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HLA-DRB and HLA-DQB Allele and Haplotype Frequencies in Iranian Patients with Recurrent Aphthous Stomatitis

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ABSTRACT

Recurrent aphthous stomatitis (RAS) is known as the most common chronic disease of the oral cavity, which affects a range of 5-25% of the population. RAS appears to be associated with some human leukocyte antigen (HLA) class II alleles and haplotypes. This study attempts to survey the distribution of HLA-DRB and -DQB alleles among Iranian RAS patients and healthy controls.

In order to evaluate the association of HLA-DR and DQ alleles and haplotypes, 54 patients with RAS and 100 unrelated healthy subjects as control group were investigated.

Our data indicated that DRB1*13:17, DRB1*15:01, and DRB5*01 were significantly more frequent in RAS patients in comparison to controls. However, DRB3:01allele frequency was higher in the controls compared to the patients. The significantly frequent allele in the patients compared with the healthy subjects was HLA-DQB1*03:02. However, both HLA-DQB1*02:01 and HLA-DQB1*03:01 alleles were most frequent in the healthy individuals rather than the patients. The DRB*04/DQB1*03:01 and DRB*01:01/DQB1*02:01 haplotypes were significantly distributed in healthy subjects compared with patients. However, DRB*07:01/DQB1*03:02 haplotype was found to be significantly frequent in patients than controls.

In respect of HLA genes, factors are involved in the incidence of RAS; various HLA-DRB and HLA-DQB1 alleles and the related haplotypes are suggested to be the three main RAS susceptibility factors in our population study.

Keywords: Allele frequency; Haplotype frequency; Human leukocyte antigen; Recurrent aphthous stomatitis

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INTRODUCTION

Recurrent aphthous stomatitis (RAS), also known as

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recurrent aphthous ulceration or canker sores, is the most common inflammatory disorder of oral mucosa in all age groups, affecting approximately 20–25% of the population worldwide. Even though RAS is globally distributed, it is an oral cavity disorder that has been least understood to date.[1,2] The main manifestations of the disease are the appearance of painful and recurrent, multiple or single ulcerations especially in the non-keratinized oral mucosa.[3] The differential diagnosis of RAS is considered to distinguish from other similar disorders, such as behcet's disease (BD), comprises complex aphthous stomatitis and variants related to systemic diseases, like the manifestations associated with hematologic, hormonal, nutritional and gastrointestinal alterations.[1]

The etiology of RAS has less been described, despite a general consensus on the role of an autoimmune response to oral mucosa or cross-reacting antigens.[4,5] However, it has been demonstrated that both humoral and cell-mediated immune responses aberrantly recognize the oral mucosal antigens and epithelial cells.[6-9] considering an autoimmune etiology in RAS It could be implied that susceptibility to the disease can be determined genetically. To support this concept, familial history of RAS has been frequently documented.[10-12]

Human major leukocyte antigen (HLA) has been reported to contribute in the genetic susceptibility to a wide range of diseases. Several studies from different populations suggested a genetic association of RAS with HLA antigens, in spite of controversial conclusions.[13-16] Considering the results of numerous previous investigations, this study was carried out in order to analyze HLA-DR and HLA-DQ allele and haplotype frequencies in Iranian patients with recurrent aphthous stomatitis to determine if the susceptibility to the disease in Iranian population is associated with certain HLA allele and haplotypes.

MATERIALS AND METHODS

Study Subjects

In this study, genetic analysis was performed on a total of 154 individuals which comprised of 54 unrelated patients with recurrent aphthous stomatitis and 100 healthy individuals with no history of autoimmune diseases or oral cavity disorders. The patients, with the mean age of 31±2.4 years, were comprised of 21 men and 33 women. The control group included 39 men and 61 women with the mean age of 35 ± 8.5 years. The patients were diagnosed to have RAS according to the criteria of Lehner[15], and were selected randomly from Children's Medical Center Hospital, Tehran University of Medical Sciences, Tehran, Iran, over the course of 2 years from 2012-2014. RAS patients had aphthous ulcerations that recurred at least once in 2 months during a 1-year period prior to this study. As a rational point, patients and healthy controls were age and sex-matched. The study was performed based upon the recommendations of the Helsinki Declaration. The local Ethical Committee of Tehran University of Medical Sciences approved the study protocol 89-04-70-11718-69315 and written informed consent was obtained from all subjects.

