



Journal of Acute Disease

Original Article



jadweb.org

doi: 10.4103/jad.jad_64_24

Impact Factor® 0.5

Expression level of interferon–stimulated genes *PKR*, *OAS1*, *MX1*, and *ISG15* in peripheral blood mononuclear cells of COVID–19 patients: A retrospective study

Elham Jafari Maskouni¹, Samaneh Abbasi², Elham Mousavi¹, Zahra Najafimemar³, Ali Mohammad Arabzadeh¹, Mehrdad Farrokhnia⁴, Saeedeh Ebrahimi¹✉

¹Department of Medical Microbiology (Bacteriology & Virology), Afzalipour Faculty of Medicine, Kerman University of Medical Sciences, Kerman, Iran

²Abadan University of Medical Sciences, Abadan, Iran

³Infectious Diseases Research Center, Golestan University of Medical Sciences, Gorgan, Iran

⁴Department of Internal Medicine, Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran

ABSTRACT

Objective: To explore expression level of interferon-stimulated genes *PKR*, *OAS1*, *MX1*, and *ISG15* in peripheral blood mononuclear cells of COVID-19 patients.

Methods: In this study, changes in the expression of four interferon-stimulated genes (ISGs), including *PKR*, *OAS1*, *MX1*, and *ISG15*, in peripheral blood mononuclear cells of 45 COVID-19 patients with different severities were evaluated by real-time PCR method.

Results: *OAS1*, *MX1*, *PKR*, and *ISG15* were differently expressed in COVID-19 patients with different severity. The results showed that the expression of *OAS1*, *MX1*, *PKR*, and *ISG15* genes was significantly ($P=0.001$) lower in severe patients.

Conclusions: Weak and defective IFN response and subsequent disruption of ISGs may be associated with COVID-19 severity.

KEYWORDS: COVID-19; SARS-CoV-2; Interferon; ISGs; Severe COVID-19; Risk factors; Interferon signaling

1. Introduction

In late 2019, the world was faced with an emerging virus from the Coronaviridae family called SARS-CoV-2, which rapidly spread throughout the world and turned into a pandemic. The resulting disease, called COVID-19, is undoubtedly considered an important health and social problem[1-3]. Although more than four years have

passed since the onset of the coronavirus disease 2019 (COVID-19) and the outbreak is almost under control, there are still major knowledge gaps in our understanding of the pathogenesis of the disease and immune response against SARS-CoV-2. The virus mainly affects the human respiratory system, but can also impact other organs, such as the kidneys, heart, and brain. The clinical manifestations of COVID-19 are characterized by cough, sore throat,

Significance

The risk factors of severe COVID-19 are not fully understood. The development of severe disease could be related to a defective interferon response. This study shows a significant increase in the expression of ISGs including *ISG15*, *OAS1*, *MX1*, and *PKR* in mild COVID-19 patients compared to severe COVID-19 patients. The data shows different expressions of immune response genes in COVID-19 patients based on the severity.

✉To whom correspondence may be addressed. E-mail: ebrahimisaedehe62@gmail.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2024 Journal of Acute Disease Produced by Wolters Kluwer-Medknow.

How to cite this article: Jafari Maskouni E, Abbasi S, Mousavi E, Najafimemar Z, Arabzadeh AM, Farrokhnia M, et al. Expression level of interferon-stimulated genes *PKR*, *OAS1*, *MX1*, and *ISG15* in peripheral blood mononuclear cells of COVID-19 patients: A retrospective study. J Acute Dis 2024; 13(3): 111-115.

Article history: Received 20 May 2024; Revision 11 June 2024; Accepted 12 July 2024; Available online 25 July 2024

