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Associations between HLA-C Alleles and Definite Meniere's Disease

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ABSTRACT

Both genetic and environmental factors seem to play role in the etiology of Meniere's disease (MD). Several genes may be involved in susceptibility of MD including Human Leukocyte Antigens (HLA). The associations between MD and HLA alleles have been previously studied in other populations and certain HLA alleles were shown to be predisposing. The aim of this study was to determine the association between HLA-C allele frequencies and definite MD in patients who refer to Amir-Alam otolaryngology tertiary referral center in Tehran.

Patients with definite MD (N=22) enrolled according to the diagnostic criteria of American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS). Cases with all 3 symptoms of MD (Vertigo, Tinnitus and lower frequency of sensory-neural hearing loss) were included and those with suspected MD were excluded from study. HLA-Cw allele frequencies were determined in patients non-related healthy controls (N=91) using PCR -SSP.

We found that the frequency of HLACw*04 was significantly higher in patients compared to the controls [$P = 0.0015$, OR; 20, 95% CI (3.7-196.9)].

Our results revealed that HLA-C is a genetic predisposing factor in definite MD in patients who refer to Amir-Alam otolaryngology tertiary referral center.

Keywords: HLA; Immunogenetics; Meniere's Disease

INTRODUCTION

Meniere's disease (MD) is an autoimmune disorder characterized by fluctuating hearing loss and tinnitus, intermittent episodes of vertigo and aural pressure. In more than 85% of patients with this disorder their

condition is improved by changing the life style, medical treatment or invasive surgical procedures. However no ultimate cure has been identified yet.¹

MD is a multifactorial disorder with both genetic and environmental factors playing role in the etiology of the disease.^{2,3} Various etiologies have been suggested for MD including infection, allergy, endocrine disturbance, sympathetic vasomotor disturbances, psychosomatic factors and disorders of immune system.⁴ Although majority of cases of MD

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are reported in sporadic forms there are some reports of familial patients with autosomal dominant pattern of inheritance suggesting genetic predisposing factors.⁵

Crucial role of autoimmunity in the pathogenesis of MD became more prominent since MacCabe et al have described the concept of autoimmune sensorineural hearing loss in 1979.⁶⁻⁸ Patients with MD have elevated levels of circulating immune complexes and circulating antibodies capable of binding to cochlear tissue. Also lymphocyte blastogenesis assays against human inner ear tissue are positive in these patients.⁹ Moreover MD is considered to be a disorder of endolymphatic sac involving type II collagen and presence of autoantibodies against type II collagen have been reported in the sera of patients with MD.^{7,10-12}

The heritable nature of MD together with the presence of abnormal immunological factors suggests the association of Human Leukocyte Antigens (HLA) in susceptibility of MD. Early studies determined an association between MD and Cw*07 allele in Caucasians population.¹³ Several reports indicate the association between various HLA-DR alleles and MD in different populations. However the results were not consistent.¹⁴ Because of the observed association between both sporadic and familial cases of MD and HLA class I haplotypes, Morrison et al suggested the presence of a MD locus lying between the HLA-C and HLA-A loci on the short arm of chromosome 6.⁵

Based on previous evidence and the potential role of HLA-Cw molecules in MD, in the present study we investigated whether there is a relation between HLA-Cw frequency and MD among patients who refer to Amir-Alam otolaryngology tertiary referral center.

PATIENTS AND METHODS

Patients

Twenty-two consecutive patients took part in this study according to the diagnostic criteria of American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS) for Definite MD. The criteria include: Lower frequency of sensory neural hearing loss, Vertigo attacks for several hours, Tinnitus, and more than 2 years history of disease. The diagnosis was confirmed in all patients by MRI when no other lesion was observed. Cases that showed all 3 symptoms of MD (Vertigo, Tinnitus and lower frequency of sensory-neural hearing loss) were included and those with possible and probable MD were excluded from study.

Patients were recruited from the outpatient clinic in Amir-Aalam Hospital, Tehran, Iran. Controls were from the same center. The study was approved by the Ethics committee of Tehran University of Medical Sciences. Informed consents were obtained from all of the patients attending the study.

HLA-Cw Typing

DNA of patients and controls were extracted from anti-coagulated blood collected in EDTA using salting out method. HLA-Cw typing was performed using Dynal AllSet+TM SSP kit. SSP Typing is a PCR based technique which uses Sequence Specific Primers for DNA based Tissue Typing. Each SSP kit consists of a panel of primer mixes that each contains a specific primer pair (i.e., the allele- and the group-specific primers) as well as a control primer pair that matches non-allelic sequences present in all samples. An internal primer which amplifies a segment of HLA-DRA was used in this kit as follows: 607 bp amplicon comprised of Forward primer 5'-GAGGTAAGTGTGCTCACGAACAGC-3' and Reverse primer 5'-CACGTTCTCTGTAGTCTCTGGG-3'. This control acts as an internal PCR control to verify the efficiency of the PCR amplification.

Statistical Analysis

HLA-Cw allele frequencies were estimated by direct count. To compare the differences between the allele frequencies in the control and MD patients, a 2X2 contingency table analysis was performed using the Pearson chi-square tests, with Fisher exact test when the expected value for an HLA-Cw allele was <5.