DNA Extraction and HLA Typing

Ten ml of venous blood was taken from each patient and control subject in EDTA-anticoagulated venoject tubes. Afterwards, genomic DNA was extracted from peripheral blood using a modified salting-out approach.[17] HLA typing was carried out through polymerase chain reaction based on sequence specific primers (PCR-SSP), as described previously.[18,19] Each reaction mixture contained a total volume of 10µl. Sample amplification was performed using the Techne Genius thermal cyclers (St. Louis, MO, USA). Briefly, PCR conditions were: initial denaturation at 94°C for 2 min, followed by 10 cycles of 94°C denaturation for 10 secend, 65°C annealing and extension for 60 secend, and finally, 20 cycles of 94°C denaturation for 10 secend, 61°C annealing for 50 secend, and 72°C extension for 30 secend. After amplification, PCR products were run on an agarose gel, and then the gel was interpreted for specific bands using a UV trans-illuminator.

Statistical Analyses

The differences in the HLA-DR and DQ allele and haplotype frequencies between the patient and healthy control groups were analyzed using the chi-square test with Yates's correction. Fisher's exact test was used when the minimum expected count was less than 5. The odds ratio (OR) and 95% confidence interval (CI) of odds ratio were calculated. The haplotypes were calculated according to Iranian population-specific linkage disequilibrium pattern among HLA-DR and HLA-DQ alleles.[20] A *p*-value was considered to be statically significant with the level of 0.05 or less.

RESULTS

HLA-DRB Allele Frequencies

HLA-DRB allele frequencies in patients with RAS and healthy subjects are presented in Table 1. Among DRB1 alleles, DRB1*13:17 and DRB1*15:01 were found to be susceptibility alleles with the respective frequency of 6.5% and 5.7% in the patients, in comparison to 2% and 1.5% in the healthy individuals (p<0.05). The Odds Ratio for the mentioned alleles were 53.39 (CI, 1.12-11.87) and 3.86 (CI, 1.10-15.76), respectively. Furthermore, DRB5*01was significantly higher in RAS patients in comparison to controls (39.8% vs. 16.6%, respectively, p=0.028, OR=2.04 CI, 1.07-3.91). However, DRB3:01allele frequency was higher in the controls compared to patients (59% vs. 36.2%, respectively, p=0.002, OR=0.42, CI, 0.23-0.74).

Table 1. HLA-DRB1 allele frequencies in RAS patients and the healthy controls

HLA-DRB1	RAS Patients (N=54)	Controls (N=100)	OR (95% CI)	*p*-Value
01:01	9 (8.33)	14 (7)	1.20 (0.50-2.88)	0.67
03:01	4 (3.7)	20 (10)	0.34 (0.11-1.04)	0.054
03:16	1 (0.9)	5 (2.5)	0.36 (0.04-3.16)	0.34
03:42	2 (1.8)	2 (1)	1.86 (0.25-13.44)	0.52
03:46	1 (0.9)	2 (1)	0.92 (0.08-10.32)	0.94
04:01	5 (4.6)	5 (2.5)	1.89 (0.53-6.69)	0.31
04:62	9 (8.3)	10 (5)	1.72 (0.67-4.38)	0.24
07:01	5 (4.6)	13 (6.5)	0.69 (0.24-2.01)	0.50
08:08	1 (0.9)	5 (2.5)	0.36 (0.04-3.16)	0.34
09:01	1 (0.9)	6 (3)	0.30 (0.03-2.54)	0.24
11:01	17 (15.7)	50 (25)	0.56 (0.3-1.03)	0.06
11:02	5 (4.6)	5 (2.5)	1.89 (0.53-6.69)	0.31
11:07	1 (0.9)	3 (1.5)	0.61 (0.06-5.97)	0.67
11:46	1 (0.9)	2 (1)	0.92 (0.08-10.32)	0.94
11:69	2 (1.8)	7 (3.5)	0.52 (0.10-2.54)	0.41
13:01	10 (9.2)	9 (4.5)	2.16 (0.85-5.50)	0.09
13:09	1 (0.9)	5 (2.5)	0.36 (0.04-3.16)	0.34
13:17	**7 (6.5)**	**4 (2)**	**3.39 (1.12-11.87)**	**0.043**
13:67	1 (0.9)	3 (1.5)	0.61 (0.06-5.97)	0.67
14:01	3 (2.8)	11 (5.5)	0.49 (0.13-1.79)	0.27
14:05	2 (1.8)	0 (0)	-	-
14:46	1 (0.9)	2 (1)	0.92 (0.08-10.32)	0.94
14:50	2 (1.8)	1 (0.5)	3.75 (0.33-41.89)	0.25
14:58	2 (1.8)	1 (0.5)	3.75 (0.33-41.89)	0.25
15:01	**6 (5.7)**	**3 (1.5)**	**3.86 (1.10-15.76)**	**0.044**
15:25	5 (4.6)	6 (3)	1.56 (0.46-5.26)	0.46
15:27	4 (3.7)	6 (3)	1.24 (0.34-4.50)	0.73
DRB3:01	**27 (36.2)**	**99 (59)**	**0.42 (0.23-0.74)**	**0.002**
DRB4:01	25 (34)	43 (25.5)	1.53 (0.84-2.78)	0.15
DRB5:01	**21 (39.8)**	**28 (16.6)**	**2.04 (1.07-3.91)**	**0.028**

