Comparing the expression levels of tripartite motif containing 28 in mild and severe COVID-19 infection

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Abstract

Background Tripartite motif-containing 28 (TRIM28) is an impressive regulator of the epigenetic control of the antiviral immune response. This study evaluated if the differential expression of TRIM28 correlates with the severity of coronavirus disease 2019 (COVID-19) infection.

Methods A total of 330 COVID-19 patients, including 188 mild and 142 severe infections, and 160 healthy controls were enrolled in this study. Quantitative real-time polymerase chain reaction (qPCR) was used to determine the expression levels of TRIM28 in the studied patients.

Results TRIM28 mRNA levels were significantly lower in both groups of patients versus the control group and in the severe group indicated further reduction in comparison to mild infection. The multivariate logistic regression analysis showed the mean age, lower levels of low-density lipoprotein (LDL), high-density lipoprotein (HDL), cholesterol, lower 25-hydroxyvitamin D, and PCR cycle threshold (Ct) value and higher levels of erythrocyte sedimentation rate (ESR) and differential expression of TRIM28 were linked to the severity of COVID-19 infection.

Conclusion The results of this study proved that the downregulation of TRIM28 might be associated with the severity of COVID-19 infection. Further studies are required to determine the association between the COVID-19 infection severity and TRIM family proteins.

Keywords Tripartite motif containing 28, Coronavirus disease 2019, Mild infection, Severe infection
Background

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is accompanied by different disease patterns from asymptomatic to mild to severe symptoms that can lead to mortality [1]. In addition to the advanced age or special health conditions, such as chronic lung disease, diabetes, or cardiovascular disease in patients, the host response to SARS-CoV-2 infection can inflict these different clinical manifestations [2]. The host response mechanisms in this disease are poorly understood, and there is a vital requirement to understand how the host’s immune system responds to this mysterious virus [3].

The innate immune response to the virus is triggered by recognizing viral RNA and glycoproteins by pathogen recognition receptors (PRRs) and toll-like receptors (TLRs) that activate signal transduction cascade to subsequently express interferons (IFNs) [4, 5]. This results in the nuclear translocation of the signal transducers and activators of transcription (STATs) and upregulation expression of numerous IFN-stimulated genes (ISGs), such as proinflammatory cytokines [6]. However, the principal mechanisms of this overexpression of IFNs and cytokines during SARS-CoV-2 infection are still not entirely understood [7].

Previous studies have shed light on tripartite motif-containing 28 (TRIM28) as impressive regulators of the epigenetic control of the antiviral immune response [8–10]. The TRIM28 is a negative immune regulator moderating IFNs and cytokine expression during infection with highly pathogenic avian influenza virus (HPAIV). It was shown that unrestrained inflammatory responses are more likely the reason for severe infection with HPAIV, which can lead to mortality [10].

The TRIM28 belongs to the subfamily VI of TRIM family proteins with ubiquitin E3 ligase activity in their N-terminal, and three unique functional domains in their C-terminal that put TRIM28 as a transcriptional regulator in this subfamily with TRIM24, TRIM33 and TRIM66. Heterochromatin protein 1 binding domain (HP1 BD) in C-terminal of TRIM28 can change the epigenetic state as a transcriptional repressor and affect the silencing of genes and retroelements. This repressing function of TRIM28 plays an important role on silencing viral transcription and replication.[11]. The TRIM proteins have a pivotal role in antiviral defense directly by restricting the replication of the virus and/or indirectly through modulating antiviral cytokine responses [12]. They are conjugated to the lysine residues of a target protein and modify them by phosphorylation, ubiquitination, and proteasome-driven degradation. Although most TRIM proteins activate as an immune enhancer, TRIM28 was reported as an immune suppressor which downregulates the activity of multiple immune-related transcription factors, including interferon regulatory factor 7 (IRF7), IRF5 and IRF1 [13]. It was demonstrated that TRIM28 can restrict the replication of human immunodeficiency virus type 1 (HIV-1) by inhibiting its integrase protein and repressing the HIV-1 L promoter. Additionally, TRIM28 can induce latency of herpesvirus, Epstein-Barr virus, and endogenous retrovirus. Recently, it has been shown that TRIM28 has co-expression with SARS-CoV-2 receptors and can impress cell entry by affecting interferon-γ (IFN-γ)-induced angiotensin-converting enzyme 2 (ACE2) gene expression [14, 15].

