Babol University Of Medical Sciences

IJMCM, Spring 2025, VOL 14, NO 2

International Journal of Molecular and Cellular Medicine

Journal homepage: www.ijmcmed.org

ORIGINAL ARTICLE

SARS-CoV-2 ORF7 subgenomic RNA and Host IFN-β Expression in COVID-19 Hospitalized Patients with Different Prognosis

Akbar Zirakjou[1], Farzin Sadeghi[2*], Arefeh Ebrahimian Shiadeh[3], Maryam Hatamibardar[1], Mohammad Ranaee[4], Hemmat Gholinia[5], Hossein Ghorbani[6,4*]

1. Student Research Committee, Babol University of Medical Sciences, Babol, Iran.
2. Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran.
3. Department of Medical Microbiology, Faculty of Medicine, Golestan University of Medical Science, Gorgan, Iran.
4. Department of Pathology, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran.
5. Social Determinants of Health Research Center, Health Research Institute,Babol University of Medical Sciences, Babol, Iran.
6. Cancer Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran.

ARTICLE INFO

Received: 2025/04/14
Revised: 2025/05/24
Accepted: 2025/05/25
DOI: 10.22088/IJMCM.BUMS.14.2.726

***Corresponding:**
Hossein Ghorbani
Address:
Cancer Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran.
E-mail:
ghorbani7958@yahoo.com

***Co-corresponding:**
Farzin Sadeghi
Address:
Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran
E-mail:
sadeghifarzin6@gmail.com

ABSTRACT

The worldwide spread of COVID-19, triggered by SARS-CoV-2, has highlighted how viral accessory proteins contribute significantly to bypassing host immune defenses and increasing illness severity. This study investigates the relationship between the levels of SARS-CoV-2 subgenomic RNA (sgRNA) for ORF7a and ORF7b and host interferon-beta (IFN-β) expression in hospitalized COVID-19 patients with different prognoses. Upper respiratory tract samples from 89 patients (49 with poor prognosis and 40 with good prognosis) were analyzed using quantitative real-time PCR to measure ORF7a, ORF7b, and IFN-β expression levels. The results revealed significantly higher expression of ORF7a and ORF7b in patients with poor prognosis compared to those with favorable outcomes ($P < 0.001$). Conversely, IFN-β expression was significantly reduced in the poor prognosis group ($P < 0.001$), suggesting a potential mechanism of immune suppression. Older age, underlying health conditions, and elevated levels of inflammatory biomarkers, such as CRP and D-dimer, were also associated with poorer outcomes. These findings underscore the potential role of ORF7 proteins in suppressing IFN-β signaling, contributing to disease severity. Targeting these viral proteins may offer promising therapeutic avenues to enhance antiviral responses and improve patient outcomes. The study was conducted from August 2022 to February 2022. Further research is warranted to better understand the interplay between viral immune evasion mechanisms and host responses across diverse patient populations.

Keywords: SARS-CoV-2, sgRNA, Interferon-beta (IFN-β), prognosis, Immune evasion

[DOI: 10.22088/IJMCM.BUMS.14.2.726]

Introduction

SARS-CoV-2 is the virus that triggered the global COVID-19 pandemic, which has resulted in significant illness and death around the world(1). As a member of the Betacoronavirus family, SARS-CoV-2 is genetically similar to SARS-CoV and MERS-CoV, however it exhibits unique mechanisms to escape immune detection and enhance its pathogenicity(2). By June 2024, more than 770 million cases had been reported worldwide, with over 7 million deaths, placing immense pressure on health systems globally(3). SARS-CoV-2 is notable for its capacity to inhibit the host's interferon (IFN) response, a key component of antiviral immunity. The viral accessory proteins ORF7a and ORF7b interfere with IFN signaling by blocking the activity of interferon-stimulated genes and hindering the phosphorylation of STAT1 and STAT2, facilitating viral replication and disease progression(4).

IFN-β, which belongs to the group of cytokines known as type I interferons, has a crucial role in regulating viral proliferation by activating antiviral pathways and shaping immune responses(5). Although interferon responses are crucial for antiviral defense, SARS-CoV-2 utilizes diverse strategies to circumvent these pathways, contributing to the progression of severe disease(6, 7). Elevated expression of ORF7a and ORF7b has been linked to higher viral loads and dysfunctional immune responses observed in patients suffering from severe COVID-19(8). ORF7a is known to promote viral replication through suppression of the host immune responses, while ORF7b is believed to interfere with IFN signaling pathways, further suppressing antiviral defenses(4). Additionally, studies suggest that SARS-CoV-2 suppresses IFN production through various non-structural and accessory proteins, making the IFN pathway a critical target for therapeutic interventions(9).

