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Original Article

Investigating the Potential Impact of CCR5-Δ32 Variant on COVID-19 Outcome: A Case-Control Study in Iranian Population

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Abstract

Background: The impact of CCR5-Δ32 on COVID-19 outcomes has been the focus of much research. This genetic variant may protect against SARS-CoV-2 infection, while others have produced conflicting results. Given the controversial results of previous research on different populations, we aimed to investigate the possible association between the CCR5-Δ32 variant and COVID-19 severity in an Iranian population.

Methods: This case-control study was conducted between 25th of April till 10th of October 2021 at Rasoul Akram Hospital of Iran University of Medical Sciences, Tehran, Iran. We investigated the association between CCR5-Δ32 genotype and COVID-19 severity in 200 unrelated Iranian patients. The patients were divided into 2 groups: 100 patients with severe COVID-19 (case group) and 100 patients with mild COVID-19 (control group). Genotyping of CCR5-Δ32 was performed using the polymerase chain reaction (PCR) technique.

Results: The frequency of CCR5-Δ32 allele was 11 in the case group and 16 in the control group. However, no significant association was found between this genetic variant and the clinical outcomes of COVID-19.

Conclusion: The CCR5-Δ32 variant cannot serve as a reliable predictive factor for identifying individuals prone to developing severe COVID-19 in Iranian population. Additionally, targeting CCR5 would not be a viable treatment approach for COVID-19 in Iranians.

Keywords: Severe COVID-19; Iranian population; Virus

Introduction

COVID-19 has emerged as a perplexing and life-threatening infectious disease globally and has become the leading cause of mortality in recent years. Since its initial identification in 2019, COVID-19 has garnered significant attention due to its diverse clinical manifestations. The clinical spectrum of COVID-19 ranges from asymptomatic cases to severe pneumonia requiring hospitalization, mechanical ventilation in the intensive care unit (ICU), and even death(1). The severity of COVID-19 is influenced by various risk factors, including older age, male sex, obesity, and

smoking, as well as underlying medical conditions such as cardiovascular disorders, diabetes, hypertension, cancer, etc. (2). The most significant risk factor for severe COVID-19 is age, as the likelihood of experiencing severe outcomes significantly rises with advancing age. The presence of specific underlying medical conditions and advancing age, particularly beyond 50 yr and significantly beyond 65 yr, escalates the likelihood of severe outcomes in individuals across all age groups (3, 4). Furthermore, there have been suggestions regarding the involvement of host genetic factors as potential risk factors for susceptibility to SARS-CoV-2 infection or the development of severe COVID-19 (5).

In the last four years, there has been an upsurge in research aimed at elucidating the underlying causes of these different clinical presentations, with a particular focus on genetic factors. Some genome-wide association studies (GWAS) have been conducted to elucidate the correlation between host genetic factors and the severity of COVID-19(6). In particular, the most significant genetic risk factors for severe COVID-19 have been shown to lie in chromosome 3, a genomic region that includes gene clusters encoding chemokine receptors (CCR1, CCR2, CCR3, CCR5, CCR9, XCR1, and CXCR6)(7, 8).

The C-C chemokine receptor 5 (CCR5) is expressed in various cell types, including immune cells. Its binding to the chemokine RANTES/CCL-5(9), reported to be elevated in COVID-19, suggests that CCR5 may be involved in the inflammatory response to coronavirus infection (10).

A well-studied variant of the CCR5 gene known for the deletion of 32 base pairs, (bp) results in the formation of the CCR5-Δ32, which introduces a premature stop codon. This genetic change significantly reduces the expression of the receptor on the cell surface, potentially affecting the individual's resistance to viral diseases (11, 12), including HIV infection and AIDS progression(13). However, this variant is not always immunologically advantageous but could have adverse side effects, such as an increased risk of fatal outcomes in influenza and West Nile virus infections (14, 15). To date, there exists a paucity of research on the population-level associations between the CCR5- Δ32 variant and the severity of COVID-19. While some studies have reported a significant association (16), others have yielded entirely contradictory findings (17).

