Association of polymorphisms in tumor necrosis factors with SARS-CoV-2 infection and mortality rate: A case-control study and in silico analyses

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Abstract
The present coronavirus disease 2019 (COVID-19) is spreading rapidly and existing data has suggested a number of susceptibility factors for developing a severe course of the disease. The current case-control experiment is aimed to study the associations of genetic polymorphisms in tumor necrosis factors (TNFs) with COVID-19 and its mortality rate. A total of 550 participants (275 subjects and 275 controls) were enrolled. The tetra-amplification refractory mutation system polymerase chain reaction technique was recruited to detect −308G>A TNFα and +252A>G TNFβ polymorphisms among the Iranian subjects. We demonstrated that carriers of the G allele of TNFβ−252A/G, rs909253 A>G were more frequent in COVID-19 subjects compared to the healthy group and this allele statistically increased the disease risk (odds ratio [OR] = 1.55, 95% confidence interval [CI] = 1.23–1.96, p < 0.0001). At the same time, the A allele of TNFα−311A/G, rs1800629 G>A moderately decreased the risk of COVID-19 (OR = 0.68, 95% CI = 0.53–0.86, p < 0.002). Also, we analyzed the various genotypes regarding the para-clinical and disorder severity; we found that in the AA genotype of TNFβ−252A/G (rs909253 A>G), the computed tomography scan pattern was different in comparison to cases carrying the AG genotype with p1 < 0.001. In addition, in the severe cases of COVID-19, leukocyte and neutrophil count and duration of intensive care unit hospitalization in the deceased patients were significantly increased (p < 0.001). Moreover, the TNFα−311A/G (rs1800629 G>A) variant is likely to change the pattern of splicing factor sites. Our findings provided deep insights into the relationship between TNFα/TNFβ polymorphisms and severe acute respiratory syndrome coronavirus 2. Replicated studies may give scientific evidence for exploring molecular mechanisms of COVID-19 in other ethnicities.

Keywords
ARDS, COVID-19, CT pattern, polymorphism, SARS-CoV-2, tumor necrosis factor
1 | INTRODUCTION

In December 2019, the coronavirus disease 2019 (COVID-19) emerged in Wuhan, China, and caused acute respiratory distress syndrome (ARDS). This new virus, later called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), rapidly spread around China and other countries, substantially affected human health, global economics, and created a worldwide crisis. In the majority of the cases, SARS-CoV-2 infection is considered an acute self-resolving disease. Yet, until April 27, 2020, COVID-19 induced death in about 6.89% of infected individuals. Initial reports have indicated that COVID-19 has a mortality rate of approximately 2%, and this highly infectious disease might result in death due to extensive alveolar damage and lung failure. Similar to the Middle East Respiratory Syndrome and SARS-CoV, the SARS-CoV-2 belongs to the family of beta-coronaviruses mainly manifested as pneumonia in humans. In addition, COVID-19 variants of concern, including alpha (B.1.1.7), beta (B.1.351), gamma (P.1), delta (B.1.617.2), and lambda (C.37) are associated with higher transmissibility while spreading across Asia, Europe, and other continents. A single cohort study by Nikpouraghdam et al. suggested that older age, male gender, and having comorbid conditions were significantly correlated with death rates among Iranian COVID-19 patients. Soon after the pandemic COVID-19 outbreak, several studies were performed on almost every aspect of SARS-CoV-2 infection, particularly the pathogenesis of this novel beta-coronavirus. It has been shown that the virus uses angiotensin-converting enzyme type 2 (ACE2), transmembrane serine protease 2 (TMPRSS2), and the viral spike protein (S-protein) for entering host cells. These receptors are abundantly expressed in lung cells, making it easier for the virus to replicate throughout the respiratory tract. Besides genetics, psychological distress, smoking, poor sleep quality, and body mass index are among the risk factors associated with COVID-19 susceptibility and incubation time. Increasing evidence has shown that single-nucleotide polymorphisms (SNPs) play an essential role in determining the case-fatality rate of COVID-19 patients and the disease severity. In this respect, Paniri et al. showed that ACE2 SNPs impact the ability of the SARS-CoV-2 virus to enter cells via altering ACE2 function and structure. By performing a case-control study, Chong et al. reported that the interferon γ (IFNγ) +874 A/T, and the tumor necrosis factor α (TNFα)-308G/A polymorphism, is associated with the onset progress of SARS-CoV-2 infection but not the progress of SARS-CoV. When the cytokine release syndrome happened in COVID-19, it caused increasing levels of TNF-α, interleukin 1 (IL-1), IL-6, IL-8, IL-12, and IFN-γ; therefore, increasing some cytokines, for example, IL-6 and TNF-α cause poor prognosis in patients with COVID-19. More recently, Kirtipal and Bharadwaj reported that IL6 SNPs could be considered an indicator of COVID-19 severity in humans. This indicates that SNPs in genes encoding inflammatory cytokines and other innate immune genes might also impact the susceptibility to acute respiratory disorders, including COVID-19.

