



Association of HLA-DRB1 with Recurrent Aphthous Stomatitis in Northeast Iranian Population

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ABSTRACT

Introduction: Recurrent aphthous ulcers are the most common pathologic conditions of the oral cavity, which despite having clear clinical features, the etiology is unknown. This study aimed to determine the relationship between one of the histocompatibility antigens (HLA DRB1) and its sub-groups with the incidence of recurrent aphthous ulcers in an Iranian population (North East of Iran).

Methods: In this case-control study, a total of 72 patients with recurrent aphthous ulcers and 70 healthy subjects in Northeast Iranian population were included. Genotyping was done by polymerase chain reaction-specific sequence primers (PCR-SSP) for each sample, according to standard kit protocol (BAG- Germany).

Results: In 72 patients with recurrent aphthous ulcers that were included in this study, 26 were male and 46 were female; of the 70 control patients, this difference not significant statistically ($P > 0.05$). The frequency of HLA -DRB1 *16 was 0.7% in the healthy subjects, however frequency of HLA -DRB1 *16 in patients with recurrent aphthous stomatitis (RAS) was 42.36%, and this difference was statistically significant ($P = 0.03$). But, this difference was not observed in other subgroups.

Conclusion: The frequency of DRB1 * 16 in the patients with RAS were higher than the group. Therefore, DRB1 * 16 can be suggested as a Predisposing factor for aphthous ulcers patients.

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Introduction

Recurrent aphthous ulcer (RAU), which also is named as recurrent aphthous stomatitis (RAS), is one of the most usual oral inflammatory diseases in adults and children, affecting about 20% to 25% of the population in the world (1-3).

This disease is characterized by frequent periods (from days to months) of painful ulcers in the non-keratinized oral mucosa, such as buccal mucosa, floor of the mouth and ventral surface of

the tongue (4, 5).

It is more common in women (6,7). Some studies have reported a higher incidence in the second decade of life (8,9). Despite the high prevalence and clear clinical features, the etiology of this disease is unknown (1). Aphthous stomatitis is a multifactorial disease (10) and the etiologic factors may be include immunological disease, hormonal disorders, genetic

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predisposition, infectious disease, allergic reaction to food, deficiency of vitamins, digestive problems, mechanical damage, and stress (3, 11).

It has been reported that family history can be related with development of RAS (12) and 24-46% of RAS cases have been reported in same family. Persons who have family history of RAS can develop ulcers sooner and show severe forms than patients do not have family history (13, 14).

The RAS etiology is less described, despite the overall agreement about the role of autoimmune reaction to oral mucosa or cross-reactive antigens. Regarding the autoimmune etiology in RAS, it can be noted that predisposition to disease can be examined genetically (15).

Major histocompatibility complex (MHC) class I and II molecules which are called human leukocyte antigens (HLA), play an important role in the immune response (16).

These molecules of cell surface play a critical role in antigen processing and T cell activity. HLA polymorphism can directly affect the bonding capacity of antigens and thus affect antigen-specific T-cell response (17).

However, the relationship between HLA-class I (HLA-A, -B and -C) and HLA-class II (DR, -DQ - DP) with oral disorder has not yet been investigated extensively (16).

Several studies have reported the presence and absence of a relationship between HLA and RAS (18-20).

A few studies have been conducted regarding the relationship between HLA alleles and the incidence of recurrent aphthous stomatitis in different human populations especially in the Iranian population (15).

The present study aimed to examine the relationship between one of the histocompatibility antigens (HLA DRB1) and its sub-groups with the incidence of recurrent aphthous ulcers in North East of Iran (Mashhad) population.

Moreover, several studies assessed the relationship between the ablation zone and pupil size with a focus on night vision problems following refractive surgeries (6-9).

This study aimed to determine the influence of age and gender on pupil size under different illuminance conditions in emmetropic and myopic healthy eyes using Keratograph 4 software.

Methods

Patients

In this case-control study, 72 patients with recurrent aphthous stomatitis (RAS) (case group) referring to the Department of Oral Medicine, Dental School, Mashhad, Iran and 70 healthy subjects (control group) were enrolled.

Aphthous stomatitis diagnosis was done by oral

Medicine specialist based on the history of single round symmetrical lesions smaller than 1 cm, appeared in non-keratinized mucosa as recurrent and had improved within ten to fourteen days without leaving scars.

To exclude aphthous-like ulcers, systemic history was collected from the patient. Also, considering obtaining a history and performing clinical tests was approved by Ethics committee of Mashhad University of Medical Sciences (MUMS), Mashhad, Iran. Aphthous-related syndromes such as Behcet's disease (eye lesions, genital and skin test) and Reiter's disease were rejected.

To select the control subjects (which matched in terms of age and sex with the case group) patients referred to periodontics department who need to apply for a blood test for periodontal surgery were enrolled. Control group had no history of oral ulcers and systemic diseases. First obtaining informed consent and a full explanation of the study.

Then 5 ml of venous blood samples were collected in EDTA tubes (BD Vacutainer, Plymouth, UK) from the participating patients and healthy controls.