Despite the established role of ORF7 proteins in immune modulation, the precise correlation between ORF7 subgenomic RNA (sgRNA) expression and IFN-β levels remains unclear. Given the essential role of IFN-β in antiviral defense, investigating this relationship could provide meaningful perspectives on the severity and prognosis of COVID-19 in affected individuals. This study aimed to quantify ORF7 sgRNA levels in upper respiratory tract samples from COVID-19 patients and analyze their relationship with IFN-β expression and disease prognosis. By addressing this gap, our results could contribute to a deeper comprehension of how SARS-CoV-2 escapes immune detection and highlight new avenues for therapeutic intervention.

Methods

Participants and Clinical Specimens

The present cross-sectional study was performed between August and February 2022. During this period, respiratory samples consisting of combined nasopharyngeal and oropharyngeal flocked swabs preserved in viral transport medium were collected from laboratory-confirmed COVID-19 patients immediately after hospital admission. The study was conducted on hospitalized patients at Ayatollah Rouhani and Shahid Yahyanejad Hospitals, Associated with Babol University of Medical Sciences, located in northern Iran. All samples were handled in a Class II biosafety cabinet following standard biosafety guidelines without dilution or heat inactivation. The specimens were aliquoted and kept at −70°C until analysis. Demographic, clinical, and para-clinical data were extracted from the electronic medical records of patients, who were assessed by specialists in infectious diseases. Clinical results observed in patients diagnosed with COVID-١٩ were used to classify them into two distinct groups. The poor prognosis group included patients with at least one of the following conditions: intubation and mechanical ventilation, intensive care unit (ICU) admission, or death due to COVID-19.

The good prognosis group consisted of patients who were discharged in stable condition without experiencing any of the aforementioned complications. Among the 89 patients fulfilling the inclusion criteria, 49 belonged to the poor prognosis group while, 40 were in the good prognosis group. The study was granted ethical approval by the Ethics Committee at Babol University of Medical Sciences (Approval Code: IR.MUBABOL.HRI.REC.1401.050), and all participants provided written informed consent prior to their inclusion. Funding for this study was provided by Babol University of Medical Sciences (grant number: 140014025). Variant identification was conducted using SARS-CoV-2 One Step RT-PCR Kit (Geneova, Iran) according to our recently published study(10).

[DOI: 10.22088/IJMCM.BUMS.14.2.726]

RNA Isolation

Extraction of total RNA (viral and cellular) was performed on 140µL of viral transport medium using the RNJia Virus Kit (ROJE Technologies, Yazd, Iran). Following the manufacturer's protocol, each sample received 560µL of BFC lysis buffer containing 1µg/µL of carrier RNA. The samples were then incubated for 10 minutes at room temperature to facilitate thorough lysis. RNA purification was performed using a silica-based mini spin column, according to the kit protocol.

RNA Quality and Quantity Assessment

The integrity and quantity of RNA were assessed prior to qPCR analysis using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and gel electrophoresis. RNA concentration was measured using the NanoDrop, while RNA integrity was evaluated through gel electrophoresis, confirming the absence of degradation. Only samples with high-quality RNA (RIN ≥ 7.0) were used for further analysis.

ORF7a and ORF7b sgRNA mRNA Quantification

The quantification of SARS-CoV-2 ORF7a and ORF7b sgRNAs was conducted using a one-step real-time reverse transcription PCR (rRT-PCR) protocol on the QIAquant 96 5plex platform (Qiagen, Hilden, Germany). Custom-designed primers and a TaqMan probe specific to the sgRNAs were synthesized by Metabion International AG (Martinsried, Germany)(11). Each 25µL reaction mixture included 5µL of 4× CAPITAL™ 1-Step qRT-PCR Probe Master Mix (Biotech Rabbit, Germany), 1µL of 20× RTase supplemented with RNase inhibitor, 0.3 µM of each primer, and 0.2 µM of a dual-labeled fluorescent probe. The thermal cycling protocol started with a reverse transcription stage of 50°C for 10 minutes, followed by an initial denaturation at 95°C for 3 minutes. Amplification then proceeded through 40 cycles, each including a 15-second denaturation at 95°C and a combined annealing/extension step at 60°C for a duration of 30 seconds. Expression levels were normalized against β2-microglobulin using the Relative Expression Software Tool (REST)(12).

