



Frequency of HLA Alleles in a Group of Severe COVID-19 Iranian Patients

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Abstract

Background: Human Leukocyte Antigen (HLA) system composed of a group of related proteins with important functions in the immune system. Several studies have reported that there is a significant association between specific HLA alleles and the susceptibility to different infectious diseases. This study aimed to detect the specific HLA alleles that cause higher susceptibility to COVID-19, we analyzed the HLA allele frequency distribution in Iranian patients with a severe form of COVID-19.

Methods: Overall, 48 severe cases of COVID-19 that were hospitalized and required intensive care unit (ICU) admission between Oct and Dec 2020 were included in this study. Genomic DNA was extracted from the peripheral blood samples and HLA typing (Locus A, B, and DR) was performed for the patients.

Results: After analyzing and comparing the results with a reference group of 500 Iranian individuals, a significant association was found for HLA-B*38, HLA-A*68, HLA-A*24, and HLA-DRB1*01.

Conclusion: These results may be valuable for studying the potential association of specific HLA alleles with susceptibility to COVID-19 and mortality due to the disease.

Keywords: Human leukocyte antigen (HLA); COVID-19; Iran

Introduction

Recently, with emerging of a new coronavirus related disease (COVID-19) caused by SARS-CoV-2 as the global pathogen, numerous studies have been completed around the world and many are in progress to elucidate the different aspects of the disease. The severity of the disease depend not only on the virus infection but also to the host immune response (1). Human leukocyte an-

tigens (HLAs) locus in the chromosomal region 6p21 encodes more than 130 proteins expressed on the cell surface and are considered as one of the main components of the human immune system (2). These antigens have an essential role in the immune system for the recognition of pathogens through binding and introducing the pathogen-derived molecules to T lymphocytes (3). Dif-



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ferent HLA alleles have known to be associated with various disorders including diabetes, rheumatoid arthritis, human immunodeficiency virus (HIV), hepatitis virus, tuberculosis, and several other autoimmune disorders (2, 4-7). In particular, the presence of HLA-B27 has been shown to greatly increase the risk of developing ankylosing spondylitis (8).

Several studies have investigated the frequency of different HLA alleles in different populations as well as the association of their polymorphisms with COVID-19. Understanding the effects of different HLA alleles on the differences in morbidity and mortality of the disease is a valuable point for the management of the disease. Among the different studies, some didn't report any significant association between susceptibility to COVID-19 and HLA alleles, while others found a significant association. In a recent study, there is a significant association between HLA-DRB1*15:01, -DQB1*06:02 and -B*27:07 and COVID-19 patients, when compared with controls (9). Using a bioinformatics approach for screening the SARS-CoV-2 epitopes with a significant affinity for different MHC class I alleles, a significant positive correlation was found between HLA-A*02, HLA-B*38, HLA-C*04, and HLA-C*08 with the disease (10). On the other hand, another study on a large cohort of COVID-19 patients did not find any significant association between specific HLA allele with the disease (11). Overall, the data in this field are still conflicting.

In the present study, we analyzed the frequency of different HLA alleles (HLA-A, B, DR) distribution in 48 Iranian patients with COVID-19, to identifying the alleles that may cause higher susceptibility to the disease and increase the mortality rate.

Materials and Methods

Patients

Overall, 48 severe cases of COVID-19 that were hospitalized and required intensive care unit (ICU) admission between Oct and Dec 2020

were included in this study. All the patients had Iranian nationality and also had at least one positive RT-PCR result for SARS-Cov-2.

We obtained informed written consent from all participants. The study protocol was approved by AJA University of Medical Sciences ethics committee.

