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Contribution of HLA-DQ2/DQ8 haplotypes in type one diabetes patients with/without celiac disease

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ABSTRACT

Background: Based on lack of data on the distribution of the related alleles in the T1D population in Iranian population, we assessed the frequency of HLA DQ2 and DQ8 haplotypes in patients with T1D with/without CD compared to healthy population.
Materials and methods: 70 patients with T1D without celiac disease, 60 T1D cases with CD were compared to 150 healthy individuals during 2016. Ten mililiter Gheparinized blood samples were collected, genomic DNA was extracted and alleles were genotyped by Real-time PCR using SYBR Green as a low-resolution method.
Results: HLA-DQ2 and/or HLA-DQ8 genotypes was presented in 51% and 23% of T1D patients without CD respectively. Twenty one percent of those patients carried both alleles and 5% were negative for both alleles. T1D patients with CD had much higher DQ2 frequency (72%) and lower DQ8 (11.6%), than T1D patients without CD and controls, 14% carried both alleles and 3% were negative for both. The frequencies of DQ2 and DQ8 alleles in Iranian healthy population were 19 and 5% respectively.
Conclusion: According to the same genetic background for CD and T1D we suggest that HLA-typing can be a very useful screening tool for CD in patients with type one diabetes.

1. Introduction

Type 1 Diabetes (T1D) is a multifactorial and complex autoimmune disease which is widely distributed due to its multiple genetic and environmental elements.[1] The prevalence of T1D in European population was estimated to be 0.4%, and it is well worth mentioning that, the risk of developing T1D among children from whom either of their parents has this disease is 6%, which is 15 times higher than normal population.[2] Most of the T1D patients are asymptomatic and they will be diagnosed by measuring serum antibodies in early stages. Furthermore, genetic screening in high-risk individuals can further improve the early diagnosis of T1D. Multiple immune-mediated complications due to having common factors in their pathogenesis pathways, have high correlation with incidence of T1D.[3]

Different studies have shown that genes within the HLA region, specially HLA class II genes including DQ and DR, have the greatest role in T1D susceptibility.[1] Generally, up to 50% risk of developing T1D has been associated with HLA genes located in 6p21.31 loci.[4] Among DR-DQ haplotypes, DR3 with DQ 2.5 and DR4 with DQ8, are conferring the highest risk to developing T1D.[5] Furthermore, it is well known that the risk is much higher in heterozygotes carrying both haplotypes than for either of them individually. Considering that, the late diagnosis of T1D can complicate the therapy and may potentially have fatal consequences; therefore, diagnosis of T1D disease in early stages is essential. In this regard, HLA-typing can be proposed as one of the early steps in T1D diagnosis protocol.

Autoimmune thyroid disease and especially celiac disease (CD) are two of the most prevalent disorders among T1D population. CD is a prevalent and complex immune mediated intestinal disorder with a broad spectrum of clinical manifestations.[6] The onset of this disease is induced by ingestion of gluten, which is mainly found in wheat, rye and barley.[7] The frequency of CD in the European and Iranian T1D patients is estimated to be 3–16% and 3–8% respectively.[8] Coexistence of T1D and CD can cause poor blood sugar control, which leads to more hypoglycemia related complications, inadequate management of dyslipidemia, retinopathy and nephropathy.[9] The prevalence of HLA DQ2

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and/or DQ8 haplotypes in CD patients is dependent on the ethnicity, which can vary in different regions. Previous study in Iranian CD has shown that 97% of patients and 58% of controls were carriers of HLADQ2 and/or HLA-DQ8 haplotypes.[10] As common genetic factors are shared between CD and T1D diseases, typing of HLA-DQ2 and HLA-DQ8 can potentially be used as a tool for CD screening among T1D patients.

The aim of this study was to assess the frequency of HLA-DQ2 and HLA-DQ8 haplotypes in patients with type one diabetes without CD, celiac disease patients with T1D and compared them to healthy Iranian population.