The strength of association between HLA-Cw allele and MD was estimated by odds ratios (OR) and 95% confidence intervals (95% CI) using the STATA v8 program. The P values were corrected by multiplying with the number of alleles tested (Bonferroni correction). Only $P < 0.05$ was considered to be statistically significant .

RESULTS

Clinical characteristics of patients are given in table 1. HLA-Cw allele frequencies were determined in patients with definite MD and controls (Table 2). The difference in HLA-Cw allele frequencies between cases and controls was statistically significant. The allele

HLA-C association in Meniere's disease

frequency of HLA-Cw*04 was 20% in the MD group, whereas it was only 1% in the control group [$P = 10^{-4}$, $P_{corr} = 0.0015$, OR; 20, 95% CI (3.7-196.9)] (Table 2). HLA-Cw*16 allele frequency was also significantly higher in patients compared to the controls however the p value did not remain significant after Bonferroni correction [$P = 0.01$, $P_{corr} = 0.15$, OR; 3.6, 95% CI (1.06-11.7) (Table 2).

DISCUSSION

In this study we demonstrated an association between HLA-Cw*04 allele and definite MD in Iranian patients. Early studies have shown association between HLA-Cw*07 antigen and MD in British.¹⁵ Melchiorri have also found a significant increase in the distribution of the HLA-Cw*07 allele in MD patients in comparison to the patients affected by other inner ear diseases (OIDs) or healthy controls.¹⁶ However these findings were not confirmed by other studies. Koyama et al have found a higher frequency of HLA-Cw*04 in Japanese MD patients; however, it did not reach a significant level.¹⁷ In another study in South Korean population significant increase in HLA-Cw* 0303, Cw*0602, HLA-B13, HLA-B39 and DRB1*15 alleles were found compared to the control groups.¹⁸ A significantly higher frequency of HLA-DRB1*1602 in

MD patients compared to the normal controls in Japanese population also has been reported.¹⁷ Whereas Lopez-Escamez et al have observed an association between HLA-DRB1 *1101 with bilateral MD.¹⁴

Various associations reported are probably due to the ethnic or geographic differences or in some cases might be due to the small sample size studied however these studies confirm a role for HLA in MD in various populations. Various criteria for definition of MD also might result in discrepancies observed in different studies whereas in our study only patients with definite MD were included. As the linkage disequilibrium is strong between certain alleles of multiple loci within the MHC it is also possible that the HLA-Cw*04 may not be a significant factor associated with the pathogenesis of MD and it is just a marker for a MD disease susceptibility gene elsewhere in the region.

Table 1. Clinical characteristics of patients

| | | |
|--------------------------|----------|-----------------|
| Sex (M/F) | | 9/12 |
| Onset time (Years) | | 9.2±11.4 (1-48) |
| Unilateral/bilateral | | 21/1 |
| Level of hearing loss | Mild | 12 |
| | Moderate | 7 |
| | Severe | 3 |
| Total number of patients | | 22 |

Table 2. Distribution of HLA-Cw Alleles in patients and controls

| HLA-C allele | MD patients (n -22) (%)# | Control group(n -91) | P value | Pc=p*15 | OR (CI 95%) |
|--------------|--------------------------|----------------------|-----------|---------|-----------------|
| Cw*01 | 2(5%) | 7(3%) | .8 | | 1.1 (0.1-6.5) |
| Cw*02 | 0(0%) | 7 (3%) | .6 | | 0.5 (0.01-4.7) |
| Cw*03 | 1(2%) | 9(4%) | .4 | | 0.4 (0.009-3.2) |
| Cw*04 | 8(20%) | 2(1%) | 10^{-4} | 0.0015 | 20 (3.7-196.9) |
| Cw*05 | 0(0%) | 0(0%) | .2 | | 4.2 (0.05-332) |
| Cw*06 | 7(19%) | 33(18%) | .6 | | 0.8 (0.2-2.0) |
| Cw*07 | 8(20%) | 25(14%) | .5 | | 1.3(0.4-3.3) |
| Cw*08 | 0(0%) | 4(2%) | .9 | | 1.0 (0.02-10.8) |
| Cw*12 | 5(13%) | 43(24%) | .07 | | 0.4 (0.1-1.1) |
| Cw*13 | 0(0%) | 3(1%) | .7 | | 1.3 (0.02-7.7) |
| Cw*14 | 3(7%) | 17(9%) | .5 | | 0.7 (0.1-2.6) |
| Cw*15 | 1(2%) | 0(0%) | .2 | | 4.2 (0.05-332) |
| Cw*16 | 7(19%) | 9(5%) | .01 | 0.15 | 3.6 (1.06-11.7) |
| Cw*17 | 2(5%) | 7(4%) | .8 | | 1.1 (0.1-6.5) |
| Cw*18 | 0(0%) | 16(9%) | .1 | | 0.2 (0.005-1.6) |
| Total | 44(100%) | 182(100%) | | | |

Pc: P with the Bonferroni correction.

#The number of alleles is twice the number of patients, as each person has two alleles—one maternal and one paternal

CONCLUSION

In conclusion, we determined the association of Definite MD with HLA-Cw* 04 allele in patients who refer to Amir-Alam otolaryngology tertiary referral center. Further studies on larger number of samples and other genes located in HLA region will be required to confirm our data and to investigate new predisposing genes related to this disorder.

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