DNA extraction and HLA typing

Genomic DNA was extracted from the peripheral blood samples of the patients with Qiacube HT kit using automated QIAxtractor. HLA typing of the patients (Locus A, B, and DR) was performed using *Olerup* sequence-specific oligonucleotide (SSO) HLA- typing kit. The PCR-SSP procedure is based on the principle that completely or almost completely matched oligonucleotide primers without 3'-end mismatches are more efficiently used in the PCR reaction, than mismatched primers. With stringently controlled PCR conditions, matched primer pairs allow amplification to occur, i.e. a positive result, while mismatched primer pairs don't allow amplification to occur, i.e. a negative result. After the PCR process, the amplified DNA fragments are size-separated by agarose gel electrophoresis and interpreted using SCORE 5 software.

In order to distinguish the probable susceptibility alleles in the population, the results were compared to a reference group of 500 normal Iranian individuals, previously reported (12).

Results

Overall, with evaluating the HLA alleles in 48 patients, 7 HLA alleles in HLA-A class, 17 in HLA-B and 11 in HLA-DR were detected. After analyzing the results, a significant association was found for 4 alleles, HLA-B*38 (18.7% vs 6.1%, $P < 0.001$), HLA-A*68 (15.6% vs 5.7%, $P = 0.001$), HLA-A*24 (20.8% vs 10.2%, $P = 0.003$), and HLA-DRB1*01 (15.6% vs 7.5%, $P = 0.009$) (Table 1). The association of different HLA alleles with mortality due to the disease also were evaluated and we have not found any significant association between a specific HLA allele and increase rate of mortality (Table 2).

Table 1: Frequency of different HLA-alleles in COVID-19 patients in comparison with normal population (*P*: value of chi-square statistical analysis)

| <i>HLA type</i> | <i>HLA subtype</i> | <i>Frequency (%)</i> | | <i>P</i> |
|-----------------|--------------------|----------------------|---------|----------|
| | | Patients | Control | |
| HLA-A | A*01 | 9.4 | 11.5 | 0.563 |
| | A*02 | 14.6 | 20.9 | 0.161 |
| | A*03 | 12.5 | 11.1 | 0.67 |
| | A*11 | 9.4 | 11.3 | 0.599 |
| | A*24 | 20.8 | 10.2 | 0.003 |
| | A*68 | 15.6 | 5.7 | 0.001 |
| HLA-B | B*35 | 18.7 | 16.4 | 0.572 |
| | B*38 | 18.7 | 6.1 | <0.001 |
| | B*51 | 9.4 | 9.2 | 0.957 |
| | B*52 | 9.4 | 3.8 | 0.058 |
| HLA-DRB1 | DRB1*01 | 15.6 | 7.5 | 0.009 |
| | DRB1*03 | 9.4 | 8.7 | 0.805 |
| | DRB1*04 | 9.4 | 12.5 | 0.402 |
| | DRB1*11 | 18.7 | 15 | 0.354 |
| | DRB1*13 | 11.5 | 16.2 | 0.239 |
| | DRB1*15 | 11.5 | 20 | 0.051 |

Discussion

There are several studies that discovered the presence of positive associations of certain HLA alleles with susceptibility to different infectious diseases. In the COVID-19 pandemic, several studies have tried to detect an association between certain genetic markers with the disease (13-16). Such studies may help to distinguish high risk people in the various population with the goal of management and prioritize of individuals for vaccination. In this study, using a PCR-based method, we have evaluated the association of different HLA alleles (HLA-A, HLA-B and HLA-DR) with susceptibility to COVID-19 infection. We found 4 specific HLA alleles with a significant association with the disease. On the other hand, in the present study, there was no significant association between a specific allele and mortality due to the disease. Although Yusuke et al., using in silico analysis, have reported a significant association between the presence of HLA-

*02:01 allele and COVID-19 morbidity and mortality (15).

Multiple issues impact the morbidity and mortality of the disease including Ethnicity, social, environmental, and genetic factors as well as the time since the beginning of the pandemic (11, 17). In the present study, we found a significant association between HLA-B*38 with susceptibility to the disease that is in agreement with Shekarkar et al. study (10). A genome-wide association study (GWAS) was conducted on a group of 1610 COVID-19 patients with 2305 control from Italy and Spain. The author showed no significant associations in HLA alleles with susceptibility to COVID-19 (11). On the other hand, using the next-generation sequencing method in 82 patients with COVID-19, an association between HLA alleles distribution in COVID-19 patients and healthy individuals were reported in Wang et al. studies in Chinese individuals (16).

