetic predisposition in children and their siblings through the HLA polymorphism.

Subjects, Materials and Methods

Subjects:

Sixty Iraqi Type 1 diabetic patients (28 males and 32 females) were subjected to this study. The patients attended the National Diabetes Center at Al-Mustansiriya University/ College of Medicine during the period May 2004 - October 2005. Their ages ranged from 3 -17 years, and they were new onset of the disease (diagnosis was from one week up to five months). All the patients were treated with daily replacement doses of insulin at the time of blood sampling. Fifty healthy siblings (25 males and 25 females) of type 1 diabetes patients were available for investigation of the HLA-typing. Their ages ranged from 3 – 16 years. For the purpose of comparisons, 80 healthy control subjects matched for age (4-17 years old), sex and ethnic back ground (Iraqi Arabs) were selected who have no history or clinical evidence of type, 1 diabetes or any chronic diseases and obvious abnormalities as a control group for HLA typing.

Serological Typing of HLA Antigens:

Ten milliliter of venous blood was drawn from each subject (patients, siblings, and controls). The collected blood was displaced into glass universal tubes containing heparin (10 IU /ml) as anticoagulant. Lymphocytes were separated from the whole blood using Ficoll- Isopaque density centrifugation (Flow-Laboratories, UK) as described by [14]. The test was carried out in histocompatibility laboratory in Al-Karama hospital. The collected cells were suspended in washing medium (RPMI-1640 free serum cultured media) (Euroclone, UK) and centrifuged three

times, then the lymphocytes were resuspended in 2 ml warm RPMI-1640 supplemented with 10% heat inactivated human type AB serum. The lymphocyte population were separated by nylon wool method to T- cells that were used in the phenotyping of HLA- class I (A, B, and C) antigens and B- cells that were used for phenotyping of class II (DR and DQ) antigens [15]. Cells were counted and their viability was determined. The viability of cells was 95% and above. Microlymphocytotoxicity assay was used for both HLA- class I and class II typing [16].

Statistical analysis

Regarding of HLA and disease association the frequency distribution for selected variables was done first. The strength of disease association with particular HLA antigen was determined by calculating the relative risk (RR) and etiological fraction (EF), and if the association is negative, therefore the preventive fraction (PF) was calculated [17]. The significance of such association was assessed by Fisher exact probability.

Results

The frequencies of HLA antigens (-A; -B; -C; -DR; and -DQ) were compared between T1DM patients and controls, siblings and controls and between T1DM patients and siblings.

HLA association with T1DM:

The distribution of HLA-A; -B; -C; -DR; and -DQ antigens with their frequencies in T1DM patients and controls are presented in (table 1), while antigens showing significant variations between patients and controls are given in (table 2).

At HLA-A locus, the antigen A9 was significantly increased (P= 0.004) in the patients and such differences were associated with RR value of 2.88 and EF value of 0.261. This positive association remained significant after correction (PC= 0.032).

At HLA-B locus three antigens were significantly increased. They were B8(28.33 vs. 8.75%), B12(11.66 vs. 2.50%), and B15(11.66 vs. 2.00%) in the T1DM patients (P=0.002, 0.032, and 0.018 respectively) in comparison to controls. Such differences were associated with RR values of 4.122, 5.150, and 9.113 respectively, and EF values of 0.214, 0.093, and 0.103 respectively. However one positive association remained significant after correction (PC= 0.032) and this was with B8. In contrast, B35 and B51 antigens were significantly decreased in the patients compared to controls (3.33 vs. 13.75% and 15.0 vs. 28.75% respectively), but such negative associations failed to remain significant after correction.

At HLA-C locus, Cw7 antigen was significantly increased in T1DM patients (31.66 v. 16.25%, P=0.026, RR=2.388, and EF= 0.183). In the other hand, the Cw4 antigen was significantly decreased in the patients compared to controls (6.66 vs. 18.75%, P=0.031). However both associations failed to reach a significant level after correction.

