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Association between IFN-γ +874T/A SNP and COVID-19 Severity

Seyyed Amin Seyyed rezaei[1], Vahid Asgharzadeh[2], Behroz Mahdavi Poor[3], Mohammad Asgharzadeh[4], Asra Poorghani[4], Mahdi Asghari ozma[5], Ahmad Ali Khalili[6], Hossein Jalaei Nobari[7], Mortaza Raeisi[8], and Jalil Rashedi[9]

[1] Department of Medical Genetics, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[2] Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
[3] Infectious and Tropical Diseases Research Center, Faculty of Paramedicine, Tabriz University of Medical Sciences, Tabriz, Iran
[4] Biotechnology Research Center, Faculty of Paramedicine, Tabriz University of Medical Sciences, Tabriz, Iran
[5] Drug Applied Research Center, Tabriz university of Medical Sciences, Tabriz, Iran
[6] Department of Cardiovascular Surgery, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[7] Department of Islamic Education, Faculty of Paramedicine, Tabriz University of Medical Sciences, Tabriz, Iran
[8] Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[9] Department of Laboratory Sciences, Faculty of Paramedicine, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT

The severity of coronavirus disease 2019 (COVID-19) varies significantly among individuals, which indicates the impact of individual differences on disease. Emerging evidence suggests that genetic factors play a crucial role in determining the severity of the disease. For instance, variants in the interferon-gamma (IFN-γ) gene, such as the +874 T/A single nucleotide polymorphism (SNP), have been linked to altered immune responses and may influence the severity of COVID-19. We aim to determine the influence of the IFN-γ +874T/A SNP on the clinical outcomes of COVID-19 patients.

We investigated the SNP at position +874 in the promoter region of the IFN-γ gene in 416 individuals (206 critically ill COVID-19 patients and 210 healthy controls) in northwestern of Iran. Genomic DNA was extracted from the blood leukocytes of the patients, and the SNP was analyzed using the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method.

The AA genotype was significantly more frequent in critically ill COVID-19 patients than in healthy controls. Conversely, the AT and TT genotypes were more common in healthy controls. Furthermore, the A allele was more frequent in critically ill patients than in healthy controls, while the T allele was more frequent in healthy controls compared to critically ill patients.

Our study identified the IFN-γ +874T/A SNP as a significant genetic factor influencing COVID-19 severity. This finding underscores the critical role of genetic factors in disease severity and highlights the importance of personalized medicine in managing COVID-19.

Keywords: COVID-19; IFN-γ; Polymorphism; SARS-CoV-2

Corresponding Author: Jalil Rashedi, PhD;
Department of Laboratory Sciences, Faculty of Paramedicine, Tabriz University of Medical Sciences, Tabriz, Iran. Tel/Fax: (+98 41) 3337 1971, Email: rashedijalil@gmail.com

INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in late 2019. SARS-CoV-2 encodes four major structural proteins: spike (S), nucleocapsid (N), membrane (M), and envelope (E). The virus utilizes its S protein to bind to the angiotensin-converting enzyme 2 (ACE2) receptors on airway epithelial cells and endothelial cells, facilitating viral entry. After replication, the virus migrates down the respiratory tract, resulting in more severe clinical manifestations.[1]The clinical spectrum of SARS-CoV-2 infection varies widely, ranging from asymptomatic cases to severe viral pneumonia with respiratory failure and mortality. Approximately 80% of COVID-19 cases are mild to moderate, While around 15% of patients develop severe disease requiring supplemental oxygen, and about 5% progress to critical conditions such as respiratory failure, acute respiratory distress syndrome (ARDS), sepsis, and multiorgan failure.[2] While established risk factors include age, sex, and comorbidities, emerging evidence suggests that host genetic variants are crucial in determining disease severity. These genetic factors can influence the body's immune response, infection susceptibility, and overall disease progression.[3]