The TRIM28, also called KRAB-associated protein-1 (KAPI) or transcriptional intermediary factor 1β (TIF1-β), is a nuclear corepressor of Kruppel-associated box zinc-finger proteins (KRAB-ZFPs) that impresses STAT1 for ubiquitination as a posttranslational modification of viral and host protein [16].

The effect of the TRIM28 gene in COVID-19 as a new disease seems to be interesting; therefore, this study aimed to evaluate the expression of the TRIM28 gene in the blood samples of patients with mild and severe COVID-19 infections.

Materials and methods

Study population

The study population included 330 blood samples from patients with COVID-19 infection, including mild infection (the patients indicating light clinical symptoms accompanied by fever and no evidence of pneumonia or mild respiratory symptoms on computed tomography examination) and severe infection (the patients with shortness of breath, oxygen saturation less than 92%, shock, acute respiratory failure requiring mechanical ventilation machine, and need to be admitted to an intensive care unit). Furthermore, 160 blood samples of healthy individuals as a control group were collected at Pasteur Institute of Iran.

The patients’ samples were collected within March 2020 to September 2020, and the samples of healthy individuals belonged to before the COVID-19 pandemic. The patients did not have any underlying medical conditions, including heart diseases, diabetes, cancer, chronic respiratory diseases, and allergic diseases.

The laboratory parameters, including real-time PCR Ct values, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), serum creatinine, uric acid, triiodothyronine (T3), thyroxine (T4), thyroid-stimulating hormone (TSH), platelets, 25-hydroxyvitamin D, C-reactive protein (CRP), white blood cells (WBC), hemoglobin, erythrocyte sedimentation rate (ESR), fasting blood glucose (FBS), low-density lipoprotein (LDL), cholesterol, triglyceride (TG), and high density lipoprotein (HDL) were extracted from the patients’ records.
Table 1 Comparison laboratory parameters between mild and severe patients infected with COVID-19