At HLA-class II region (DR-loci), three antigens showed different frequencies in patients and controls, and these were DR2, DR3, and DR4. Increased frequencies of DR3 (53.33 vs. 26.25%) and of DR4 (50.0 vs. 12.5%) were observed in patients. The two positive associations were associated with RR values of 3.210 and 7.00 respectively and EF values of 0.366 and 0.428 respectively. Such positive associations were highly significant ($P=9.7x10^{-3}$ and $1x10^{-5}$ respectively) even after correction (PC=0.008 and

$9x10^{-5}$ respectively). In contrast DR2 antigen was significantly decreased in the patients (6.66 vs. 25.0%). Such negative association was significant before (P=0.003) and after correction (PC=0.027).

At HLA-DQ loci, two antigens DQ2 and DQ3 were significantly increased in the patients compared with controls. DQ2: (33.33 vs. 15.0%, P=0.009, RR=2.833, EF=0.215; DQ3: 40.0 vs. 20.0%, P=0.008, RR=2.666 and EF=0.249). The two positive associations remained significant after correction (PC=0.027and 0.024 respectively). The antigen DQ1 was significantly decreased in T1DM patients (6.66 vs. 22.5%) compared to controls, and such negative association (P=0.008) remained significant after correction (PC=0.024).

HLA Association with T1DM in Siblings:

The distribution of HLA-A; -B; -C; -DR and -DQ antigens in siblings and controls are given in (table 1), while antigens showing significant variations between siblings and controls are listed in (table 2).

At HLA-A locus, the antigen A2 showed a decreased frequency in siblings of T1DM patients (22.0 vs. 37.5%). This negative association was significant (P=0.047) before correction, but not after.

At HLA-B locus, the antigen B12 showed a significant (P=0.036), increased frequency (12.0 vs. 2.5%) with RR value of 5.318 and EF value of 0.097. Correcting the probability of this antigen failed to reach a significant level. In contrast, the B51 antigen showed negative association with the disease in the siblings. The antigen had a frequency of 12.0% in the siblings, while in the controls, the frequency was 28.75%. Although the association was significant (P=0.019), the corrected probability failed again to attain a significant level (PC=0.304).

At HLA-class II region (DR loci), an increased frequency of antigen DR4 (34.0 vs. 12.5%, P=0.003) was observed in the siblings. The RR value of such positive association was 2.428, and the EF value was 0.176. This association was significant (P=0.003) before correction, and after correction (PC=0.027). On the other hand, DR5 antigen showed a decreased frequency in the siblings as compared to controls (2.0 vs. 11.25% respectively). Such negative association was significant before correction (P=0.049) but not after (PC=0.441).

T1DM Patients vs. Siblings

As listed in (table 2), both the T1DM patients and their siblings shared the A2 and DQ1, may be considered as protective antigens, while A9, B8, DR3 and DR4 were susceptible ones. No other antigen in the present study was found to be common between the patients and their siblings. Such associations were significant before correction but not after.

Table 1: HLA antigen frequencies in control, T1DM patients and Siblings groups.

HLA-antigens	Control (Number = 80)		TIDM patients (Number =60)		Siblings (Number = 50)	
HLA-A locus	**No.**	**%**	**No.**	**%**	**No.**	**%**
A1	15	18.75	12	20.00	5	10.0
A2	30	37.50	26	43.33	11	22.0

A3	7	8.75	5	8.33	2	4.00
A9	15	18.75	24	40.00	10	20.0
A10	10	12.50	12	20.00	5	10.0
A11	16	20.00	0	ND	0	ND
A19	32	40.00	16	26.66	11	22.0
A28	8	10.00	7	11.66	3	6.00
HLA-B locus						
B7	6	7.50	6	10.00	7	14.00
B8	7	8.75	17	28.33	5	10.00
B12	2	2.50	7	11.66	6	12.00
B13	2	2.50	2	3.33	1	2.00
B14	4	5.00	0	ND	0	ND
B15	1	2.00	7	11.66	1	2.00
B16	2	2.50	6	10.00	1	2.00
B17	1	1.25	0	ND	1	2.00
B18	4	5.00	2	3.33	5	10.00
B27	5	6.25	2	3.33	2	4.00
B35	11	13.75	2	3.33	2	4.00
B37	4	5.00	7	11.66	1	2.00
B40	2	2.50	3	5.00	3	6.00
B41	8	10.00	5	8.33	2	4.00
B51	23	28.75	9	15.00	6	12.00
B73	2	2.50	2	3.33	4	8.00
HLA-C_W locus						
Cw**2**	3	3.75	3	5.00	3	6.00
Cw4	15	18.75	4	6.66	8	16.00
Cw5	2	2.50	2	3.33	0	ND
Cw7	13	16.25	19	31.66	10.	20.00
HLA-DR locus						
DR1	18	22.50	21	35.00	12	24.00
DR2	20	25.00	4	6.66	7	14.00
DR3	21	26.25	32	53.33	18	36.00
DR4	10	12.50	30	50.00	17.0	34.00
DR5	1	11.25	2	3.33	1	2.00
DR6	3	3.75	6	10.00	3	6.00
DR7	12	15.00	14	23.33	10	20.00
DR8	8	10.00	11	18.33	6	12.00
DR10	0	ND	4	6.66	6	12.00
HLA-DQ locus						
DQ1	18	22.5	4	6.66	11	22.00
DQ2	12	15.00	20	33.33	11	22.00
DQ3	16	20.00	24	40.00	15	30.00