HLA, human leukocyte antigen; RAS, recurrent aphthous stomatitis; CI, confidence interval; OR, odds ratio. The bold values are significant at the level of ≤0.05

Table 2. HLA-DQA1 allele frequencies in RAS patients and the healthy controls

HLA-DQB1	RAS Patients N=54(%)	Controls N=100(%)	OR(95% CI)	*p*-Value
02:01	**11 (10.2)**	**38 (19)**	**0.48 (0.23-0.98)**	**0.043**
02:03	6 (5.5)	10 (5)	1.12 (0.39-3.16)	0.83
03:01	**22 (20.4)**	**62 (31)**	**0.56 (0.32-0.99)**	**0.045**
03:02	**16 (14.8)**	**11 (5.5)**	**2.98 (1.33-6.69)**	**0.006**
03:03	9 (8.3)	8 (4)	2.18 (0.81-5.82)	0.11
03:17	6 (5.5)	4 (2)	2.88 (0.79-10.44)	0.09
04:01	2 (1.9)	2 (1)	1.86 (0.25-13.44)	0.52
05:01	16 (14.8)	44 (22)	0.61 (0.32-1.15)	0.13
06:01	11 (10.2)	14 (7)	1.50 (0.65-3.44)	0.32
06:29	9 (8.3)	7 (3.5)	2.50 (0.90-6.93)	0.07

HLA, human leukocyte antigen; RAS, recurrent aphthous stomatitis; CI, confidence interval; OR, odds ratio. The bold values are significant at level of ≤0.05

HLA-DQB1 Allele Frequencies

The allele frequencies of HLA-DQB1 in RAS patients and control group are shown in Table 2. In DQB1 region, HLA-DQB1*03:02 was the significantly frequent allele in the patients in comparison to the healthy subjects (14.8% vs. 5.5%, respectively, p=0.006, OR=2.98, CI, 1.33-6.69). Nonetheless, both HLA-DQB1*02:01 and HLA-DQB1*03:01 alleles were most frequent in the healthy individuals compared to the patients (19 and 31% vs. 10.2 and 20.4%, respectively $p\leq0.05$). The respective OR of these alleles were 0.48 (0.23-0.98) and 0.56 (0.32-0.99).

HLA-DRB and HLA-DQB1 Haplotype Frequencies

A summary of the frequencies for HLA-DRB1/HLA-DQB1 haplotypes are listed in Table 3. The DRB*04/ DQB1*03:01 haplotype had the highest frequency in controls and was significantly (p=0.003) distributed in healthy subjects compared with patients (31% vs. 19.5%; OR=0.64; CI, 0.20-0.61). Furthermore, DRB*01:01/DQB1*02:01 haplotype was significantly (p=0.008) prevalent in healthy subjects in comparison to RAS patients (20% vs. 10.1%; OR=0.49; CI, 0.50-0.88). However, DRB*07:01/ DQB1*03:02 haplotype was found to be significantly (p=0.01) more frequent in the patients than the control group (16.6% vs. 7.5%; OR=1.95; CI, 0.32-0.57).

Table 3. HLA class II haplotype frequencies in RAS patients and the healthy controls