Cytokines are key regulators of the immune response, and their dysregulation can lead to severe outcomes in COVID-19. Interferon-gamma (IFN-γ) is a crucial cytokine that plays a significant role in the immune defense against viral infections, including SARS-CoV-2. IFN-γ is produced by various immune cells, including T cells and natural killer cells, and it helps activate macrophages and enhance antigen presentation, which are essential for controlling viral replication.[4] However, in severe COVID-19 cases, an imbalance in cytokine production, including elevated levels of IFN-γ, can contribute to a hyperinflammatory state known as a cytokine storm. This excessive inflammatory response can cause widespread tissue damage, particularly in the lungs, leading to ARDS and other severe complications.[5] Given the critical role of IFN-γ in the immune responses, genetic variations in the IFN-γ gene may significantly influence COVID-19 severity. Variations in the genetic sequence of the IFN-γ gene can lead to differences in the production and activity of this cytokine. Specifically, the single nucleotide polymorphism (SNP) at position +874 (rs2430561) in the promoter region of the IFN-γ gene is known to affect cytokine production and activity. This SNP affects the binding affinity of transcription factors to the promoter region, altering the expression of IFN-γ and impacting the host's ability to control viral replication and manage the inflammatory response.[6] In the context of COVID-19, elucidating the relationship between this specific SNP and disease severity could provide critical insights into the mechanisms underlying severe disease outcomes. Such knowledge may also facilitate the development of personalized therapeutic strategies aimed at modulating the immune response in patients with severe COVID-19.[7] This study aims to investigate the association between the +874 T/A polymorphism in the promoter region of the IFN-γ gene and the severity of COVID-19 in northwest Iran.

MATERIALS AND METHODS

Study Population

Samples were collected from 206 critically ill patients with COVID-19 admitted to the intensive care unit (ICU) of Imam Reza Hospital in Tabriz over a one year period from March 21, 2020, to March 21, 2021. Additionally, 210 healthy individuals without COVID-19 were selected as controls. Patients were confirmed positive for COVID-19 using real-time RT-PCR.

DNA Extraction and Polymorphism Analysis

Genomic DNA was extracted from blood by using sodium dodecyl sulfate (SDS), proteinase Kand cetyltrimethylammonium bromide (CTAB) method.

Using the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method, previously described by Farhat and colleagues,[8] SNPs at position +874 in the promoter region of the IFN-γ gene were investigated. The amplification of the target DNA was carried out using three specific primers. The generic antisense primer was 5'-TCA ACA AAG CTG ATA CTC CA-3'. Additionally, two allele-specific sense primers were employed: 5'-TTC TTA CAA CAC AAA ATC AAA TCT-3' (primer T, allele1) and 5'-TTC TTA CAA CAC AAA ATC AAA TCA-3' (primer A, allele2). PCR was performed in a total volume of 30 µL, which included genomic DNA, 0.5 µL of each primer,

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PCR buffer, 1.5 mM MgCl2, and two units of recombinant Taq DNA polymerase (Cinaclon, Iran). The PCR steps were conducted in a Techne-Cyclogene Thermocycler as follows: initial denaturation at 94°C for 7 minutes, followed by cycles of 94°C for 1 minute, 59°C for 1 minute, and 72°C for 1 minute (total of 35 cycles), with a final extension at 72°C for 7 minutes. The PCR products underwent electrophoresis in a 1.5% agarose gel.

Statistical Analysis

We compared the allele and genotype frequencies between critically ill COVID-19 patients and healthy control individuals using the chi-square test (X^2-test), considering a p-value less than 0.05 as statistically significant.

RESULTS

The frequency of the AA genotype in critically ill COVID-19 patients (59.2%) was significantly higher than that in healthy controls (44.7%) (p value = 0.003). In contrast, the frequencies of the AT and TT genotypes were higher among healthy controls compared to critically ill COVID-19 patients (Table 1).

A significant difference in the frequency of IFN-γ alleles at position +874 in the promoter region was observed for both the A and T alleles. The A allele was more frequent in critically ill COVID-19 patients (74.8%) compared to healthy controls (65%) (*p* value=0.002). Conversely, the T allele was more frequent in healthy controls (35%) compared to critically ill COVID-19 patients (25.2%) (*p* value=0.002) (Table 2).

Table1. Genotype frequencies of IFN-γ +874 gene in COVID-19 patients and healthy controls

Gene	Genotype	COVID-19 patients (%)	Healthy controls (%)	*p*
IFN-γ	AA	122 (59.2)	94 (44.7)	0.003
	AT	64 (31.1)	85 (40.5)	0.052
	TT	20 (9.7)	31 (14.8)	0.135
Total		206	210	

Table 2. Allele frequencies of IFN-γ +874 gene in COVID-19 patients and healthy controls

Gene	Allele	COVID-19 patients (%)	Healthy controls (%)	*p*
IFN-γ	A	308 (74.8)	273 (65)	0.002
	T	104 (25.2)	147 (35)	0.002
Total		412	420	