Table 2: Antigens of HLA-class I and class II regions showing significant variations between T1DM patients, siblings and controls.

HLA	T1DM vs. control					Siblings vs. control					TIDM vs siblings	
	RR	EF	PF	P	PC	RR	EF	PF	P	PC	P	PC
A2	–	–	–	–	–	0.470	–	0.198	0.047	NS	0.014	NS
A9	2.88	0.261		0.004	0.032	–	–	–	–	–	0.019	NS
B8	4.122	0.214	–	0.002	0.032	–	–	–	–	–	0.014	NS
B12	5.150	0.093	–	0.032	NS	5.318	0.097	–	0.036	NS	–	–
B15	9.113	0.103	–	0.018	NS	–	–	–	–	–	–	–
B35	0.216	–	0.107	0.031	NS	–	–	–	–	–	–	–
B51	0.437	–	0.162	0.041	NS	0.337	–	0.191	0.019	NS	–	–
Cw4	0.309	–	0.128	0.031	NS	–	–	–	–	–	–	–
Cw7	2.388	0.183	–	0.026	NS	–	–	–	–	–	–	–
DR2	0.214	–	0.195	0.003	0.027	–	–	–	–	–	–	–
DR3	3.210	0.366	–	$9.7x10^{-3}$	0.008	–	–	–	–	–	0.051	NS
DR4	7.00	0.428	–	$1x10^{-5}$	$9x10^{-5}$	2.428	0.176	–	0.003	0.027	0.026	NS
DR5	–	–	–	–	–	0.160	–	0.095	0.049	NS	–	–
DQ1	0.246	–	0.168	0.008	0.024	–	–	–	–	–	0.019	NS
DQ2	2.833	0.215	–	0.009	0.027	–	–	–	–	–	–	–
DQ3	2.666	0.249	–	0.008	0.024	–	–	–	–	–	–	–

RR: relative risk; EF: Etiological fraction; PF: Preventive fraction; P: Fisher exact probability; PC: Corrected probability

Discussion

The present study demonstrated that immunogenetic predisposition may be considered as an important factor for the development of T1DM in association with the HLA antigens in which markers of human HLA showed different distributions in patients, siblings and controls.

At HLA class I region, significant increased frequencies of antigen A9 and B8 were observed in the patients. Other HLA T1DM association studies carried out in other world populations revealed an association with other HLA-class I antigens; B8 and B15 in Finnish population [18], in addition to A1, A2, B56, B62, Cw3 and Cw7 [19], A24 in Japanese [20]. Such differences can be explained in the ground of racial differences, especially if we consider that HLA antigens show different frequencies in different populations including Iraqis. HLA-A1 and B8 were found to be associated with T1DM in Basrah population (11) while Al-Samarria, found a very high significant association between HLA-A24, B8 and B15 and T1DM in her study that was conducted in Baghdad [12]**.** The EF value can range from 0 (no association) to 1 (maximum association). That means a value of 1 for an antigen is interpreted that this antigen is fully responsible for the development of the disease other wise if the value is in between 0 and 1, it indicates that this marker is partially involved in the disease development [17],