Real-Time PCR for IFN-β Expression

Quantification of IFN-β expression was carried out using the QIAquant 96 5plex real-time PCR instrument (Qiagen, Hilden, Germany) with gene-specific primers for IFN-β (13) and β2-microglobulin (14) as the internal control, the one-step rRT-PCR was conducted in a final reaction volume of 25µL. The reaction setup included 12.5µL of 2× SYBR® RT-PCR buffer, 0.5µL of TaKaRa Ex Taq HS (5 U/µL), 0.5µL of reverse transcriptase (Takara, Shiga, Japan), and 10µM of each primer per reaction. The initial step involved synthesizing complementary DNA through reverse transcription at 42°C for 5 minutes, then the reaction proceeded with a 10-second denaturation at 95°C. The PCR amplification involved 40 cycles, each cycle featuring a 5-second denaturation at 95°C and a 30-second annealing/extension at 60°C. The specificity of the amplification was validated through melting curve analysis and agarose gel electrophoresis, confirming the absence of nonspecific products. Gene expression data for IFN-β were normalized to β2-microglobulin using the REST(12).

Statistical Evaluation

Data analysis was conducted using IBM SPSS Statistics, version 23. Associations between categorical variables were assessed employing the chi-square test, and continuous variables were analyzed using the independent t-test or Mann-Whitney U test. Gene expression was normalized using the REST version 2.0, following Pfaffl method(12) with β2-microglobulin as the internal control. Logistic regression was used to assess the association between ORF7 expression and clinical outcomes (poor vs. good prognosis) using a stepwise approach. A p-value of <0.05 was considered statistically significant.

Results

This cross-sectional study included 89 samples obtained from patients diagnosed with COVID-19. Patients were categorized into two groups based on prognosis: good prognosis (n=40) and poor prognosis (n=49). Participants' ages spanned from 23 to 90 years, with a mean age of 59.65 ± 17.54 years (40 males, 49 females). All demographic and underlying diseases of the patients is provided in Table 1. Notably, the majority of patients with a good prognosis were women, whereas the majority of those with a poor prognosis were men. Hypertension was the most common comorbidity, affecting 36 patients. Overall, individuals with a poor prognosis had a higher prevalence of underlying diseases compared to those

[DOI: 10.22088/IJMCM.BUMS.14.2.726]

with a good prognosis. Regarding the SARS-CoV-2 variants, the included patients were infected with 4 different variants of concern (Table 2). There was a strong correlation between the patients' prognosis and their admission to the intensive care unit. Specifically, nearly 96% of individuals classified under the poor prognosis category needed ICU care, while patients with a favorable prognosis did not require ICU admission ($P < 0.001$). Likewise, survival rates were significantly higher in the good prognosis group compared to the poor prognosis group, where 40.8% of patients did not survive ($P < 0.001$).

Laboratory parameters showed significant differences between the two groups. Statistically significant differences were observed in pro B-type natriuretic peptide (NT-proBNP) ($P = 0.001$), erythrocyte sedimentation rate (ESR) ($P = 0.001$), CRP ($P = 0.004$), creatinine ($P = 0.007$), blood urea nitrogen (BUN) ($P = 0.006$), and platelet count ($P = 0.032$) (Table 3). However, the use of Remdesivir and corticosteroids (Cortone) did notshow a statistically significant association with disease prognosis (Remdesivir: $P = 0.797$; Cortone: $P = 0.196$). The expression levels of viral ORF7a and ORF7b sgRNAs and the cellular IFN-β gene were analyzed in both groups using quantitative PCR (qPCR). Relative gene expression levels were normalized to the β2-microglobulin housekeeping gene. As shown in Figure 1, the median fold change expression of ORF7a was significantly higher in the poor prognosis group (9.68; interquartile range: 1.21–387.19) compared to the good prognosis group (1.21; interquartile range: 0.09–4.23) ($P < 0.001$) (Figure 1A). Similarly, ORF7b expression was markedly elevated in the poor prognosis group (71.01; interquartile range: 0.83–1704.29) relative to the good prognosis group (0.55; interquartile range: 0.13–4.43) ($P < 0.001$) (Figure 1B). Additionally, a significant reduction in IFN-β expression was observed in the poor prognosis group (0.46; interquartile range: 0.23–0.92) compared to the good prognosis group (0.92; interquartile range: 0.45–1.83) ($P < 0.001$) (Figure 1C).

Table 1. Demographic and underlying diseases of COVID-19 hospitalized patients with different prognosis.