Therefore, considering the impact of host genetic factors on the variability of the clinical presentation of infectious disease, and in light of the inconsistent findings regarding the effect of the CCR5-Δ32 genetic variant on COVID-19 severity across various populations, this study aimed to investigate the association between the frequency of the CCR5-Δ32 variant and COVID-19 severity in the Iranian population.

Materials and Methods

Study design and sample collection

This case-control study investigated 2 groups of COVID-19 patients, categorized as mild and severe, each comprising 100 individuals with a mean age of 52. All participants were of Iranian nationality and tested positive for SARS-Cov-2 through RT-PCR. The study was conducted between 25 Apr and 10 Oct 2021 at Rasoul Akram Hospital of Iran University of Medical Sciences, in Tehran, Iran. Demographic information was collected from participants using a Persian questionnaire and summarized in Table 1. The severe group (case group) consisted of hospitalized patients, while the mild group (control group) included outpatients. Both groups were selected randomly from patients between the ages of 22 and 65. Patients with chronic comorbidities that could have serious or fatal consequences were excluded from the study. Two mL blood samples were collected from hospitalized patients who met WHO criteria for severe COVID-19 disease and from each mild patient using vacutainers containing ethylenediaminetetraacetic acid (EDTA).

Table 1: General characteristics of subjects

Number (total 200)		
Parameter	Case Group	Control Group
Age, yy (mean ± SD)	55.4 ± 1.4	49 ± 1.2
Male	71	54
Female	29	46
Smoker	27	31

Confirmation of SARS-CoV-2 Infection

Confirmation of SARS-CoV-2 infection was achieved through utilizing the highly reliable PowerChek™ SARS-CoV-2 Real-time PCR Kit (KogeneBiotech, South Korea) on the StepOnePlus Real-Time PCR Systems (Applied Biosystems, USA). The diagnostic process involved testing nasal, nasopharyngeal, and oropharyngeal swab samples from mild and severe patients.

DNA extraction

Following the lysis of the buffy coat, genomic DNA was extracted using Exgene™ Clinic SV (GenAll, South Korea) following the manufacturer's guidelines. The DNA quantity and purity were determined using a NanoDrop spectrophotometer (Thermo Scientific™, USA).

To evaluate DNA purity, the absorbance ratio at 260/280 nm was employed. A ratio ranging from 1.8 to 2.0 was widely acknowledged as indicative of DNA purity. Additionally, the absorbance ratio at 260/230 nm was utilized as a supplementary measure of DNA purity. Acceptable 260/230 values were within the range of 2.0 to 2.2.

Genotyping

The genotyping of samples was conducted through PCR amplification of the CCR5-Δ32 region. The primer sequences and PCR protocol utilized in this investigation were consistent with those employed in a prior study (18).

The genotypes were identified based on the final size of the PCR products; 169 bp products were related to wild genotypes and 137 bp products corresponded to the mutant genotypes. In this study, each Polymerase Chain Reaction (PCR) reaction was conducted in a 25 µl volume, which included 50–100 ng/µl genomic DNA, 1.5-unit Taq DNA polymerase, 2.5 µl of PCR Buffer (10X), 10 pmol/µl of the reverse primer, and 10 pmol/µl of the forward primer for detecting the CCR5-Δ32. The amplification was done using the Applied Biosystems PCR (Life Technologies Company; United States) according to the following thermal conditions: initial denaturation for 5 min at 95 °C, followed by 35 cycles at 95 °C for 30 sec, 60 °C for 30 sec, 72 °C for 30 sec, and a final elongation at 72 °C for 10 min. Then, PCR products were visualized by electrophoresis on a 2.5% agarose gel using a gel documentation system (Bio-Rad, USA).