The host genetics plays a fundamental role in the immune response to the SARS-CoV-2 virus and influences the risk of COVID-19, severity, and outcome in affected patients. Herein, we aimed to study the relationship between TNFβ-252A/G, rs909253 A>G and TNFα-311A/G, rs1800629 G>A polymorphisms, susceptibility, lesions in computed tomography (CT) scan, and duration of hospitalization to COVID-19 in an Iranian population.

2 | MATERIALS AND METHODS

2.1 | Characteristics of patients

The current study involved 550 participants (275 subjects and 275 controls) who were admitted to Bu-Ali Hospital Lab in Zahedan between June 2020 and January 2021. The subject group involved 275 hospitalized patients in the infectious units or intensive care units (ICU) and laboratory-confirmed for the SARS-CoV-2 test. The healthy participants were selected among subjects with a high probability of exposure to the SARS-CoV-2 virus, which had a family history of COVID-19 and/or health care workers in high exposure with COVID-19 cases, but tested multiple times and showed a negative result for SARS-CoV-2 RNA based on quantitative reverse transcription-polymerase chain reaction (RT-qPCR) test in the routine lab of our hospital. The selection criteria for COVID-19 diagnosis were based on suggestive clinical features and confirmation via positive RT-qPCR results in the oro-/nasopharyngeal swab. On the basis of clinical features, we subcategorized COVID-19 cases as follows: mild/moderate (nonsevere) cases manifested with respiratory distress and oxygen saturation with less than 93%; and severe cases with SpO2 less than 90% and one of the following conditions: respiratory failure occurs and/or ICU admission is required for mechanical ventilation (severe/critical cases). Clinical and paraclinical characteristics of all participants and signs/symptoms of severe/nonsevere cases are indicated in Table 1.

2.2 | Real-time RT-PCR assay

Viral ribonucleic acid was extracted from the oro- and nasopharyngeal swab samples using the COVID-19 ORF1ab/N Gene Nucleic Acid Detection Kit. The sequences were as follows: forward primer 5'-TCAGAATGCCAATCTCCACG-3'; reverse primer 5'-AAAGGTC CACCCGATACATTGAC-3'; and the probe 5'-CY5-CTAGTTACATTAC 1503 GAGCCATCTTACTGC-3' BHQ1. Also, the reaction procedure was as follows: 50°C for 30 min, predenaturation at 95°C for 10 min, followed by five cycles of 94°C for 15 s, 50°C for 30 s and 72°C for 30 s, and 40 cycles of 94°C for 10 s and 58°C for 30 s for fluorescence detection. According to the cycle threshold (Ct) analysis, if the Ct values are less than 37, the test result sample is positive.