nasal discharge, headache, and malaise in mild and moderate disease, shortness of breath, acute respiratory distress syndrome, pneumonia, and multi-organ failure in severe and critical disease[1-3]. Since the beginning of the COVID-19 pandemic, one of the main questions has been why some infected people develop severe disease while others can be asymptomatic or experience mild or moderate forms of COVID-19. Studies show that the severity of the disease depends on viral factors including virus strain, viral load, and host factors including genetics, age over 50 years, gender, various underlying conditions, and immune system characteristics[4-6]. Currently, little is known about the immune characteristics and molecular mechanisms involved in the severity of COVID-19 however, evolving pieces of evidence show that dysregulated immune responses to SARS-CoV-2 are thought to underlie the progression of COVID-19. The host immune response to SARS-CoV-2 infection involves innate immune system and adaptive immune system. The innate immune system recognizes pathogens and induces production of pro-inflammatory cytokines and chemokines that trigger the adaptive immune system. SARS-CoV-2 infection activates the innate immune response through pattern recognition receptors. Recognition of viral components, especially the viral genome, with pattern recognition receptors, activates the NF- κ B and IRF3 signaling pathway, which ultimately leads to induction and production of inflammatory cytokines or interferons (IFNs) respectively. The activation of the IFN system subsequently leads to the activation of hundreds of interferon-stimulated genes (ISGs). On the other hand, disruption in the regulation of the innate immune response leads to excessive production of inflammatory cytokines and cytokine storm and severe disease[4,7-10]. It has been reported that defects of TLR3 and IRF7 dependent type- I IFN pathway and type- I IFN may be a hallmark of severe COVID-19. ISGs are one of the most important genes induced by the activation of interferons, which play a vital role in the antiviral response and control of viral infections. ISGs have a wide range of functions and can target the key stages of a viral infection to fight viral infection. Examples include ISGs that modulate nucleic acid integrity (OAS/RNase L, ADAR1, and APOBEC family members), prevent virus entry (IFITM3), disrupt protein translation (PKR, IFIT family members), and prevent virus egress (BST2/tetarin) [7,11-14]. Hence, ISG deficiency may be a risk or increase factor of developing severe or critical COVID-19. To better understand the role of ISGs in the severity of COVID-19 disease, this study investigated mRNA expression of ISGs in COVID-19 patients.

2. Patients and methods

2.1. Study population

This study was performed among patients with COVID-19 referred

to Afzalipour Hospital in Kerman City, Iran, from February to April 2021.

2.2. Inclusion and exclusion criteria

The inclusion criteria were as follows: Persons ≥ 18 years old with laboratory-confirmed COVID-19 infection. The exclusion criteria were as follows: individuals with any immunodeficiency (for example, those with HIV, individuals receiving immunosuppressant medication, and those with cancer), tuberculosis, and other respiratory diseases.

2.3. Grouping

According to disease severity, the study population was divided into mild/moderate cases (15 COVID-19 patients with mild clinical symptoms *e.g.*, cough, sore throat, with or without fever, and no pneumonia on lung CT or lung CT with mild pneumonia) and severe group (15 COVID-19 patients with respiratory rate, ≥ 30 breaths per minute, and hypoxemia (oxygen saturation, $\leq 93\%$)).

2.4. RNA extraction, cDNA synthesis, and quantitative real-time PCR

Peripheral blood mononuclear cells (PBMCs) were isolated from the whole blood samples of patients and healthy controls. PBMCs were stored by suspending in RPMI with 20% fetal bovine serum in liquid nitrogen at -196°C . Total RNA was extracted from PBMCs using the Trizol reagent (Yekta Tajehiz, Iran). RNA concentration and purity were determined by Nano DROP ND1000 Nanodrop spectral photometer (PqLab, Erlangen, Germany). cDNA synthesis was performed by reverse transcriptase kit from Yekta Tajehiz, according to the manufacturer's guidelines. mRNA expression of the ISGs genes was tested by SYBR green master mix (AMPLICON) using ABI 7500 StepOnePlus Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) via specific primer sets (Table 1). Quantitative Real-time PCR technique was conducted to measure the relative amounts of target genes in each sample, based on the $2^{-\Delta\Delta\text{Ct}}$ method for data analysis. The mean of *GAPDH* gene expression was considered as an internal control gene to normalize the expression levels of target genes.

2.5. Statistical analysis

Statistical analyses were conducted in the SPSS software 19.0 version and Graph pad prism 9. The data were presented as mean $\pm$ SD or frequency and analyzed by *t*-test, chi-square test, and Fisher's exact test. The differences were regarded as statistically significant when $P < 0.05$.

Table 1. Primer sets used to determine the mRNA expression levels of ISGs by Real-time PCR.