2. Materials and methods

2.1. Patients and control

During January to December 2016, two hundred and eighty individuals were invited to participate in this study. The population sample consisted in 70 T1D patients without CD, 60 celiac disease patients with T1D and 150 healthy individuals. All T1D patients without celiac disease had fasting plasma glucose level of ≥126 mg/dL and random plasma glucose of ≥200 mg/dL and all were negative for tTGA (IgA) and EMA (IgA) antibodies for celiac disease. All CD patients had positive serum antibodies (tTGA and EMA) and were confirmed by histology according to the Marsh classification (Marsh I-III).[11] Demographic and clinical information of all participants including age, gender, duration of disorder, serology results in CD patients, HbA1c and daily insulin requirement in T1D patients were collected using questionnaire. The ethics committee of Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, approved this study (IR SBMU.RIGLD.1395.14) and consent forms were collected from all participants.

2.2. HLA-DQ typing

10 ml heparinized blood sample was collected from each patient, genomic DNA was extracted using salting out method[12] and stored in −80 °C prior to the testing. Real-time PCR based on SYBR Green, as a reporter dye to achieve a low-resolution method, was conducted to allele discrimination. After amplification cycles, the melting curve analysis was performed as the main tool to determine the aforementioned alleles. All primers that are used in this study have been designed and confirmed in pervious study.[13] Real-time PCR reaction has been conducted according to QIAGEN Rotor-Gene Q48 user protocol. Following alleles were examined for determination of HLA-DQ haplotypes: HLA-DQB1*02 for HLA-DQ2 and HLA-DQB1*0302 for HLA-DQ8. Beta 2 micro-globulin was used as the internal control in the multiplex setup for each set of primers.

2.3. Statistically analysis

In our study, we have used Fisher exact test and *t*-test for gender distribution frequency and differences in the mean age among groups respectively. In T1D patients with CD, the association between Marsh classification and HLA-typing results was measured using chi-square test. p values <0.05 was considered statistically significant.

3. Results

In this study, 280 individuals were examined for HLA DQ2 and DQ8 haplotypes. Out of 70 T1D patients without CD [mean age of 26, ranging from 8 to 45 years old], 57% (n = 40) were female. In 60 T1D patients with CD [mean age of 31, ranging from 3 to 51 years old], 64% (n = 38) were female. In healthy control group [mean age of 29, ranging from 21 to 37 years old] like other two groups, the majority of participants were female (56%, n = 84). Fisher's exact test confirmed that there is no significant difference between CD and T1D group regarding the gender (p = 0.41) and age (p = 0.24).

According to descriptive analysis, HLA typing results confirmed that 51% of T1D patients without CD were carrying HLA DQ2, 23% were positive for DQ8, 21% were positive and 5% were negative for both predisposing alleles. In contrast, T1D patients with CD had higher frequency of DQ2 (72%), lower DQ8 (11.6%), 14% carried both alleles, and 3% were negative for both. The results for healthy control group showed that allele frequencies of DQ2 and DQ8 were 19% and 5% respectively, 7% were positive for both alleles and the rest were negative for both studied alleles (Fig. 1). The results confirmed that HLA-DQ2 are frequent in people with both T1D and CD than control group and this difference was statistically significant (p = 0.001).

Among T1D patients, polyphagia (95% vs 64%), polyuria (91% vs 70%), fatigue (90% vs 72%) and renal related problems (45% vs 18.5%) were reported more prominently in compare to T1D patients with active CD. Moreover, gastrointestinal complications like diarrhea (40% vs 11.4%), bloating (80% vs 7%) and gas passing (61.6% vs 8%) were more prominent in T1D patients with CD comparing to T1D group without CD. Most frequent symptoms in both groups of patients are presented in Table 1.