DRB/DQB1	RAS Patients N=54 (%)	Controls N=100 (%)	OR (95% CI)	*p*-value
DRB*0101/DQB1*0201	**11 (10.1%)**	**41 (20%)**	**0.49 (0.50-0.88)**	**0.008**
DRB*03/ DQB1*0203	9 (8%)	21 (10.5%)	0.94 (0.50-3.18)	0.12
DRB*04/ DQB1*0301	**21 (19.5%)**	**62 (31%)**	**0.64 (0.20-0.61)**	**0.003**
DRB*0701/ DQB1*0302	**18 (16.6%)**	**15 (7.5%)**	**1.95 (0.32-0.57)**	**0.01**
DRB*0808/ DQB1*0303	7 (6.4%)	15 (7.5%)	0.91 (0.70-4.34)	0.23
DRB*0901/ DQB1*0317	4 (3.7%)	11 (5.5)	0.97 (0.10-1.54)	0.38
DRB*11/ DQB1*0401	6 (5.5%)	14 (7%)	0.91 (0.71-3.54)	0.51
DRB*13/ DQB1*0501	9 (8.3%)	22 (11%)	0.89 (0.51-5.18)	0.41
DRB*1317/ DQB1*0601	6 (5.5%)	13 (6.5%)	0.96 (0.35-7.53)	0.34
DRB*14/ DQB1*0629	4 (3.7%)	7 (3.5%)	1.12 (0.82-6.81)	0.56
DRB*15/ DQB1*0201	7 (6.4%)	13 (6.5)	1.00 (0.82-5.41)	0.42
DRB*1501/ DQB1*0302	2 (1.8%)	5 (2.5%)	0.92 (0.34-4.82)	0.16

HLA, human leukocyte antigen; RAS, recurrent aphthous stomatitis; CI, confidence interval; OR, odds ratio. The bold values are significant at level of ≤0.05

DISCUSSION

This study, provides an exhaustive report of the association of HLA class II, DRB and DQB1 alleles and haplotypes with RAS in the Iranian population, resulting in a similar genetic characteristics with other geographically adjacent populations.

According to the observation of the families suffering from RAS, Ship[21] and Miller et al.[22] for the first time reported the role of genetic background in the development of RAS and suggested the autosomal recessive or multigene form of inheritance alongside with modifying influence of the environmental factors. Furthermore, the positive family history of RAS has been reported in 24–46 % of cases from relatives and twins with RAS, providing even more validation for the role of genetic factors in the etiopathogenesis of recurrent aphthous.[23,24] To enumerate the genetic risk factors that impress the individual susceptibility to RAS, there are various polymorphisms in the genome, particularly those associated with the modulations in the metabolism of interleukins (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12), tumor necrosis factor (TNF)-α and interferon (IFN)-γ.[25-28] Gene polymorphisms in other genes such as serotonin transporter gene, endothelial nitric oxide synthase gene and cell adhesion molecule genes have also been considered as possible variations predisposing to RAS.[29-32] Although the etiopathology of RAS remains unclear, a role for disrupted immunologic response is proposed. Aphthous ulcer was found to develop in response to the enhanced immunologic response against particular regions of the oral mucosa. This reaction is the result of aberrant initiated cascade of cytokines.[33] Furthermore, a bulk of studies suggest that Th1-type immunologic response plays a crucial role in the development of RAS.[34,35]

On the other hand, several investigations indicated an association between the alleles and the increased risk of RAS. According to previously performed studies in patients with RAS, a higher distribution of HLA-A33, HLA-B35 and HLA-B81,[36] HLA-B12,[16] HLA-B51,[37] HLA-DR7 and HLA-DR5 and lower incidence of HLA-B5 and HLA-DR4[38,39] were observed in comparison to healthy controls. Previous cross-sectional studies from UK and Greece populations have shown the linkage of a certain HLA antigens with RAS, especially the class I antigens A2 and B12[16] and the class II antigen DR5.[38] Nonetheless, the associations have not been found in all populations studied.[40] In the current study, associations between individual HLA class II alleles, haplotypes and RAS were assessed. Our study revealed that among DRB1 alleles, DRB5*01, DRB1*13:17 and DRB1*15:01 were observed to be susceptible alleles with high prevalence in the patients compared with healthy individuals. However, DRB3:01allele frequency was higher in the controls compared to patients, suggesting a protective effect of this locus in RAS. In DQB1 region, HLA-DQB1*03:02 was the more frequent allele in the patients in comparison to the healthy subjects. On the contrary, both HLA-DQB1*02:01 and HLA-DQB1*03:01 alleles were found to be more frequent in the healthy individuals compared to the patients.

This study is the first of its type, to the best our knowledge, which demonstrates HLA-DRB and HLA-DQB allele and haplotype frequencies in a replicated case-control survey of Iranian patients with RAS. The contribution of HLA to RAS would be beneficial if evaluated with regard to ethnic background. Our results confirmed the association of specific HLA-DR and HLADQ alleles and haplotypes with RAS, which were previously observed in other populations. Moreover, some distinctive haplotypes, which may underlie the characteristic differences distinguishing Iranian RAS patients form other populations, were observed. Last but not least, it is of an equally paramount necessity to gain closer insight into the implication of other susceptible genes in RAS; nevertheless, either determination of miscellaneous indices alongside with genetic analysis and investigation of various populations would be beneficial.

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