Primer	Forward (5'–3')	Reverse (5'–3')	Annealing temperature (°C)	Reference
<i>ISG15</i>	CTCTGAGCATCCTGGTGAGGAA	AAGGTCAGCCAGAACAGGTCGT	60	[15,16]
<i>OAS1</i>	AGGAAAGGTGCTTCCGAG	GGACTGAGGAAGACAACCAGGT	58	[15,16]
<i>MX</i>	GGCTGTTTACCAGACTCCGACA	CACAAAGCCTGGCAGCTCTCTA	60	[15,16]
<i>PKR</i>	TCTTCATGTATGTGACACTGC	CACACAGTCAAGGTCCTTAG	58	[17]
<i>GAPDH</i>	CCCCTCCTCCACCTTTGAC	CATACCAGGAAATGAGCTTGACA	60	[18]

2.6. Ethical statement

Sample collection was performed according to the rules and regulations set by the ethical committee of Kerman University of Medical Sciences (ethical number: IR.KMU.AH.REC.1400.196).

3. Results

3.1. Demographics characteristics

A total of 30 COVID-19 patients were included. They were divided into two groups (mild/moderate and severe). Demographic information of patients is collected, including sex, age, symptoms, and the presence or absence of underlying diseases such as diabetes, hypertension, or heart diseases (Table 2). The average age of all subjects was (50.4±15.5) years and there were no significant differences in age or sex ($P>0.05$).

3.2. mRNA expression of ISGs

Expression levels of *PKR*, *MX1*, *OAS1*, and *ISG15* were significantly lower ($P<0.05$) in the severe group (Table 3).

Table 2. Patients characteristics.

Variables	Mild/moderate(n=15)	Severe (n=15)	t/χ^2	P
Age (years, mean±SD)	47.4 ± 6.0	53.4 ± 15.5	1.397	0.173
Male (n)	7	6	0.135	0.712
Symptoms (n)				
Fever	1	3	1.154	0.597
Cough	2	5	1.677	0.389
Sore throat	2	4	0.833	0.561
Pneumonia	1	3	1.154	0.597
Underlying diseases (n)				
Diabetes	2	4	0.833	0.561
Heart diseases	1	3	1.154	0.597
Hypertension	2	5	1.677	0.389

Table 3. mRNA expression of ISGs.

Gene (fold)	Mild/moderate(n=15)	Severe (n=15)	t	P
<i>PKR</i>	1.00±0.11	0.15±0.02	31.108	<0.01
<i>MX1</i>	1.00±0.11	0.26±0.03	24.699	<0.01
<i>ISG15</i>	1.00±0.14	0.46±0.06	13.722	<0.01
<i>OAS1</i>	1.00±0.16	0.43±0.11	11.382	<0.01

4. Discussion

Like other viral diseases, in SARS-CoV-2 infection, the host's innate immune system is activated as the first line of defense to fight this foreign agent, and by recognizing the pathogenic patterns of SARS-CoV-2 (mainly RNA genome), leads to the production of cytokines and chemokines, including interferons and inflammatory cytokine. Cytokines released from innate immune moreover to fight the virus in the early stages of infection, also stimulate the acquired immune system. Interferons are one of the most important cytokines produced by innate immunity in viral infections and SARS-CoV-2 infection. The activation of interferons in virus-infected cells leads to the expression of interferon-stimulated genes (ISGs) in nearby cells, which have strong antiviral effects. ISGs are thought to be the primary effectors against many viral infections including human coronaviruses[7,8,12,14,19]. In various studies, the decrease in the expression level of ISGs has been mentioned as one of the reasons for the death of severe COVID-19 patients and the severity of the disease. In this study, the expression level of 4 ISG genes including *ISG15*, *OAS1*, *MX1*, and *PKR* was investigated. The data of the study showed different expressions of immune response genes in COVID-19 patients based on the severity of the disease. In line with the results of our study, several studies have reported increased expression of ISGs in patients with mild form of the disease compared to severe form. For example, in a study by Li *et al.*, the expression of interferon alpha, as well as several genes stimulated by interferon ISGs such as *APOBEC3G*, *MX1*, and *ISG15*, which plays an important role in antiviral activities, were significantly lower in severe /critical COVID-19 patients compared to mild and moderate COVID-19 patients[7]. Another study described the clinical characteristics of COVID-19 patients including high viral load, low expression of type 1 interferon, low expression of ISGs, lymphopenia, and increase of TNF- α , NF- κ B, IL-6, CXCR2, CCL2/CCR2. They showed by examining PBMC in patients with different severity of COVID-19, that the responses of type 1 interferon in patients with severe or critical COVID-19 were very damaged and the expression level of ISGs was significantly lower in severe patients compared to mild patients[12]. Also, these findings, *i.e.* a regular pattern of interferon-stimulated ISG gene expression in patients with mild COVID-19 compared to severe COVID-19 patients, have been confirmed in the study of Combes *et al*[11]. Several factors can disrupt the interferon signaling pathway and the