Genomic DNA Extraction and PCR-SSP Technique

DNA was extracted from 5ml Complete Blood Sample by the QIAamp DNA Mini Kit manufactured by QIAGEN Germany according to the manufacturer's protocol. (Qiagen, Hilden, Germany), 35 DNAs obtained are measured by NanoDrop 100TM to determine the quality and quantity of DNA and all samples are adjusted to 25-40 ng / μ l.

HLA typing was performed for DRB1 gene alleles using the BAG HEALTH CARE kit based on the PCR-SSP molecular method according to the manufacturer's protocol (BAG HEALTH CARE-Germany). In brief, the DNA was amplified in reaction to a volume of 11.60 μ l including 8.0 μ l dH₂O, 1.10 μ l 10 x PCR buffer, 0.1 μ l Taq DNA polymerase enzyme and 2.30 μ l DNA.

Primers and nucleotides were covered on to PCR wells by Histo Type Kit DR Low (BAG Health Care, Germany). The reaction mixture was covered with strip-caps and PCR amplifications were performed using the following situations: one cycle of primary denaturation at 94° C for 5 min, then 5 cycles of denaturation at 95° C for 30 seconds, and annealing at 68° C for 30 seconds and extension 69° C for 30 seconds.

Then 10 cycles were completed using the following situations: denaturation at 96° C for 30 seconds, annealing at 64° C for 55 seconds, and extension at 73° C for 55 seconds. After this section the PCR-SSP program was followed.

This program performed 15 added cycles with the following conditions: denaturation at 95° C for 20 seconds, annealing at 61° C for 55 seconds, and an extension step at 73° C for 50 seconds. The final extension continued for 5 minutes at 72° C (21).

The amplification products were detected by electrophoresis on 2 – 2.5% (horizontal) agarose gel. Buffer for electrophoresis was 0.5x TBE (45 mM of Tris, 0.5 mM of EDTA, 45 mM of boric acid).

For size comparison apply 5 µl of the DNA length standard. Electrophoretic separation is done at 10 - 12 V/cm (by 25 cm space among the electrodes approx. 200 - 240 V), for 25 - 45 min (22) (Figure 1)

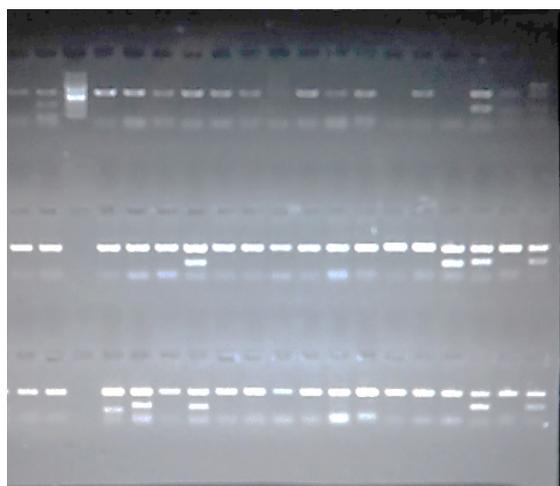


Figure 1. Gel electrophoresis of HLA-DRB1* PCR-SSP from recurrent aphthous stomatitis patients DNA. Internal control Bands are in all samples.

Statistical Analysis

Statistical analyses such as descriptive statis-

tics and odds ratio were performed by SPSS V 13 (SPSS Inc., Chicago, IL, USA). P values less than 0.05 were considered as statistically significant.

Result

In this study, 72 patients with Recurrent Aphthous Stomatitis (RAS) and 70 healthy subjects were studied to determine HLA type II (HLADRB1) and its subgroups was determined in 72 patients with RAS. In this study, 57 males (40.1%) and 85 females (59.9%) with a mean age of 36.38 ± 10.11 years and aged 21-62 years participated.

The subjects were divided into two groups: recurrent aphthous ulcers (72) and healthy (70). There were no significant differences between the two groups regarding age and sex ($p=0.656$ and $p=0.515$ respectively). In total 72 patients with RAS, 28 of them was male (38.89%) and 44 were female (61.11%), and of 70 subjects of the control group, 30 were male (42.85%) and 40 were female (57.14%). There was not statistically different between the two groups in this regard (Fisher's Exact test) ($P > 0.05$). Also, the mean age of people with RAS was 26.14 and in the control group was 32.47 that was not significant ($P > 0.05$).

Table 1 shows the distribution of different alleles of HLA class II (DRB1) in the two groups of patients and healthy people.

As shown in table, only DRB1 *16 allele frequency was statistically significant between the case and control groups; DRB1 *16 allele was positive in 1 people of the control group, but this factor was 61 cases in patients with RAS. ($P=0.03$, CI: 1.015-54.811).