and other factors like environment factors can be involved. The EF value of A9 (0.261) and B8 (0.214) support the previous hypothesis and so other factors in association with these antigens contribute the rest percentage required in the development of T1DM**.** At HLA-class II region, further antigens had positive associations with T1DM. These were DR3, DR4, DQ2 and DQ3. The polymorphism of HLA-class II loci has gained much interest in the HLA disease association studies. However, multiple studies have reported association between HLA-DR and DQ phenotypes and T1DM. DQ2.DR3 and DQ3.DR4 haplotypes reported as high risk alleles in Caucasians [21], DR4, DQ4 but not DR3 were found to be dominant in Japanese [22], while DR3, DR4, DR9 and DQ2 were the only alleles positively associated with T1DM in Koreans [23]. In Finland, DQ2/DQ3 genotype was found to be associated with genetic susceptibility and was more frequent in children diagnosed <5 years of age [24], and in diabetes-associated autoantibodies emerged in children with predisposing HLA-DQ alleles after 3 months of age [25]. In Lebanese, 77% and 40% of T1DM patients were positive for DQ2 and DQ3 respectively (10). Others reported high significant association of HLA-DR3, DR4, DQ2 and DQ3 with T1DM in Iraqi patients (12). Studies of HLA genes at the molecular levels showed that this association with HLA-DR is secondary to a stronger link with certain HLA-DQ variants.

The critical factor is the amino acid at position 57 in the HLA-DQB chain. Genetic variants of DQB which encode the amino acid aspartate at this position seem to confer protection against T1DM, whereas variants encoding other amino acids increase the risk. Hence the HLA-DR3 and DR4 association arises because these DR-alleles are linked to DQB alleles which do not encode aspartate [4]. It is worthy to note that amino acid 57 in HLA-DQB lies in the "antigen binding groove". It was reported that class I HLA-24 gene promotes pancreatic β-cells destruction in an additive manner in the patients with T1DM susceptible HLA-class II genes [26]. Antigens B35, B51, Cw4, DR2 and DQ1 showed a negative association with the disease, but after correction only the DR2 and DQ1 antigens remained significant. These antigens may have protective effect especially if we consider PF values to be 0.195 for DR2 and 0.163 for DQ1 antigens.

In siblings, a significant increased frequency of antigens B12 and DR4 was observed in comparison with control subjects, but this positive association remained significant only for DR4 antigen after correction with RR value of 2.428 and EF value of 0.176. Concerning other world population studies, HLA-DR4 was found to be associated with the presence of ICA (7%) in siblings of T1DM Mexican-American patients [27]. This locus is known to be associated with T1DM risk particularly with in type 1 diabetes families [28]. Thus it may be much more useful for predicting T1DM in affected families than in population. A highly diabetogenic subset of DR4 haplotypes was detected among T1DM patient's sibling, suggesting that DR typing is 6-10 times less powerful as predictor of T1DM in the population than among patients siblings [7].

Clearly, the structural differences seen between the predisposing and protective HLA molecules will affect

their ability to bind or interact with diabetogenic antigens and the T- cell receptor (TCR) of autoreactive β-cell specific T-cells [29]. Several mechanisms have been proposed to explain how this might influence the risk of developing autoimmune T1DM. The protective HLA molecules may form stable complexes with self antigens in the thymus, leading to efficient deletion of potentially autoreactive T cells (negative selection). In contrast, the less stable complexes formed by the predisposing HLA-molecules may result in inefficient T-cell removal and the release of autoreactive T cells into the periphery [30]. The predisposing and protective HLA molecules may interact differently with the TCRs of autoreactive T cells, affecting the phenotype of the T cells (proinflammatory versus regulatory) [29] or their activation status (Proliferative versus anergised) [31]. This immunomodulatory hypothesis is supported by the observation that DQ1 can protect against the development of diabetes, even after the onset of β-cell autoimmunity [32].

The HLA- class I (-A9 and -B8) and class II (-DR3, -DR4, -DQ2, -DQ3) antigens were significantly increased in T1DM patients and they may played an important role in the etiology of the disease, while -DR2 and -DQ1 antigens were significantly decreased in those patients. In siblings a significant increase was observed in HLA-DR4 antigen compared to controls.

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