Variable	Good Prognosis (n=40)	Poor Prognosis (n=49)	P-value
Gender			
Male	15	25	0.202
Female	25	24	
Age (years)			
Mean ± SD	54.62 ± 16.79	63.75 ± 17.21	0.014
Underlying Disease			
Diabetes	11	19	0.263
Hypertension	13	23	0.167
Ischemic heart disease	6	9	0.673
Cerebrovascular accident (CVA)	4	2	0.268
Asthma	3	2	0.486
Hypothyroidism	4	3	0.499
Hyperlipidemia	1	7	0.053
Chronic kidney disease	1	2	0.603

Table 2. Frequency of SARS-CoV-2 Variants in COVID-19 hospitalized patients with different prognosis.

Variant	Good Prognosis (n=40)	Poor Prognosis (n=49)	Total (n=89)
Wuhan	9	24	33
Alpha	19	7	26
Delta	9	11	20
Omicron	3	7	10

[DOI: 10.22088/IJMCM.BUMS.14.2.726]

Table 3. Laboratory Parameters in COVID-19 hospitalized patients with different prognosis.

Laboratory Variable	Good Prognosis (n=40)	Poor Prognosis (n=49)	P-value
NT-proBNP(pg/mL)	16	4	0.001
ESR (mm/h)	18	7	0.001
CRP (mg/L)	34	28	0.004
Creatinine (mg/dL)	34	30	0.007
BUN (mg/dL)	30	18	0.006
Platelet Count	36	20	0.032

*Percentages represent the proportion of patients with abnormal laboratory values in each group

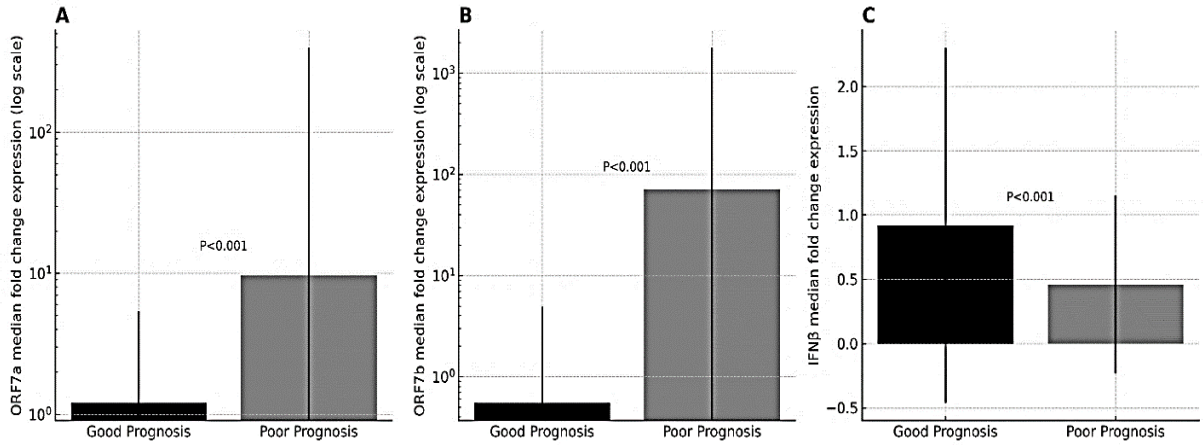

Figure 1. Comparison of SARS-CoV-2 ORF7a (A), ORF7b (B) subgenomic RNAs and host IFN-β (C) median fold change expression levels between good prognosis and poor prognosis groups. Bars represent median values, and error bars indicate interquartile ranges (IQR). Logarithmic scale is used for panels A and B. Statistically significant differences (P < 0.001) were observed between the two groups for all three targets.

Analysis using Pearson's correlation showed that IFN-β expression did not significantly correlate with ORF7a or ORF7b sgRNAs levels in both prognosis groups (r = 0.216, P = 0.181; r = 0.167, P = 0.303, respectively). Tables 4 to 6 provide a detailed analysis of the association between ORF7a, ORF7b, and IFN-β expression levels with disease prognosis. Among the patients older than 50 years, those classified with poor prognosis exhibited a notably higher median expression level of ORF7a compared to the group with favorable prognosis, as detailed in Table 4 (P = 0.004). Gender analysis revealed that this difference was statistically significant only among females (P = 0.006). Additionally, considering factors such as comorbidities, CRP, ESR, PCT, IL6, NT-proBNP, and D-dimer, the results indicate that the median fold change in ORF7a expression increased in the poor prognosis group relative to the good prognosis group. Considering demographic and clinical factors, ORF7b expression was found to be significantly greater in the poor prognosis group than in the good prognosis group (Table 5, P < 0.05). As demonstrated in Table 6, IFN-β expression was consistently lower in the poor prognosis group across all studied variables, with notable differences reaching statistical significance (P < 0.05) in individuals above 50 years of age, both genders, individuals with comorbidities, and those with abnormal ESR, IL-6, NT-proBNP, and D-dimer levels.