expression of ISGs, including autoantibodies against interferons, deficiency of interferon proteins due to inherited mutations in the genes of the interferon signaling pathway, and disruption of the interferon response due to escape mechanisms of SARS-CoV-2. Zhang *et al*, found the presence of autoantibodies against interferons, especially interferon α , in at least 10% of patients with severe COVID-19[6]. These autoantibodies lead to the reduction of type 1 interferons, thereby reducing the production of ISGs and weakening the host's immune response, facilitating the viral replication cycle and increasing the chance of the patient suffering from a severe or critical form of the disease[6,20]. Other studies indicate deficiencies in interferon proteins, specifically, type 1 interferon through inherited mutations in genes of the type 1 interferon signaling pathway. Zhang *et al*, also showed that the mutation in these genes of signaling pathways such as TLR3 and TLR7 is much higher in patients with severe COVID-19 than in people with mild disease[6,20]. In addition, an impaired type 1 interferon response can occur due to the escape mechanisms of SARS-CoV-2, which allow the virus to escape the host's immune response. It seems that the proteins of SARS-CoV-2 can interfere with the interferon pathways and inhibit some of the interferon signaling pathways and the production of ISGs[6,20-22]. The limitations of this study are its small sample size and also miss detection of more ISGs. In the future, more studies will be included and more ISGs will be detected.

In conclusion, this study demonstrated the low expression level of ISGs, *PKR*, *OAS1*, *MX1*, and *ISG15* in severe and mild patients. These data suggest ISG activation could contribute to controlling SARS-CoV-2 infection, while impairment of IFN responses and subsequent downregulation of ISGs could be associated with progression to severe COVID-19.

Conflict of interest statement

The authors report no conflict of interest.

Funding

This work was financially supported by the research council of Kerman University of Medical Sciences, Kerman, Iran (Grant Number: 400000232).

Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

SE, EM, SA, and AMA performed concepts, and SE and EJM performed design. EJM and ZN performed the literature search and clinical studies. EJM and EM performed experimental studies. ZNM, EJM, SE, and SA performed manuscript preparation. SE, EJM, and ZN performed statistical analysis. SE, SA, and EM analyzed the data. SE, EM, AMA, and MF performed manuscript editing.