Table 1. Comparison of HLA-DRB1 alleles genotyping in RAS cases and healthy control group

HLA-DRB1 ype	RAS Patients N (%)	Control patients N (%)	P-Value	Odds	CI
01	11 (7.64)	8 (5.71)	0.32	1.36	0.47-2.94
03	9 (6.25)	12 (8.57)	0.51	0.72	0.35-2.12
04	7 (4.86)	13 (9.29)	0.47	0.49	0.29-1.91
07	5 (3.47)	7 (5.0)	0.42	0.68	0.26-2.74
10	4 (1.39)	7 (5.0)	0.54	0.54	0.22-2.68
11	11 (7.64)	21 (15.0)	0.36	0.47	0.33-1.55
12	2 (1.39)	4 (2.86)	0.41	0.49	0.13-4.03
13	9 (6.25)	31 (22.14)	0.21	0.26	0.25-1.23
14	7 (4.86)	10 (7.14)	0.43	0.66	0.31-2.26
15	16 (11.11)	19 (13.57)	0.21	0.79	0.44-1.84
16	61 (42.36)	1 (0.7)	0.03	102.16	1.02-54.81
18	2 (1.39)	7 (5.0)	0.42	0.29	0.11-2.76

HLA: Human Leukocyte Antigen, RAS: Recurrent Aphthous Stomatitis, OR: Odds Ratio, CI: Confidence Interval. *This value is significant at the level of ≤ 0.05

Discussion

Recurrent aphthous stomatitis is one of the common oral diseases with unknown etiology (6). One of the important factors in this context is the genetic factor.

Today, several studies have focused on genetic predisposing markers of this disease (4). such as modulations in the metabolism of interleukins (IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12), tumor necrosis factor (TNF)- α and interferon (IFN)- γ (15). However, the association between HLA antigens and pathogenesis of recurrent aphthous stomatitis remains unclear (2).

In this study, different HLA-DRB1 alleles by SSP-PCR method and its relationship with recurrent aphthous stomatitis ulcers have been discussed.

According to the results of this study, age of RAS patients were between 20 and 30 years old and the average of patients were 26.14 years. Also, in our study most patients were female (61.11%). Some other studies have similar results (9, 23). but in few studies , results were not similar with our result (6).

More research conducted in different countries has reported the positive relationship between different HLA alleles and the incidence of aphthous ulcers (24). Association DRB1 alleles, DRB5*01, DRB1*13:17 and DRB1*15:01 were found to be disposed alleles with high frequency in the subjects with RAS compared with healthy persons in the Iranian population (15).

However, DRB3:01 allele frequency in the control subjects was higher compared to patients, suggesting a protective effect of this factor in RAS. Another study in an Iranian population (60 person) found higher incidence of HLA-DQW3, HLA-A28, HLA-B12 in control group than patients and it was suggested this antigens can be used as antigens for resistance to recurrent aphthous stomatitis (25).

A study performed on Japanese population showed a significant positive association of the HLA alleles B*5801 and CW*0302 with resistance to RAS, but this correlation was observed for HLA-A and HLA-DRB1 alleles. They suggested as HLA region involved in the RAS development, it may be also contain alleles that inhibit the onset of disease (26).

In Turkish population, HLA-A24, B13 was significantly higher in patients with aphthous than the control group; however, HLA-DR 10 and DR17 in patients with aphthous was less than the control group. As a result, it was suggested that certain antigens may be responsible for recurrent aphthous stomatitis (2). Positive relationship founded between HLA-A33, HLA-B81

and HLA-A35 with recurrent aphthous ulcers in a Brazilian population (6). Also positive relationship founded between the presence of HLAB52 and HLA-B44 and the frequency of aphthous ulcers in Arab Israeli teenagers (5, 27).

HLA-DR-W9 antigen as a genetic marker has shown in patients with recurrent aphthous ulcers in a Chinese population (20). Other studies, (28) on an English population reported a higher frequency of HLA-B12 in patients with herpetiform ulcers.

All these studies show the relationship between the frequencies of different HLA alleles in the human population with the appearance of various subtypes of recurrent aphthous stomatitis, which is not consistent with our study. This may be due to genetic differences and diversity between different human populations, the differences in the studied HLA alleles, in the choice of controls and disease diagnostic. Also, the number of samples highly affects the results; the frequency of different HLA alleles particularly in each population must be determined, so that we can identify genetic factors that may be involved in causing this disorder.

In some no relationship was found between HLA-B * 51 and patients with RAS and concluded that other environmental or genetic factors may be responsible for the RAS development (1).

Consistent with the current study, HLA-DR10 and HLA-DR17 allele frequencies were significantly lower in patients with aphthous ulcers than the control group (2).

Also, HLADR4 antigen was higher in the control group and this ratio was significantly different compared with patients with recurrent aphthous ulcers. The mentioned researchers suggested HLA-DR4, HLA-DR 17 and HLA-DR10 alleles as protective alleles against aphthous (27).

Conclusion

In the current study, relationship was observed between the incidence of recurrent aphthous stomatitis and different HLA-DRB1 alleles. We found significant difference between the case and control groups in our study was associated with HLA-DRB1 * 16 (P=0.03).

Therefore, this factor can be suggested as a Predisposing factor for aphthous ulcers; however, larger studies on HLA antigens and other subgroups with larger sample size from other populations of Iranian subgroup is required to confirm the protective role of this allele and other similar alleles against recurrent aphthous ulcers.

Proving this claim and more definitive statement depends on further studies with larger sam-

ple size and more diverse alleles and comparison with similar populations.

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Conflicts of interest

The authors declare no conflicts of interest.

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