DISCUSSION

IFN-γ is one of the main pro-inflammatory cytokines and the only member of the type 2 class of interferons. It is primarily secreted by adaptive immune cells, such as T-helper 1 (Th1) and natural killer (NK) cells. It binds directly to the type 2 interferon-gamma receptor (IFNGR1) and activates Janus kinase 1 (JAK1) and Janus kinase 2 (JAK2), leading to signal transducer and activator of transcription 1 (STAT1) phosphorylation, nuclear translocation, and initiation of gene transcription for immuno-related genes.[9] Therefore, IFN-γ possesses a direct antiviral effect in infections such as SARS-CoV-2. However, uncontrolled expression of this cytokine can lead to increased disease severity, hyperinflammation, and cytokine storm, causing damage to various organs such as the lungs and bone marrow.[5] This study investigates the SNP of the IFN-γ gene at position +874 in the promoter region among 416 individuals (206 critically ill COVID-19 patients admitted to the ICU and 210 healthy controls) in northwest Iran to elucidate the possible association

between this SNP and susceptibility to severe COVID-19. Our findings indicate that the frequency of the AA genotype is significantly higher in critically ill COVID-19 patients compared to healthy controls (*p* value=0.003). This suggests a potential predisposition of individuals with the AA genotype to severe manifestations of COVID-19. Conversely, the AT and TT genotypes were more common in the healthy control group, suggesting a potential protective effect against severe COVID-19. Furthermore, the A allele was significantly more frequent in critically ill patients than in healthy controls (0.002). This finding indicates that the A allele may be linked to an increased risk of severe COVID-19. Conversely, the T allele was markedly more prevalent in the healthy control group than in the critically ill patients (0.002), suggesting a possible protective role of the T allele. However, a study by Kevin Matheus Lima de Sarges and colleagues found no significant differences in the frequencies of IFN-γ +874T/A SNP alleles and genotypes between non-severe and severe COVID-19 patients.[10] The observed differences in the results are likely due to differences in the racial origins of the studied populations, linkage disequilibrium with other functional polymorphisms in specific populations, and differences in environmental factors that can cause variations in the results through gene-environment interactions.

The underlying mechanisms of these associations can be attributed to the pivotal role of the IFN-γ gene in the immune response. IFN-γ is a critical cytokine that plays a multifaceted role in the immune system. It primarily activates macrophages, which are essential for phagocytosing pathogens and presenting antigens to T cells. Additionally, IFN-γ induces antiviral responses by enhancing the expression of major histocompatibility complex (MHC) molecules, thereby facilitating the recognition and elimination of infected cells by cytotoxic T lymphocytes.[11] The A allele at this position has been associated with lower production of IFN-γ, while the T allele is linked to higher IFN-γ production.[12] In COVID-19, a robust IFN-γ response is essential for controlling viral replication and preventing severe disease. Higher IFN-γ production associated with the T allele may enhance the antiviral response, providing protection against severe COVID-19. On the other hand, individuals with the AA genotype, associated with lower IFN-γ production, may have an impaired ability to mount an effective immune response against SARS-CoV-2.

In addition to the association of allele A with increased severity of COVID-19 symptoms and allele T with protection against severe COVID-19, these alleles also influence the risk and clinical severity of other infectious diseases. For instance, a study by Don Vu et al demonstrated that the AA genotype could elevate the risk of Cytomegalovirus (CMV) infection by up to 3.4 times in renal transplant recipients (RTRs). This study also indicated that the TT genotype might confer protection against CMV in RTRs.[13] In addition to the role of IFN-γ and its gene polymorphism in COVID-19 severity, polymorphisms in other genes can also exacerbate or mitigate COVID-19 severity. It has been shown that alleles of the MHC, known as human leukocyte antigens, HLA, particularly HLA-A*30 and HLA-A*33, can reduce the risk of contracting COVID-19 and significantly decrease the risk of severe COVID-19.[14]

Genetic polymorphisms can significantly influence susceptibility to viral infections, including COVID-19. Genotypes that reduce the production of IFN-γ and subsequently impair the establishment of a robust immune response against viral infections like SARS-CoV-2 can be considered risk factors for severe COVID-19. Consequently, genetic variations can lead to differences in disease response and change the risk of developing severe forms of COVID-19. This underscores the critical importance of personalized medicine in treating various diseases. However, our study had several limitations. Firstly, the study population, comprising patients and healthy individuals, was exclusively selected from Imam Reza hospital in Tabriz City. Secondly, the amount of interferon was not measured. Thirdly, this study focused solely on critically ill patients without considering other stages of the disease. Lastly, other polymorphisms in genes related to inflammatory and anti-inflammatory cytokines were not examined. We recommend conducting broader studies on patients at various stages of the disease and simultaneously examining polymorphisms in adjacent cytokine-related genes. These considerations will facilitate a more comprehensive statistical analysis of the impact of individual genetics on COVID-19 severity, yielding broader and more precise insights into the relationship between genetics and disease severity.