| Variables                          | Mild patients (n = 188) | Severe patients (n = 142) | Control group (n = 160) | p-value |
|------------------------------------|------------------------|---------------------------|-------------------------|---------|
| Mean age ± SD                      | 53.7 ± 11.7            | 61.2 ± 12.8               | 54.2 ± 12.1             | 0.217   |
| Gender (male/female)               | 108/80 (57.4/42.6%)    | 66/76 (46.5/53.5%)        | 82/78 (51.2/48.8%)      | 0.187   |
| ALT, IU/L (mean ± SD) (Reference range: 5–40) | 29.7 ± 19.1            | 32.5 ± 20.3               | 29.5 ± 20.4             | 0.761   |
| AST, IU/L (mean ± SD) (Reference range: 5–40) | 31.1 ± 16.9            | 34.6 ± 19.7               | 28.6 ± 16.7             | 0.367   |
| ALP, IU/L (mean ± SD) (Reference range: up to 306) | 180.4 ± 98.9           | 172.8 ± 82.6              | 212.8 ± 99.6            | 0.901   |
| Cholesterol, mg/dL (mean ± SD) (Reference range: 50–200) | 171.9 ± 53.9           | 109.1 ± 29.4              | 167.9 ± 49.8            | <0.001* |
| TG, mg/dL (mean ± SD) (Reference range: 60–165) | 173.1 ± 62.4           | 125.9 ± 63.4              | 162.1 ± 58.4            | <0.001* |
| LDL, mg/dL (mean ± SD) (Reference range: up to 150) | 125.1 ± 49.1           | 56.4 ± 19.3               | 110.5 ± 28.7            | <0.001* |
| HDL, mg/dL (mean ± SD) (Reference range: >40) | 31.0 ± 12.1            | 33.1 ± 12.9               | 39.1 ± 13.1             | 0.287   |
| WBC, 10^3/L (mean ± SD) (Reference range: 4000–10,000) | 7443.6 ± 2524.5        | 7823.1 ± 2906.9           | 6842.1 ± 2102.1         | 0.882   |
| CRP, mg/L (mean ± SD) (Reference range: <10 mg/L Negative) | 58.8 ± 21.5            | 743 ± 14.9                | 93.2 ± 29               | <0.001* |
| ESR, mm/1st h (mean ± SD) (Reference range: 0–15) | 51.0 ± 17.4            | 61.5 ± 12.4               | 11.5 ± 4.7              | <0.001* |
| FBS, mg/dL (mean ± SD) (Reference range: 70–100) | 103.5 ± 40.0           | 102.5 ± 30.1              | 99.5 ± 28.2             | 0.751   |
| Platelets × 1000/cumm (mean ± SD) (Reference range: 140,000–400,000) | 179 ± 67               | 188 ± 84                  | 185 ± 81                | 0.689   |
| T3, ng/dL (mean ± SD) (Reference range: 2.3–4.2) | 2.4 ± 1.3              | 1.7 ± 0.9                 | 2.1 ± 1.1               | 0.752   |
| T4, mcg/dL (mean ± SD) (Reference range: 5.6–13.7) | 8.6 ± 7.1              | 9.9 ± 7.2                 | 10.1 ± 7.9              | 0.284   |
| TSH, mu/L (mean ± SD) (Reference range: 0.4–4.5) | 3.1 ± 1.7              | 3.3 ± 1.8                 | 2.8 ± 1.1               | 0.456   |
| Hemoglobin, g/dL (mean ± SD) (Reference range: 12–18) | 13.1 ± 2.5             | 12.2 ± 1.7                | 14.2 ± 2.7              | 0.417   |
| BUN, mg/dL (mean ± SD) (Reference range: 15–45) | 38.1 ± 7.1             | 36.9 ± 6.9                | 32.1 ± 5.9              | 0.086   |
| Creatinine, mg/dL (mean ± SD) (Reference range: 0.6–1.4) | 1.1 ± 0.6              | 1.0 ± 0.4                 | 1.2 ± 0.7               | 0.258   |
| 25-hydroxy vitamin D, ng/mL (mean ± SD) (Sufficiency: 21–150) | 34.7 ± 13.8            | 19.1 ± 9.7                | 31.2 ± 11.7             | <0.001* |
| Real-time PCR Ct values            | 28.8 ± 8.9             | 12.8 ± 7.4                | -                      | 0.018*  |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TG, triglyceride; LDL, low density lipoprotein; HDL, high density lipoprotein; WBC, white blood cells; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; FBS, fasting blood glucose; T3, triiodothyronine; T4, thyroxine; TSH, Thyroid-stimulating hormone; BUN, Blood urea nitrogen; Ct, cycle threshold; SD, standard deviation. *Statistically significant (<0.05)

Evaluation of the Expression of TRIM28 mRNA by Real-Time PCR.

Total RNA was extracted using Trizol Reagent (QIAGEN, USA) according to the manufacturer’s instructions and quantified by a NanoDrop spectrophotometer. The complementary DNA (cDNA) was synthesized according to the manufacture of the kit (Yekta Tajhiz, Iran) by (1 µg) of total RNA treated by the DNase I enzyme (Thermofisher, USA). The cDNA was amplified using a real-time polymerase chain reaction (PCR) machine (Corbett Rotor-Gene 6000, QIAGEN, USA) with SYBR Green qPCR Master Mix (Ampliqon, Denmark) and hypoxanthine phosphoribosyltransferase 1 (HPRT1) gene as a reference. The primer sequences were designed as an exon junction or separate exon by Beacon designer software (Version 8 Primer, Biosoft, USA). The primer sequences for the TRIM28 gene were: 5’-GGACCAC-CAGTACAGTTC-3’ (forward), and 5’-CCATCCT-TGACATCCACTTG-3’ (reverse) and for HPRT1 gene was: 5’-TGCTGAGGATTGGAAGGGG-3’ (forward), and 5’-ACAGAGGGCTACAATGTGATG-3’ (reverse).