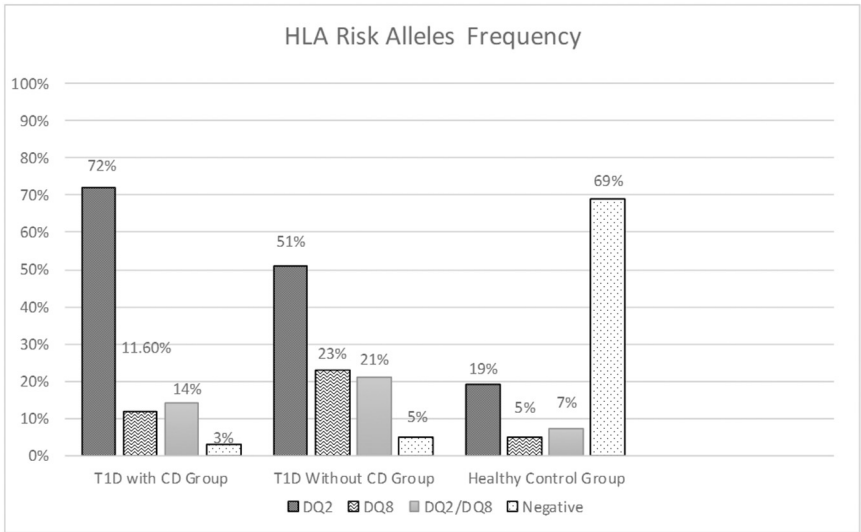

Fig. 1. Comparing the frequency of HLA risk alleles (DQ2, DQ8, DQ2/DQ8) in T1D patients with CD, T1D without CD and healthy control.

Table 1
Comparing of symptoms in T1D with and without CD.

Symptoms	T1D with CD group	T1D without CD group
Polydipsia	48 (80%)	51 (72%)
Polyphagia	57 (95%)	45 (64.2%)
Polyuria	55 (91.6%)	49 (70%)
Fatigue	54 (90%)	51 (72%)
Renal problems	27 (45%)	13 (18.5%)
Vision problems	29 (48.3%)	33 (47%)
Diarrhea	24 (40%)	8 (11.4%)
Bloating	48 (80%)	5 (7%)
Gas	37 (61.6%)	6 (8%)
Muscle ache	31 (51.6%)	21 (30%)
Weight loss	38 (63.3%)	36 (51%)
Nausea	24 (40%)	19 (27%)
Anemia	32 (53.3%)	6 (8%)
Bone disease	29 (48.3%)	11 (15.7%)
Neurological problems	23 (38.3%)	28 (40%)
Menstrual problems	17 (28.3%)	11 (15.7%)
Sterility	2 (3.3%)	1 (0.14%)
Abortion	9 (15%)	1 (0.14%)
Skin rash	23 (38.3%)	5 (7%)
Skin disease	10 (16.6%)	6 (8%)

Table 2
Association between Marsh classification and HLA risk alleles in T1D patients with CD.

Marsh classification	DQ2	DQ8	DQ2/8	Negative[a]	Total	p value
Marsh I	0	0	2	2	4	0.19
Marsh II	11	2	3	0	16	0.22
Marsh III	32	6	2	0	40	0.12
Total	43	8	7	2	60	0.43

[a] Patients were positive for both tTG and EMA.

Patients who were simultaneously affected by T1D and CD have been classified according to the Marsh classification and descriptive analysis have shown that 6.6% were classified as Marsh I, 26.6%, and 66% Marsh II and Marsh III respectively. The association between different Marsh classifications and HLA groups in T1D patients with CD has been calculated using chi-square test and we have found no significant association between different classes of Marsh and HLA groups (Table 2) ($p = 0.43$). The results showed that there is no association between the type of DQ alleles and the amount of HbA1c and the age of T1D onset in T1D without CD group.