2.3 | Genomic DNA isolation and genotyping

According to protocol, genomic DNA was isolated using a simple salting-out procedure from 500μl of venous whole blood of each participant. The polymorphisms in TNFβ-252A/G, rs909253 A>G
and TNFα-311A/G, rs1800629 G>A were genotyped using the tetra amplification refractory mutation system PCR method. In summary, the DNA of each participant was amplified for SNPs using 1 µl of DNA (~60 ng/ml), 1 µl of each primer (6 pmol), 12 µl of Taq 2X Master Mix Red-MgCl₂ 1.5 mM (Ampliqon Inc.), and 5 µl of distilled water. Each reaction mixture was heated to 95°C for 5 min for initial denaturation and underwent 30 cycles at 95°C for 45 s, annealing at different temperatures (according to Supporting Information Table for each SNP) for 45 s with an extension at 72°C for 45 s, followed by a final extension at 72°C for 5 min. For each reaction, we used a common reverse primer, and one of the two allele-specific forward primers was shown in the Supporting Information Table. The products were analyzed on 1.5% agarose gel stained with safe stain dye and recorded using a gel doc system (Figure 1).

### Data collection

Fasting venous blood was collected from all patients and participants for laboratory measurements, and complete cell blood count, C-reactive protein, and chest CT scan were performed. Symptoms/signs and duration of hospitalization were also recorded.

| Parameter evaluated | Controls (N, %) (mean ± SD) | COVID-19 cases (N, %) (mean ± SD) | p nonsevere/severe |
|---------------------|-----------------------------|----------------------------------|--------------------|
| Age (year)          | 53.86 ± 15.45               | 54.93 ± 14.19                   | 0.268              |
| Gender (female/male)| 122/153                     | 112/163                          | 0.005*             |
| Leukocytes count (×10⁹ /L) | 8.09 ± 5.32               | 9.51 ± 4.91                      | <0.001*            |
| Plt count (×10⁹ /L) | 272.87 ± 73.23              | 245.56 ± 100.70                  | 0.504              |
| Lymph count (×10⁹ /L) | 2.85 ± 2.33                 | 1.02 ± 0.55                      | <0.001*            |
| Neut count (×10⁹ /L) | 4.50 ± 2.72                 | 7.87 ± 4.70                      | <0.001*            |
| CRP (mg/L)          | 4.29 ± 0.70                  | 15.27 ± 4.77                     | 0.996              |
| Temperature (°C)    | 37.3 ± 0.5                   | 37.33 ± 2.17                     | 0.796              |
| Hospitalization (Day) | 0                          | 7.69 ± 5.53                      | <0.001*            |
| Saturation (%)      | 98.1 ± 1.4                   | 85.08 ± 8.16                     | <0.001*            |
| Density pattern     |                             |                                  |                    |
| No lesion           | 275 (100%)                  | 6 (2.2%)                         | <0.001*            |
| GGO                 | 0 (0%)                       | 140 (50.9%)                      | <0.001*            |
| Consolidation       | 0 (0%)                       | 37 (13.5%)                       | <0.001*            |
| Mixed               | 0 (0%)                       | 92 (33.5%)                       | <0.001*            |
| Hospitalizations ward |                          |                                  |                    |
| Infectious ward     | 0 (0%)                       | 247 (89.8%)                      | <0.001*            |
| ICU ward            | 0 (0%)                       | 28 (20.2%)                       |                    |
| Signs and symptoms  |                             |                                  |                    |
| Febrile             | 0 (0%)                       | 137 (49.8%)                      | 0.089              |
| Cough               | 0 (0%)                       | 166 (60.4%)                      | 0.025*             |
| Myalgia             | 0 (0%)                       | 89 (32.4%)                       | 0.441              |
| Respiratory distress| 0 (0%)                       | 208 (75.6%)                      | 0.037*             |
| Tracheal intubation | 0 (0%)                       | 26 (9.5%)                        | <0.001*            |
| Status              |                             |                                  |                    |
| Death               | 0 (0%)                       | 26 (9.5%)                        | <0.001*            |
| Survived            | 275 (100%)                   | 249 (90.5)                       |                    |

Abbreviations: COVID-19, coronavirus disease 2019; CRP, C-reactive protein; GGO, ground-glass opacity; ICU, intensive care unit; lymph, lymphocyte; neut, neutrophil; Plt, platelet; saturation, oxygen saturation measured by pulse oximetry; WBC, white blood cell.