References

- [1] Rabi FA, Al Zoubi MS, Kasasbeh GA, Salameh DM, Al-Nasser AD. SARS-CoV-2 and Coronavirus Disease 2019: What we know so far. *Pathogens* 2020; **9**(3). DOI: 10.3390/pathogens9030231.
- [2] Wang MY, Zhao R, Gao LJ, Gao XF, Wang DP, Cao JM. SARS-CoV-2: Structure, biology, and structure-based therapeutics development. *Front Cell Infect Microbiol* 2020; **10**: 587269. DOI: 10.3389/fcimb.2020.587269.
- [3] Wu YC, Chen CS, Chan YJ. The outbreak of COVID-19: An overview. *J Chin Med Assoc* 2020; **83**(3): 217-220. DOI: 10.1097/jcma.0000000000000270.
- [4] Jordan RE, Adab P, Cheng KK. Covid-19: Risk factors for severe disease and death. *BMJ* 2020; **368**: m1198. DOI: 10.1136/bmj.m1198.
- [5] Yahya BA, Saad DN, Ali SH. The effect of age and gender on the severity of coronavirus infection. *Mosul J Nurs* 2022; **10**(2): 169-174.
- [6] Zhang Q, Bastard P, Cobat A, Casanova JL. Human genetic and immunological determinants of critical COVID-19 pneumonia. *Nature* 2022; **603**(7902): 587-598. DOI: 10.1038/s41586-022-04447-0.
- [7] Li S, Duan X, Li Y, Li M, Gao Y, Li T, et al. Differentially expressed immune response genes in COVID-19 patients based on disease severity. *Aging* 2021; **13**(7): 9265-9276. DOI: 10.18632/aging.202877.
- [8] Park A, Iwasaki A. Type I and type III interferons - Induction, signaling, evasion, and application to combat COVID-19. *Cell Host Microbe* 2020; **27**(6): 870-878. DOI: 10.1016/j.chom.2020.05.008.
- [9] Vincent JL, Taccone FS. Understanding pathways to death in patients with COVID-19. *Lancet Respir Med* 2020; **8**(5): 430-432. DOI: 10.1016/s2213-2600(20)30165-x.
- [10] Zhou Z, Ren L, Zhang L, Zhong J, Xiao Y, Jia Z, et al. Heightened innate immune responses in the respiratory tract of COVID-19 patients. *Cell Host Microbe* 2020; **27**(6): 883-890. DOI: 10.1016/j.chom.2020.04.017.
- [11] Combes AJ, Courau T, Kuhn NF, Hu KH, Ray A, Chen WS, et al. Global absence and targeting of protective immune states in severe COVID-19. *Nature* 2021; **591**(7848): 124-130. DOI: 10.1038/s41586-021-03234-7.
- [12] Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Smith N, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science* 2020; **369**(6504): 718-724. DOI: 10.1126/science.abc6027.
- [13] Munnur D, Teo Q, Eggermont D, Lee HH, Thery F, Ho J, et al. Altered

- ISGylation drives aberrant macrophage-dependent immune responses during SARS-CoV-2 infection. *Nat Immunol* 2021; **22**(11): 1416-1427. DOI: 10.1038/s41590-021-01035-8.
- [14]Wang Z, Pan H, Jiang B. Type I IFN deficiency: An immunological characteristic of severe COVID-19 patients. *Signal Transduct Target Ther* 2020; **5**(1): 198. DOI: 10.1038/s41392-020-00306-4.
- [15]Lv LL, Yang YX, Shi TD. Effect of haptoglobin on the treatment of chronic hepatitis B with interferon. *Exp Ther Med* 2019; **18**(2): 1417-1425.
- [16]Chen M, Hu P, Ling N, Peng H, Lei Y, Hu H, et al. Enhanced functions of peripheral $\gamma\delta$ T cells in chronic hepatitis B infection during interferon α treatment *in vivo* and *in vitro*. *PloS One* 2015; **10**(3): e0120086.
- [17]Ranganath N, Sandstrom TS, Fadel S, Côté SC, Angel JB. Type I interferon responses are impaired in latently HIV infected cells. *Retrovirology* 2016; **13**: 1-9.
- [18]Lechler P, Balakrishnan S, Schaumburger J, Grässel S, Baier C, Grifka J, et al. The oncofetal gene survivin is re-expressed in osteoarthritis and is required for chondrocyte proliferation *in vitro*. *BMC Musculoskelet Disord* 2011; **12**: 1-8.
- [19]King C, Sprent J. Dual nature of type I Interferons in SARS-CoV-2-induced inflammation. *Trends Immunol* 2021; **42**(4): 312-322. DOI: 10.1016/j.it.2021.02.003.
- [20]Zhang Q, Meng Y, Wang K, Zhang X, Chen W, Sheng J, et al. Inflammation and antiviral immune response associated with severe progression of COVID-19. *Front Immunol* 2021; **12**: 631226. DOI: 10.3389/fimmu.2021.631226.
- [21]Clark LK, Green TJ, Petit CM. Structure of nonstructural protein 1 from SARS-CoV-2. *J Virol* 2021; **95**(4). DOI: 10.1128/jvi.02019-20.
- [22]Niranjan V, Setlur AS, Karunakaran C, Uttarkar A, Kumar KM, Skariyachan S. Scope of repurposed drugs against the potential targets of the latest variants of SARS-CoV-2. *Struct Chem* 2022; **33**(5): 1585-608. doi: 10.1007/s11224-022-02020-z.

Publisher's note

The Publisher of the Journal remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Edited by Tan BJ, Chen SR