Statistical analysis

Processing real-time PCR data was performed by the ΔΔCt method. Statistical analysis was applied via the GraphPad Prism statistical software (version 9.3.1) and SPSS for Windows version (version 22.0.) (SPSS, Inc., Chicago, IL, USA). Nonparametric data are expressed as medians and interquartile ranges (IQR). The Shapiro-Wilk test was used to evaluate the normality of ordinal variables. Pearson’s Chi-square test and Mann-Whitney U test were also used to assess quantitative and continuous variables, respectively. A multivariate logistic regression analysis was carried out by the Hosmer-Lemeshow test to assess the association between COVID-19 resistance and several risk factors for susceptibility. The area under the receiver-operating characteristic curve (AUC-ROC) analysis was applied to evaluate the impact of TRIM28 gene expression on resistance and susceptibility to COVID-19. A p-value less than 0.05 was considered statistically significant.

Results

Baseline characteristics of COVID-19 patients

Table 1 shows the patients’ laboratory and clinical characteristics. The studied subjects were divided into three groups, including mild patients (n=188), severe patients (n=142), and healthy controls (n=160). The mean age values of mild patients, severe patients, and healthy controls were 53.7 ± 11.7, 61.2 ± 2.8, and 54.2 ± 12.1 years,
The severity of COVID-19 infection was associated with various factors. The present study evaluated the relationship between TRIM28 gene expression and the progression of COVID-19 infection. Our results showed a meaningful reduction of TRIM28 in patients with COVID-19 in both studied groups and proposed a possible relationship between TRIM28 expression and the rate of COVID-19 severity. Growing data indicate that TRIM28 showed a reduction in COVID-19 disease [17–19]. Moreover, the co-culture of lung epithelial cells and NK cells showed that TRIM28 can be inhibited by NK cell-derived factors and causes the induction of ACE2 expression in lung epithelial cells [19]. In addition, Yinfang Wang et al. reported that in vitro TRIM28 knockdown enhanced interferon-gamma-induced ACE2 expression via upregulating interferon-gamma receptor 2 (IFNGR2) in both A549 and primary alveolar epithelial cells. Therefore, the promotion of ACE2 expression due to NK cell function and decrease in TRIM28 raise the possibility of infection with SARS-CoV-2 in lung epithelial cells and intensify lung inflammation and infection [19]. It seems that the low expression of TRIM28 causes an increase in the ACE2 expression and SARS-CoV-2 entry in the cells. As a result, the decrease in the expression of TRIM28 in both studied groups compared to the control group may be due to the increase in the expression of ACE2 and the entry of the virus into the cell.

The TRIM28 gene significantly decreased in children with severe COVID-19 in comparison to children with mild infection in the blood samples [17]. The RNA-sequencing analysis of transcriptional response to SARS-CoV-2 in cells, animal models, and lung tissue clinical samples of COVID-19 patients also showed a reduction in TRIM28 gene [18].

Recently, Blanco-Melo D et al. characterized immune cells by analyzing a single-cell RNA sequencing dataset from bronchoalveolar fluid in patients with severe COVID-19 and revealed that different types of immune cells were identified in patients [18]. Among various identified cells, natural killer (NK) cells lead to a balanced immune response by removing infected cells quenching dendritic cell and T cell activity due to SARS-CoV-2 infection [20]. Krischuns and et al. reported that inactive TRIM28 due to phosphorylation of TRIM28 at serine

Discussion

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Factors related to COVID-19 infection severity

The multivariate logistic regression analysis evaluated the factors associated with COVID-19 infection severity. The severity of COVID-19 infection was associated with mean age (OR 0.911, 95% CI 0.875–0.998, P=0.021), LDL (OR 0.917, 95% CI 0.856–0.981, P=0.012), cholesterol (OR 0.919, 95% CI 0.845–0.998, P=0.046), HDL (OR 1.283, 95% CI 1.070–1.539, P=0.007), 25-hydroxyvitamin D (OR 1.180, 95% CI 1.106–1.912, P=0.018), ESR (OR 0.801, 95% CI 0.679–0.945, P=0.009), real-time PCR Ct values (OR 2.181, 95% CI 1.129–2.925, P=0.033), and TRIM28 gene expression (OR 0.6456, 95% CI 0.457–0.911, P=0.013) (Table 2).