[DOI: 10.22088/IJMCM.BUMS.14.2.726]

Table 4. Association between median expression levels of ORF7a and clinical variables in patients with different prognosis.

Variable	Good Prognosis (Median [Q1–Q3])	Poor Prognosis (Median [Q1–Q3])	P-value
Age			
≤ 50 years	1.21 (0.15–10.88)	82.27 (0.75–619.51)	0.071
> 50 years	1.21 (0.07–4.84)	9.68 (1.21–154.87)	0.004
Sex			
Male	2.42 (0.15–19.35)	9.68 (0.91–387.19)	0.100
Female	1.21 (0.07–2.42)	77.44 (1.21–503.36)	0.006
Underlying Disease			
Present	1.21 (0.07–2.42)	9.68 (0.61–154.87)	0.005
Absent	2.42 (0.15-14.52)	82.27 (1.51-1084.15)	0.027
CRP			
Normal	1.81 (0.15–9.68)	7.26 (0.75–503.36)	0.030
Abnormal	0.34 (0.07–4.22)	77.43 (0.61–1316.47)	0.012
ESR			
Normal	2.42 (0.61–9.68)	9.68 (0.91–1239.04)	0.144
Abnormal	1.21 (0.07–4.22)	19.36 (0.76–387.19)	0.007
PCT			
Normal	2.42 (0.61–9.68)	9.68 (0.75–619.52)	0.019
Abnormal	1.21 (0.07–4.22)	77.44 (0.61–154.87)	0.320
IL6			
Normal	0.91 (0.15–3.67)	7.26 (0.75–503.36)	0.017
Abnormal	2.42 (0.07–9.68)	77.44 (1.21–619.51)	0.013
NT-proBNP			
Normal	2.42 (0.15–4.22)	0.91 (0.07–7.56)	0.385
Abnormal	1.81 (0.21–7.86)	77.43 (1.21–619.52)	0.005
D-dimer			
Normal	9.68 (0.91–619.52)	77.44 (0.61–619.52)	0.308
Abnormal	1.21 (0.07–4.22)	9.68 (0.61–154.87)	0.016

Table 5. Median expression levels of ORF7b and clinical variables in patients with different prognosis.

Variable	Good Prognosis (Median [Q1–Q3])	Poor Prognosis (Median [Q1–Q3])	P-value
Age			
≤ 50 years	1.11 (0.21–9.98)	142.02 (4.64–1988.35)	0.034
> 50 years	0.55 (0.14–4.43)	71.01 (0.83–1704.29)	<0.001
Sex			
Male	0.55 (0.14–8.87)	35.51 (1.38–825.15)	0.007
Female	1.11 (0.10–4.44)	142.02 (0.69–2272.39)	0.001
Underlying Disease			
Present	1.11 (0.14–9.98)	142.02 (1.11–1988.35)	0.034

[DOI: 10.22088/IJMCM.BUMS.14.2.726]

Variable	Good Prognosis (Median [Q1–Q3])	Poor Prognosis (Median [Q1–Q3])	P-value
Absent	0.55 (0.07–4.43)	71.01 (0.55–1704.29)	<0.001
CRP			
Normal	0.92 (0.23–4.43)	53.26 (1.11–994.17)	0.001
Abnormal	0.55 (0.15–3.88)	142.02 (0.14–3408.59)	0.003
ESR			
Normal	0.92 (0.21–4.43)	71.01 (0.14–1704.29)	0.002
Abnormal	1.66 (0.17–7.76)	35.51 (0.55–1704.29)	0.002
PCT			
Normal	0.92 (0.14–8.87)	35.51 (0.14–568.09)	0.004
Abnormal	1.66 (0.15–4.43)	71.01 (0.55–568.09)	0.115
IL6			
Normal	0.92 (0.21–9.88)	35.51 (0.55–7953.39)	0.032
Abnormal	2.21 (0.14–8.87)	71.01 (0.55–2272.39)	0.002
NT-proBNP			
Normal	0.92 (0.24–3.88)	35.51 (0.21–2272.39)	0.962
Abnormal	1.66 (0.08–7.76)	35.51 (0.55–2272.39)	0.001
D-dimer			
Normal	0.92 (0.17–5.32)	35.51 (0.72–568.09)	0.15
Abnormal	1.66 (0.14–4.43)	35.51 (0.14–2272.39)	0.016

Table 6. Median fold change expression levels of IFN-β and clinical variables in patients with different prognosis.