Ethical approval

This research was carried out in compliance with the guidelines outlined in the Declaration of Helsinki and the Ethics Committee of Pasteur Institute of Iran (IR-PII.REC.1400.001) Tehran, Iran, granted ethical clearance for the project. Furthermore, before sampling, all subjects were provided with a written consent form and given thorough explanations regarding the project.

Statistical Analysis

The analysis was conducted using the free R-project software available at www.freerproject.org. The impact of the CCR5-Δ32 variant on the risk of severe COVID-19 was tested using a logistic regression model. After calculating raw odds ratios, adjusted odds ratios were calculated using a multiple logistic regression model to control for potential confounding factors such as age, sex, and smoking status. Statistical significance was determined at a *P*-value of 0.05.

Results

Frequency of CCR5-Δ32 variant in mild and severe COVID-19 patients

Wild-type alleles were identified as 169-bp bands and the mutant alleles as 137-bp bands, shown in Fig. 1. Eleven mutated alleles were identified in the case group, including 11 heterozygotes and no homozygotes. In addition, the control group consists of 16 mutant alleles, including 16 heterozygotes and no homozygotes (Table 2).

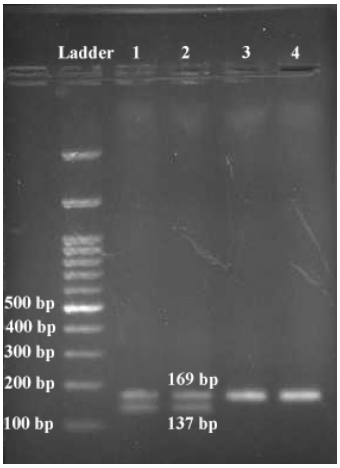

Fig. 1: CCR5-Δ32 allele genotyping by polymerase chain reaction (PCR) technique
Lanes 1 and 2: heterozygous (CCR5 Wild/Δ32); lanes 3 and 4: wild type (CCR5 Wild/Wild); ladder 100 bp DNA size marker

Associations of CCR5-Δ32 variant with severe COVID-19

The prevalence of the CCR5-Δ32 variant was examined in relation to the severity of the COVID-19. CCR5-Δ32 allele frequency in the severe and mild groups were 0.05 and 0.08 respectively. (Table 2)

Table 2: Frequency of CCR5-Δ32 variant in case and control groups

	Homozygous wild-type	*Heterozygous*	*Homozygous CCR5-Δ32*	*CCR5-Δ32 allele frequency*
Case Group%	89	11	0	0.05
Control Group%	84	16	0	0.08

There was no statistical difference in the prevalence of this variant between mild and severe COVID-19 patients (OR 0.581, 95% CI 0.25-1.35, P=0.21). However, there was a significant association between severe COVID-19 disease and age (P<0.001) and sex (P<0.024) of patients, but not with smoking status (P<0.508). After adjusting for potential confounding factors (age, sex, and smoking status), the findings once again indicated a lack of statistically significant difference between the severe and mild groups (adjusted OR 0.523, 95% CI 0.213–1.281, P=0.156). Deletion-homozygotes were not detected among the patients

Discussion

Considering the significant geographic variability in prevalence and mortality rates of the COVID-19 pandemic, besides the diversity of clinical presentation within the population, the host genetic variations may explain the different outcomes of SARS-CoV-2 infection. Extensive re-

search has been conducted in this field, but there remains an urgent need for further efforts to understand the causal association between host genetics and COVID-19 susceptibility and severity. The results could help identify biomarkers that can effectively identify individuals at risk and also identify potential therapeutic targets.

Several genes and genetic variations have been associated with susceptibility, severity, and clinical outcomes of COVID-19 (19). Among these, the CCR5-Δ32 deletion, located in the gene cluster on chromosome 3, has been extensively evaluated as a genetic variant that affects susceptibility to infectious diseases, including COVID-19 (20). CCR5-Δ32 can affect various aspects of the immune system, including risk of infection and response to inflammation (12, 21).