Discussion

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473 in a signaling cascade leads to an intensification of persistent immune response by boosting IFN-β, IL-6 and IL-8 expression which may result to extreme immune cells activity and cytokine-mediated tissue damage during HPAIV infection [18]. On the other side, it showed TRIM28 co-expression with ACE2 and transmembrane serine protease 2 (TMPRSS2) in type II pneumocystis. Since binding the spike glycoprotein of Coronavirus to their cellular receptors and then fusion between viral and host cell membranes is the first step of viral infection, increasing the expression of these receptors can impress on the rate of viral infection [7]. Ubiquitination alterations in both viral and host proteins were observed in SARS-CoV-2-infected cells and the degradation of ACE2 receptor by E3 ligase ubiquitination was improved [7]. As regards TRIM28 is an E3 ligase, and had higher expression in patients with mild symptoms compared to severe cases, it may contribute to the downregulation of the major SARS-CoV-2 entry receptor and decreasing the severity of infection in mild group.

On the other hand, it was shown that TRIM28 could restrict HIV-1 replication by inhibiting the activation of HIV-1 integrase [21]. Moreover, TRIM28 was reported to inhibit Moloney murine leukemia virus (M-MLV) in pluripotent cells as an integral component of the silencing complex by binding to the primer binding site of M-MLV and repressing transcription from the viral promoter [22].

The current study demonstrated a significant reduction in patients with severe infection compared to patients with mild infection and healthy control group. More TRIM28 expression in mildly symptomatic individuals may suppress inflammatory genes in this group, while its more defective activation in severe group causes the

### Table 2 Factors associated with severe patients infected with COVID-19

| Factors                        | Baseline Predictors | OR (95% CI)       | P-value |
|--------------------------------|---------------------|-------------------|---------|
| Mean age ± SD                  | 0.911 (0.875–0.998) | 0.021*            |
| LDL (mg/dL)                    | 0.917 (0.856–0.981) | 0.012*            |
| Cholesterol, mg/dL             | 0.919 (0.845–0.998) | 0.046*            |
| HDL (mg/dL)                    | 1.283 (1.070–1.539) | 0.007*            |
| 25-hydroxyvitamin D, (ng/mL)   | 1.180 (1.106–1.912) | 0.018*            |
| ESR, (mm/1st h)                | 0.801 (0.679–0.945) | 0.009*            |
| Real-time PCR Ct values        | 2.181 (1.129–2.925) | 0.033*            |
| TRIM28 gene expression         | 0.645 (0.457–0.911) | 0.013*            |

LDL, low density lipoprotein; HDL, high density lipoprotein; ESR, erythrocyte sedimentation rate; Ct, cycle threshold; TRIM28, tripartite Motif Containing 28; SD, standard deviation; *Statistically significant (<0.05)
high inflammation in cells. It seems that TRIM28 more likely has an important role in the control of high inflammatory response and might inhibit the replication of SARS-CoV-2 by the restriction of ACE2 receptor in lung epithelial cells.

The results of this study indicated that the severity of COVID-19 infection was significantly correlated with the low levels of lipid profiles, including LDL, HDL, and cholesterol, and real-time PCR cycle threshold (Ct) value. A meta-analysis study demonstrated significantly decreased levels of LDL, HDL, and total cholesterol in the severe patients, compared to those of nonsevere patients in a random effect model (REM). Additionally, REM results indicated significantly lower levels of lipid profiles in the dead patients, compared to those of the survivors [23]. However, the main mechanism responsible for the decrease in lipid profile levels in severe COVID-19 patients is not clearly understood. There are several hypotheses in this regard. Firstly, the liver has a vital role in lipid metabolism, and SARS-CoV-2 might damage the liver, thereby impairing the absorption and biosynthesis of lipoproteins [24]. Secondly, an important feature of COVID-19 patients is hyperinflammation, especially in patients with severe infection or dead patients, which alters lipid metabolism. Interleukin (IL)-6, IL-1β, and tumor necrosis factor-alpha as proinflammatory cytokines have been indicated to modulate lipid metabolism by altering the liver's function and reducing cholesterol efflux and transport [14, 15]. Thirdly, the inflammatory response caused by the virus can also alter vascular permeability, leading to the leakage of cholesterol molecules into tissues, such as the alveolar spaces for secretion. Secretions contain high levels of protein and cholesterol [25].