Variable	Good Prognosis (Median [Q1–Q3])	Poor Prognosis (Median [Q1–Q3])	P-value
Age			
≤ 50 years	0.92 (0.34–1.83)	0.92 (0.16–0.92)	0.269
> 50 years	0.92 (0.45–3.66)	0.46 (0.23–0.92)	0.001
Sex			
Male	0.92 (0.46–3.67)	0.46 (0.17–0.92)	0.026
Female	0.92 (0.69–1.83)	0.46 (0.23–0.92)	0.033
Underlying Disease			
Present	0.92 (0.46–1.83)	0.46 (0.11–0.92)	0.001
Absent	0.92 (0.34–2.75)	0.92 (0.46–0.92)	0.264
CRP			
Normal	0.92 (0.46–2.29)	0.46 (0.11–0.92)	0.001
Abnormal	0.92 (0.46–3.21)	0.92 (0.34–0.92)	0.263
ESR			
Normal	0.37 (0.80–3.67)	0.46 (0.57–1.83)	0.091
Abnormal	0.92 (0.28–1.83)	0.46 (0.23–0.92)	0.033
PCT			
Normal	0.92 (0.46–3.67)	0.46 (0.11–0.92)	0.041
Abnormal	0.92 (0.52–3.83)	0.46 (0.23–1.83)	0.743
IL6			
Normal	1.83 (0.28–3.67)	0.46 (0.28–1.49)	0.339
Abnormal	0.92 (0.46–1.83)	0.46 (0.23–0.92)	0.03
NT-proBNP			
Normal	0.68 (0.28–3.83)	0.14 (0.05–0.74)	0.086
Abnormal	0.83 (0.92–3.67)	0.46 (0.23–0.92)	0.003
D-dimer			
Normal	0.92 (0.28–3.83)	0.23 (0.23–0.46)	0.086
Abnormal	1.83 (0.46–3.67)	0.46 (0.23–0.92)	0.013

[DOI: 10.22088/IJMCM.BUMS.14.2.726]

Discussion

This study examined the expression levels of ORF7a and ORF7b sgRNAs in upper respiratory tract samples from hospitalized COVID-19 patients and assessed their relationship with IFN-β expression and clinical outcomes. The results indicated that patients with poor prognosis exhibited higher expression levels of ORF7a and ORF7b compared to those with a better prognosis. Furthermore, IFN-β expression was reduced in patients with poor prognosis, suggesting a potential role of this factor in disease severity. Previous studies have demonstrated that ORF7a and ORF7b play a crucial role in suppressing antiviral immune responses. For instance, García-García et al. reported that these two proteins impair immune processes and inhibit key antiviral pathways(8). Additionally, Shemesh et al. showed that ORF7b suppresses IFN-β production, facilitating immune evasion(9). These findings align with our results, suggesting that increased ORF7a and ORF7b expression may be associated with reduced IFN-β response and, consequently, increased disease severity. However, despite this apparent inverse trend, we did not observe a statistically significant correlation between IFN-β expression and ORF7a/b sgRNA levels.

This unexpected finding may be due to inter-patient variability in immune response timing, as well as the influence of confounding factors such as corticosteroid or antiviral treatments that were not accounted for in this study. Additionally, ORF7a and ORF7b might exert their immunosuppressive effects through mechanisms that do not directly modulate IFN-β transcription. Further studies with larger cohorts and controlled clinical variables are warranted to elucidate this relationship. Moreover, recent comprehensive reviews have emphasized that SARS-CoV-2 accessory proteins, including ORF7a and ORF7b, contribute significantly to immune evasion by interfering with key antiviral pathways, such as the interferon signaling cascade. According to a 2022 review article by Zandi et al., these proteins suppress interferon production and signaling, disrupt JAK-STAT pathways, and interfere with other immune regulators, facilitating viral persistence and pathogenesis(١٥).

Recent evidence also has indicated that ORF7a interacts with the NF-κB pathway, promoting inflammation while simultaneously inhibiting type I IFN responses(16). This dual mechanism may explain the exaggerated inflammatory response observed in critically ill COVID-19 patients. Furthermore, structural analysis of ORF7a by Kim et al. revealed that this protein binds to BST2/Tetherin, preventing virus recognition by the immune system and facilitating viral dissemination(17). Unlike earlier studies that primarily focused on ORF7a's role in immune suppression, our research highlights that ORF7b is also significantly upregulated in patients with poor prognosis, especially in individuals infected by the Delta and Omicron variants.