Our study found a significant association between IFN-γ gene polymorphisms and COVID-19 severity. The AA genotype and A allele at position +874 were significantly more prevalent in critically ill patients, while the T allele was markedly more common in

healthy controls. Also, the AT and TT genotypes were more prevalent in healthy controls. These findings suggest that genetic variations in the IFN-γ gene may influence the immune response to SARS-CoV-2, highlighting the importance of using personalized medicine to manage COVID-19 in the future.

STATEMENT OF ETHICS

This study was approved by the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1399.1172).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY

All necessary data will be readily upon request. For any research reviews or contributions, please do not hesitate to contact Jalil Rashedi at the following email address: Rashedijalil@gmail.com

AI ASSISTANCE DISCLOSURE

Not applicable.

REFERENCES

1. Li H, Liu SM, Yu XH, Tang SL, Tang CK. Coronavirus disease 2019 (COVID-19): current status and future perspectives. Int J Antimicrob Agents. 2020;55(5):105951.
2. Srivastava P, Gupta N. Clinical manifestations of corona virus disease. Clinical Synopsis of COVID-19: Evolving and Challenging. 2020:31-49.
3. van der Made CI, Netea MG, van der Veerdonk FL, Hoischen A. Clinical implications of host genetic variation and susceptibility to severe or critical COVID-19. Genome Med. 2022;14(1):96.
4. Kak G, Raza M, Tiwari BK. Interferon-gamma (IFN-γ): Exploring its implications in infectious diseases. Biomol Concepts. 2018;9(1):64-79.
5. Coperchini F, Chiovato L, Croce L, Magri F, Rotondi M. The cytokine storm in COVID-19: An overview of the involvement of the chemokine/chemokine-receptor system. Cytokine Growth Factor Rev. 2020;53(14):25-32.
6. Xu H, Li W, Fu H, Jiang G. Interferon-gamma gene polymorphism+ 874 (A/T) in Chinese children with Henoch-Schönleinpurpura. Iran J Allergy Asthma Immunol. 2014:184-9.
7. Zhang Y, Qin L, Zhao Y, Zhang P, Xu B, Li K, et al. Interferon-induced transmembrane protein 3 genetic variant rs12252-C associated with disease severity in coronavirus disease 2019. J Infect Dis. 2020;222(1):34-7.
8. Farhat K, Hassen E, Gabbouj S, Bouaouina N, Chouchane L. Interleukin-10 and interferon-gamma gene polymorphisms in patients with nasopharyngeal carcinoma. Int J Immunogenetics. 2008;35(3):197-205.
9. Ivashkiv LB. IFNγ: signalling, epigenetics and roles in immunity, metabolism, disease and cancer immunotherapy. Nat Rev Immunol. 2018;18(9):545-58.
10. Sarges KM, Póvoa da Costa F, Santos EF, Cantanhede MH, da Silva R, et al. Association of the IFNG+ 874T/A Polymorphism with Symptomatic COVID-19 Susceptibility. Viruses. 2024;16(4):650.
11. Castro F, Cardoso AP, Gonçalves RM, Serre K, Oliveira MJ. Interferon-gamma at the crossroads of tumor immune surveillance or evasion. Fron Immunol. 2018;9(3):847.
12. Teker E, Akadam-Teker AB, Ozturk O, Eronat AP, Yalin K, Golcuk SE, et al. Association between the interferon gamma 874 T/A polymorphism and the severity of valvular damage in patients with rheumatic heart disease. Biochem Genetics. 2018;56(12):225-34.
13. Vu D, Shah T, Ansari J, Sakharkar P, Yasir Q, Naraghi R, Hutchinson I, Min D. Interferon-gamma gene polymorphism+ 874 A/T is associated with an increased risk of cytomegalovirus infection among Hispanic renal transplant recipients. Trans Infect Dis. 2014;16(5):724-32.
14. Dieter C, Brondani LD, Leitão CB, Gerchman F, Lemos NE, Crispim D. Genetic polymorphisms associated with susceptibility to COVID-19 disease and severity: A systematic review and meta-analysis. PLoS One. 2022;17(7):e0270627.