4. Discussion

T1D and CD are both immune mediated disorders with considerable pathogenic and clinical overlaps. Previous studies have shown that the majority of T1D patients with CD have mild gastrointestinal symptoms compared to T1D patients without CD.[14] But it is worth mentioning that, whether symptomatic or silent, CD in children with T1D may be accompanied by delayed puberty and growth failure.[15] Regarding to the recent genome wide associated studies (GWAS), multiple single nucleotide polymorphisms (SNPs) have been identified as associated with autoimmune conditions such as T1D and CD.[16] Overlaps of genetic variants between CD and T1D with common pathogenic mechanisms likely explains the increase in prevalence of concomitant diseases. Although the prevalence of CD and T1D in Caucasians is thoroughly studied, there is little information about their association in Iranian population. Polymorphisms related to DQ and DR alleles in HLA class II encoding regions, are contributing 40–50% to the risk of developing T1D. DR3 and DQ2 (DRB1*0301 and DQB1*0201) and DR4 and DQ8 (DRB1*0401 and DQB1*0302) especially in heterozygote individuals, are known to confer the highest risk in developing T1D.[17] In contrast, MHC class I alleles showed to affect only T1D susceptibility.[18] Previous studies showed that 90% of CD and 55% of T1D patients were HLA-DQ2 carriers, and HLA-DQ8 is found in about 10% of CD and 70% in Caucasian T1D patients.[19] Our finding suggests that HLA-DQ2 is more prevalent in both T1D with and without CD in Iranian patients than control, with noticeably higher frequency in T1D patients with CD. We have also examined whether DQ2 and DQ8 can potentially participate in time of the T1D onset. Although DQ2 and DQ8 double positive patients usually manifest T1D symptom 2–3 years sooner than DQ2 or DQ8 single positive patients, no significant correlation between time of the T1D onset and the type of HLA has been found (Fig. 2). We have also used the HbA1c as the indicator T1D manifestation, and found that there is no correlation between the type of DQ alleles and the amount of HbA1c in T1D without CD group (Fig. 3). Similar to CD, T1D as a complex disease, is not exclusively dependent on HLA-DQ2 and HLA-DQ8.[4] Although HLA class II molecules are considered to be main protagonists in the scenario of onset of both diseases, there are multiple factors involved in the progression and the severity of both disorders.[20]

The result of this study showed that DQB1* 0302 has a higher association with frequency of Type one diabetes than CD. We identified that patients with T1D who are carrying DQB1* 0302 allele have a lower

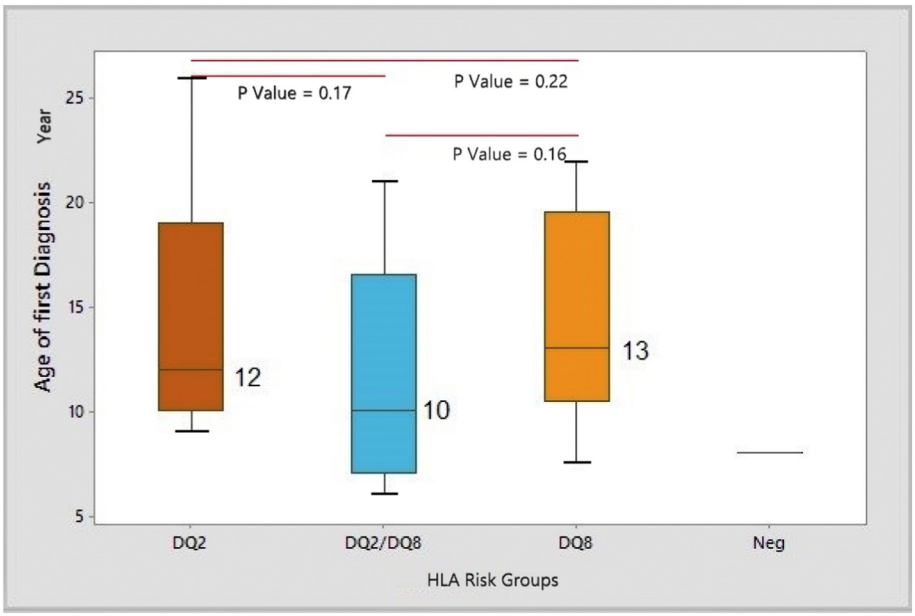

Fig. 2. Association between HLA risk alleles and the age of T1D onset.