This suggests that these newer variants may have developed enhanced mechanisms of immune evasion, potentially through upregulation of ORF7b, which could play a key role in modulating immune responses and influencing disease severity. These findings highlight the importance of considering variant-specific differences in viral immune modulation and provide a basis for future research into how viral evolution impacts immune escape mechanisms.This finding indicates that more recent variants may have evolved mechanisms that enhance the immunosuppressive effects of ORF7 proteins(18).

In addition to investigating ORF7a and ORF7b expression, this study examined the influence of host factors such as age and gender on disease severity. Consistent with previous reports, advanced age was significantly associated with worse outcomes, likely due to weakened immune responses and increased comorbidities in older individuals(19). Although no significant difference was observed between genders in this study, some reports suggest that hormonal and genetic factors might influence immune responses differently in males and females, warranting further investigation(20).

Another key aspect analyzed in this study was the relationship between laboratory markers and disease prognosis. Laboratory markers such as CRP, NT-proBNP, and D-dimer exhibited significant differences between the two groups. Elevated CRP levels in the poor prognosis group support the role of systemic inflammation in worsening disease outcomes(21). Similarly, increased levels of D-dimer and NT-proBNPlikely indicate greater involvement of the coagulation and cardiovascular systems in these patients. Our results align with earlier research that identified these biomarkers as critical indicators of COVID-19 severity(22). Similarly, Cr and PLT levels showed significant variation across prognosis groups. Elevated Cr levels are indicative of renal dysfunction,

[DOI: 10.22088/IJMCM.BUMS.14.2.726]

while abnormal PLT counts suggest hematologic involvement, both of which are associated with worse clinical outcomes. On the other hand, markers such as IL-6, NT-proBNP, D-dimer, ESR, AST, and Hb were mostly abnormal in both prognosis groups,with NT-proBNPand ESR showing noteworthy differences.Elevated IL-6 levels, characteristic of cytokine release syndrome, have been associated with severe COVID-19 and poorer prognosis. High NT-proBNP levels suggest cardiac involvement, while elevated D-dimer levels are indicative of coagulopathy, both of which are markers of severe disease. These findings underscore the complex nature of COVID-19 pathology, which involves multiple organ systems and inflammatory processes. Given the potential role of ORF7a and ORF7b in IFN suppression, targeting these proteins could serve as a promising therapeutic approach to enhance the immune response against SARS-CoV-2.

Developing inhibitors that block the interaction of ORF7a and ORF7b with key immune regulators such as STAT2 may help mitigate disease severity and improve clinical outcomes. Additionally, regular monitoring of laboratory markers like CRP and D-dimer could aid in identifying high-risk patients and optimizing treatment strategies. There are several limitations in this study, including a small sample size that can limit the generalization of the results. Although we determined the SARS-CoV-2 variant types in our study population, we did not perform a comparative analysis of ORF7a, ORF7b, or IFN-β expression across different variant groups. This was due to the limited number of samples within each variant subgroup, which precluded meaningful statistical evaluation and increased the risk of inaccurate conclusions. Future studies with larger, well-stratified cohorts are needed to investigate whether specific SARS-CoV-2 variants differentially modulate the expression of these immune-related genes. Furthermore, the effects of therapeutic interventions such as corticosteroids and antiviral drugs on IFN-β expression were not assessed, which could influence the observed trends. Additionally, other SARS-CoV-2 ORFs involved in immune evasion were not investigated, and their role remains to be explored in future research.

To validate our findings and expand the scope of this study, future research should be conducted on larger cohorts, considering the effects of antiviral and anti-inflammatory treatments on IFN-β and ORF7a/b sgRNAs expression.In conclusion, this study highlights the crucial role of SARS-CoV-2 accessory proteins ORF7a and ORF7b in inhibiting type I IFN signaling and their implications for disease severity. The findings suggest that targeting these viral factors could enhance antiviral defenses and improve clinical outcomes. Additionally, the identification of key laboratory markers associated with disease prognosis provides valuable insights for clinical management. Further research into the intricate interplay between viral immune evasion mechanisms and host immune responses will be essential for developing effective therapeutic strategies against COVID-19.

Acknowledgements

The authors would like to thank the Clinical Research Development Unit of Rouhani Hospital, Babol University of Medical Sciences, provided support for this study.