There is now considerable evidence to suggest a significant correlation between the insufficiency and deficiency of 25-hydroxyvitamin D and COVID-19 severity [26]. 25-hydroxyvitamin D is correlated with the immunity of both B and T cells. In general, T cell responses play a key role in fighting viral infections. The evidence suggests that patients with severe COVID-19 are characterized by functional T cells exhaustion, and the improvement of vitamin D status can reduce this process via immunomodulation [27].

A low Ct of real-time PCR Ct values was observed in the present examined severe patients. Some reports have indicated that this parameter increases the hospitalization risk and mortality [28]. Furthermore, a study showed a significant correlation between real-time PCR Ct values and secondary transmission. It was suggested that by real-time PCR Ct values or viral load calculations can help modify decision-making, such as shorter isolation [29].

The limitations of this study were the relatively small sample size, lack of evaluation of TRIM28 expression in patients' tissues, and lack of evaluation of the amount of TRIM28 protein in the patient's serum.

**Conclusion**

The current study's findings revealed a potential correlation between the downregulation of TRIM28 gene expression with the severity of COVID-19 infection, the lower levels of 25-hydroxyvitamin D, lipid profiles, and real-time PCR Ct values. Further studies are recommended to confirm the aforementioned findings.

**Abbreviations**

TRIM28 Tripartite motif-containing 28.
COVID-19 coronavirus disease 2019.
PCR polymerase chain reaction.
LDL low-density lipoprotein.
HDL high-density lipoprotein.
Ct cycle threshold.
ESR erythrocyte sedimentation rate.
SARS-CoV-2 Severe acute respiratory syndrome coronavirus-2.
PRRs pathogen recognition receptors.
TLR Toll-like receptors.
IFNs interferons.
STATs signal transducers and activators of transcription.
ISGs IFN-stimulated genes.
HPAIV highly pathogenic avian influenza virus.
IRF7 interferon regulatory factor 7.
KAP1 KrAB-associated protein-1.
TIF1-β transcriptional intermediary factor 1β.
KRAZFPs Kruppel-associated box zinc-finger proteins.
ALT alanine aminotransferase.
AST aspartate aminotransferase.
ALP alkaline phosphatase.
BUN blood urea nitrogen.
T3 triiodothyronine.
T4 thyroxine.
TSH thyroid-stimulating hormone.
CRP C-reactive protein.
WBC white blood cells.
FBG fasting blood glucose.
TG triglyceride.
cDNA complementary DNA.
HPRT1 hypoxanthine phosphoribosyltransferase 1.
IQR interquartile range.
AUC-ROC area under the receiver-operating characteristic curve.
NK natural killer.
ACE2 angiotensin-converting enzyme 2.
TMIPRSS2 transmembrane serine protease 2.
HIV-1 human immunodeficiency virus type 1.
M-MLV Moloney leukemia virus.
REM random effect model.

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**Authors’ contributions**

RT and MH: Performed the experiments and manuscript preparation; IA and EA: Clinical sample and data acquisition; DD and SE: analyzed and interpreted data; PR, MRA, and AF: designed and supervised clinical study, interpreted data, read and approved manuscript. All authors reviewed the manuscript.

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Data availability
All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethical Review Committee of Pasteur Institute of Iran, (Identification No:IR.PH.REC.1400.029). Informed consent was obtained from all participants included in the study.

Consent for publication
Not applicable.

Competing interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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