Table 2: Association of different HLA alleles with mortality (*P*: value of chi-square statistical analysis)

| <i>HLA type</i> | <i>HLA sub-type</i> | <i>Total number</i> | <i>Alive</i> | <i>Dead</i> | <i>Mortality rate (%)</i> | <i>P</i> |
|-----------------|---------------------|---------------------|--------------|-------------|---------------------------|----------|
| HLA-A | A*01 | 9 | 3 | 6 | 66.7 | 0.4 |
| | A*02 | 14 | 6 | 8 | 57.1 | |
| | A*03 | 12 | 6 | 6 | 50 | |
| | A*11 | 9 | 6 | 3 | 33.3 | |
| | A*23 | 2 | 2 | 0 | 0 | |
| | A*24 | 20 | 8 | 12 | 60 | |
| | A*26 | 6 | 2 | 4 | 66.7 | |
| | A*32 | 3 | 3 | 0 | 0 | |
| | A*33 | 6 | 2 | 4 | 66.7 | |
| HLA-B | A*68 | 15 | 6 | 9 | 60 | 0.9 |
| | B*07 | 2 | 1 | 1 | 50 | |
| | B*08 | 3 | 1 | 2 | 66.7 | |
| | B*14 | 6 | 2 | 4 | 66.7 | |
| | B*15 | 2 | 1 | 1 | 50 | |
| | B*18 | 5 | 2 | 3 | 60 | |
| | B*27 | 3 | 2 | 1 | 33.3 | |
| | B*35 | 18 | 8 | 10 | 55.6 | |
| | B*38 | 18 | 7 | 11 | 61.1 | |
| | B*39 | 2 | 1 | 1 | 50 | |
| | B*44 | 2 | 1 | 1 | 50 | |
| | B*49 | 6 | 2 | 4 | 66.7 | |
| | B*50 | 3 | 2 | 1 | 33.3 | |
| | B*51 | 9 | 5 | 4 | 44.4 | |
| | B*52 | 9 | 4 | 5 | 55.6 | |
| | B*53 | 2 | 1 | 1 | 50 | |
| | B*55 | 3 | 2 | 1 | 33.3 | |
| B*67 | 3 | 2 | 1 | 33.3 | | |
| HLA-DRB1 | DRB1*01 | 15 | 7 | 8 | 53.3 | 0.9 |
| | DRB1*03 | 9 | 5 | 4 | 44.4 | |
| | DRB1*04 | 9 | 4 | 5 | 55.6 | |
| | DRB1*07 | 8 | 3 | 5 | 62.5 | |
| | DRB1*08 | 3 | 2 | 1 | 33.3 | |
| | DRB1*09 | 3 | 2 | 1 | 33.3 | |
| | DRB1*11 | 18 | 6 | 12 | 66.7 | |
| | DRB1*13 | 11 | 5 | 6 | 54.5 | |
| | DRB1*14 | 3 | 2 | 1 | 33.3 | |
| | DRB1*15 | 11 | 6 | 5 | 45.5 | |
| | DRB1*16 | 6 | 2 | 4 | 66.7 | |

These results may be valuable for studying the potential association of specific HLA alleles with susceptibility to COVID-19 and mortality due to the disease. An important limitation in the present study was the small sample size, which might lead to false positive results, particularly for systems with multiple alleles such as HLA and other

studies with large sample size are needed to validate our findings.

Conclusion

The results of this study may be valuable for future studies about the potential association of

specific HLA alleles with susceptibility to COVID-19 and mortality due to the disease.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Founding

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Conflicts of interest

The authors declare that they have no conflict of interest.

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