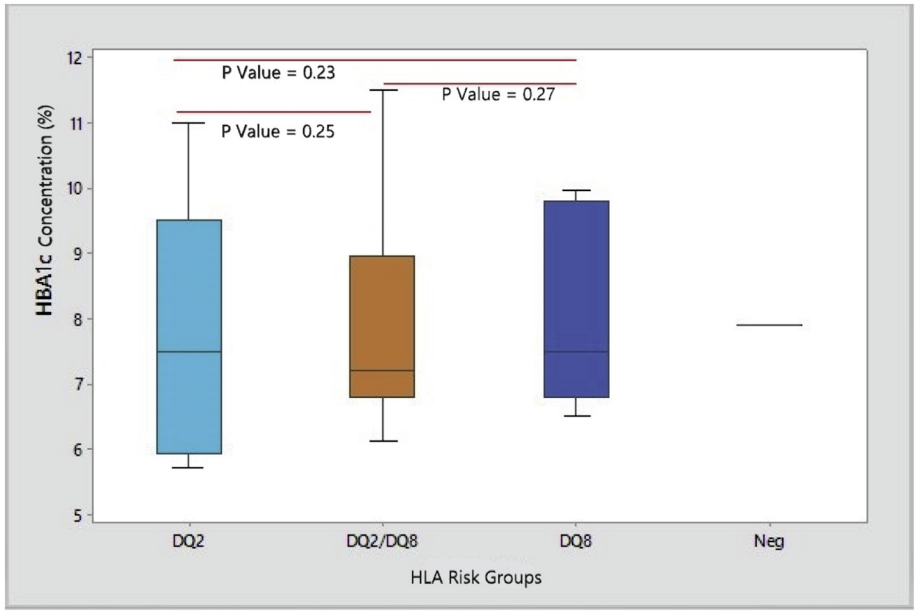

Fig. 3. Association between HLA risk alleles and the concentration of HbA1c in T1D patients without CD.

chance to be affected by celiac disease after initial diagnosis of T1D. We propose therefore, the use of HLA-typing as a reliable screening tool to identify CD among T1D patients. On the other hand, DQB1*0201 in both diseases has a much higher frequency than healthy population which can be explained by similarities in pathological pathways in both CD and T1D.[21] The higher prevalence of DQB1* 0302 in T1D patients without CD compared to healthy individuals can be explained by considering the underlying biochemical pathway of T1D and CD regarding corresponding proteins. In CD, DQ2 heterodimers bind to the side chain of gliadin peptides at P4, P6 and P7 positions while DQ8 molecules have a preference for charged residues at P9 and P1 positions. The higher avidity explains the prominent role of DQ2 comparing to DQ8 in triggering CD.[22]

Severity of T1D symptoms in the presence of active CD was shown in different studies and Iranian patients with CD are no exception with the emphasis on renal complications. In T1D patients, poor lipid profiling and higher need of daily insulin intake are evident when active CD is present.[23]

Although HLA susceptibility is known to be a common risk factor, multiple non-HLA genetic variants and environmental factors appear to contribute to the risk of developing both conditions. Considering the higher frequency of CD in patients with T1D, and the fact that the increased prevalence of obesity among children impact negatively the discrimination of T2D from T1D; we suggest that HLA-typing for DQ2 and DQ8 alleles not only increases the accuracy of screening for T1D among high-risk population but, it can be used as a reliable biomarker for distinguishing of CD and type one diabetes. On the other hand, given the increased prevalence of developing both conditions in Iranian population, HLA-typing can be a very useful screening tool for CD in patients with type one diabetes.

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