Some studies investigated the association of CCR5-Δ32 variant with susceptibility and clinical outcomes of COVID-19(16, 22, 23). However, the results of these studies have been contradictory, underscoring the necessity for additional research. The CCR5-Δ32 variant may protect against SARS-CoV-2 infection and could predict the risk and severity of COVID-19(22-24). However, other studies have not found a substantial association between the CCR5-Δ32 variant and the severity of COVID-19(25).

Given the controversial results of previous studies in different populations, the present study investigated the potential association between the CCR5-Δ32 variant and the severity of COVID-19 in a group of Iranian population. Our results suggest that the prevalence of the CCR5-Δ32 variant was not significantly different between the severe and mild groups of COVID-19 Iranian patients.

Our data does not support the previously proposed association between CCR5-Δ32 and the risk of severe COVID-19. An ecological study demonstrated a significant negative correlation between the allelic frequency of CCR5-Δ32 in a European population and COVID-19 deaths. CCR5-Δ32 may protect against SARS-CoV-2 infection and could predict the risk and severity of COVID-19(22).

Additionally, further investigation was conducted to gather information on COVID-19 and mortality rates across 107 countries. The study revealed a noteworthy correlation between the CCR5-Δ32 variant with susceptibility and severity of SARS-CoV-2 (16). Furthermore, Gomez et al. genotyped 294 Spanish patients hospitalized for COVID-19 and 460 healthy controls. They found a significantly lower frequency of CCR5-Δ32 in patients with severe disease than in control patients as to control (23).

However, some studies have encountered a lack of association between the CCR5-Δ32 variant and the severity of COVID-19. For example, a cross-sectional study was conducted in Germany among stem cell donors aged 18-61 yr to investigate the potential impact of CCR5-Δ32deletion on the risk of severe COVID-19. The findings suggested that the CCR5-Δ32 variant did not appear to play a significant role in determining the disease course (26).

Moreover, a comprehensive study of CCR5-Δ32 mutation frequency across 39 European countries revealed that this mutation cannot be considered a reliable indicator of COVID-19 prevalence or mortality in the European population (25).

These conflicting results highlight the need for further research to understand fully the role of this genetic variant in the context of COVID-19 in different populations.

Since genetic variations interact with different genes and environmental factors, these interactions should be considered in future studies to understand further the association between the CCR5-Δ32 variant and COVID-19 severity.

Various factors such as age, health status, and immunological responses influence the severity of the disease (27, 28). Therefore, depending on the specific circumstances, the CCR5-Δ32 variant may have different effects on the severity of COVID-19. Our results indicate that older people and men are more likely to experience severe COVID-19 disease, compared to their younger counterparts and females, which is consistent with the results of previous studies. Age and sex were important predictors of disease severity in

the Arab population (29). Another similar study in the Iranian population demonstrated that male gender and advanced age were the predominant risk factors significantly linked to the severity of COVID-19 (30). Besides, one meta-analysis revealed that male patients and elderly individuals, specifically those aged 50 yr or older, were more susceptible to experiencing severe COVID-19 (31).

Conclusion

This study represents the first report on the involvement of the CCR5-Δ32 variant in the COVID-19 pandemic in Iran. Although advanced age and male gender are the main risk factors for disease severity, the CCR5-Δ32 variant cannot be considered a reliable indicator of COVID-19 severity in the Iranian population. Furthermore, targeting CCR5 may not be a viable treatment option for COVID-19 in Iranians. However, it is important to acknowledge that various other genetic and environmental factors may influence disease severity, considered in future investigations. Additionally, certain limitations such as study design, sample size, and confounding factors may affect the results of investigations on CCR5-Δ32 and COVID-19. Therefore, to ensure the reliability and consistency of the results, further research with larger sample sizes and robust study designs in diverse populations is necessary.

Journalism 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.

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Conflict of interest

The authors declare that there is no conflict of interests

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