References

1. Singhal T. A review of coronavirus disease-2019 (COVID-19). IJP. 2020;87(4):281-6.
2. Sevajol M, Subissi L, Decroly E, et al. Insights into RNA synthesis, capping, and proofreading mechanisms of SARS-coronavirus. Virus Res. 2014;194:90-9.
3. Zhu Z, Lian X, Su X, et al. From SARS and MERS to COVID-19: a brief summary and comparison of severe acute respiratory infections caused by three highly pathogenic human coronaviruses. Respir Res. 2020;21:1-14.
4. Rashid F, Xie Z, Suleman M, et al. Roles and functions of SARS-CoV-2 proteins in host immune evasion. Front immunol. 2022;13:940756.
5. Viox EG, Bosinger SE, Douek DC, et al. Harnessing the power of IFN for therapeutic approaches to COVID-19. J Virol. 2024;98(5):e01204-23.
6. Thierry AR, Roch B. SARS-CoV2 may evade innate immune response, causing uncontrolled neutrophil extracellular traps formation and multi-organ failure. Clin Sci. 2020;134(12):1295-300.
7. Zheng Y, Zhuang M-W, Han L, et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) membrane (M) protein inhibits type I and III interferon production by targeting RIG-I/MDA-5 signaling. Signal Transduct. 2020;5(1):299.

[DOI: 10.22088/IJMCM.BUMS.14.2.726]

8. García-García T, Fernández-Rodríguez R, Redondo N, et al. Impairment of antiviral immune response and disruption of cellular functions by SARS-CoV-2 ORF7a and ORF7b. IScience. 2022;25(11).
9. Shemesh M, Aktepe TE, Deerain JM, et al. SARS-CoV-2 suppresses IFNβ production mediated by NSP1, 5, 6, 15, ORF6 and ORF7b but does not suppress the effects of added interferon. PLoS Pathog. 2021;17(8):e1009800.
10. Sadeghi F, Halaji M, Shirafkan H, et al. Characteristics, outcome, duration of hospitalization, and cycle threshold of patients with COVID-19 referred to four hospitals in Babol City: a multicenter retrospective observational study on the fourth, fifth, and sixth waves. BMC Infectious Diseases. 2024;24(1):55.
11. Chen Z, Ng RWY, Lui G, et al. Profiling of SARS-CoV-2 subgenomic RNAs in clinical specimens. Microbiol Spectr. 2022;10(2):e00182-22.
12. Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res. 2002;30(9):e36-e.
13. Cheng J, Liao Y, Zhou L, et al. Amplified RLR signaling activation through an interferon-stimulated gene-endoplasmic reticulum stress-mitochondrial calcium uniporter protein loop. Sci Rep. 2016;6(1):20158.
14. Dawson HJ, Hibbert AP, Chantler PD, Botham KM. Myosin VI and Associated Proteins Are Expressed in Human Macrophages but Do Not Play a Role in Foam Cell Formation in THP- 1 Cells. Int J Vasc Med. 2013;2013(1):516015.
15. Zandi M, Shafaati M, Kalantar-Neyestanaki D, et al. The role of SARS-CoV-2 accessory proteins in immune evasion. Biomed Pharmacother. 2022;156:113889.
16. Su C-M, Wang L, Yoo D. Activation of NF-κB and induction of proinflammatory cytokine expressions mediated by ORF7a protein of SARS-CoV-2. Sci Rep. 2021;11(1):13464.
17. Petrosino M, Stellato F, Chiaraluce R, et al. Zn-Induced Interactions Between SARS- CoV- 2 orf7a and BST2/Tetherin. ChemistryOpen. 2021;10(11):1133-41.
18. Arshad N, Laurent-Rolle M, Ahmed WS, et al. SARS-CoV-2 accessory proteins ORF7a and ORF3a use distinct mechanisms to down-regulate MHC-I surface expression. PNAS. 2023;120(1):e2208525120.
19. Singhal S, Kumar P, Singh S, et al. Clinical features and outcomes of COVID-19 in older adults: a systematic review and meta-analysis. BMC Geriatr. 2021;21(1):321.
20. Roved J, Westerdahl H, Hasselquist D. Sex differences in immune responses: Hormonal effects, antagonistic selection, and evolutionary consequences. Horm Behav. 2017;88:95-105.
21. Smilowitz NR, Kunichoff D, Garshick M, et al. C-reactive protein and clinical outcomes in patients with COVID-19. Eur Heart J. 2021;42(23):2270-9.
22. Chi L, Wang S, Wang X, et al. Predictive value of C-reactive protein for disease severity and survival in COVID-19 patients: a systematic review and meta-analysis. CLIN EXP MED. 2023;23(6):2001-8.

[DOI: 10.22088/IJMCM.BUMS.14.2.726]