*p < 0.05 was considered statistically significant, between severe and nonsevere.
2.5 | Statistical analysis

SPSS version 22.0 for the windows package was recruited for statistical analysis. Quantitative data were described as mean ± standard deviation for parametric data. In terms of qualitative data, number and percent were the basis of analysis. Qualitative data were analyzed by $\chi^2$ and logistic regression wherever it is appropriate. Student t‐test and one‐way analysis of variance tests were used to compare parametric quantitative data. The distribution of genotypes in all groups and that of in general population was compared using the Hardy–Weinberg equilibrium (HWE) model.

2.6 | Computational analyses

SpliceAid2 server (available at https://onlinelibrary.wiley.com/doi/abs/10.1002/humu.21609) was recruited to determine the effect of the TNFα‐311A/G, rs1800629 G>A variant on the splicing site pattern. Moreover, the web logo server (available at https://genome.cshlp.org/content/14/6/1188.short) assisted the analysis of interested sequences related to studied variants in terms of conservation.

3 | RESULTS

3.1 | Clinical and demographic findings

Both subject and control groups were adjusted regarding age and gender parameters ($p = 0.076$ and $p = 0.388$, respectively). Of the 275 patients with SARS-CoV-2 infection (hospitalized in infectious or ICU ward) included, 96 patients as nonsevere cases and 179 patients as severe cases were diagnosed on admission in hospital, and 26 (14.5%) patients (from severe cases) expired (death group, [see Table 1]). The mean age of two cases (severe vs. nonsevere), 57.56 and 50.21 years old, respectively, was statistically significantly different ($p < 0.001$). Most of the death cases (25 cases) were hospitalized in the ICU ward. The amount of leukocyte and neutrophil count, and duration of hospitalization time, were markedly higher in severe cases suffering from COVID-19 when compared to nonsevere cases ($p < 0.001$), and lymphocytes count was reduced in severe when compared to non‐severe cases ($p = 0.005$). The oxygen saturation was about 81.75% in severe cases ($p < 0.001$). In admission in the severe cases, the COVID-19 related lesions affected glass‐ground opacity (GGO) pattern in 84 (46.9%) patients and consolidation pattern and mix pattern of 30 (16.8%) and 65 (36.3%) patients when compared with non‐severe cases (56 [58.3%], 7 [7.3%], 27 [28.1%]), respectively ($p < 0.001$). At the same time, six cases showed no lesion in the CT scan of non‐severe patients. The $\chi^2$ test indicated that the signs/symptoms such as cough, respiratory distress, and tracheal intubation were significantly elevated in patients with severe COVID-19 compared with the nonsevere cases (Table 1). Unfortunately, 26 (9.5%) of affected subjects with COVID-19 were deceased.

3.2 | Genotypic distribution of the TNF SNPs

We found no deviation from HWE in our population. Table 2 shows the distribution of alleles and genotypes in COVID-19 and control subjects. The G allele of TNFβ‐252A/G, rs909253 A>G was more frequent in COVID-19 subjects compared to the healthy group, statistically (OR = 1.55, 95% CI = 1.23–1.96, $p < 0.0001$). GG versus AA and GG versus AA plus AG increase the risk of COVID-19 in our study population significantly (OR = 1.89, 95% CI = 1.15–3.11, $p = 0.011$ and OR = 1.71, 95% CI = 1.13–2.58, $p = 0.010$, respectively). On the other hand, the A allele of TNFα‐311A/G, rs1800629 G>A caused a moderate decrease in risk of COVID-19 (OR = 0.68, 95% CI = 0.53–0.86, $p = 0.002$). Our results showed that the AA genotype compared to the GG genotype decreased the risk of studied disorder by (OR = 0.44, 95% CI = 0.26–0.73, $p < 0.001$). In addition, the AA genotype versus GG plus GA genotype had a protective role in the risk of COVID-19 (OR = 0.54, 95%...
CI = 0.34–0.85, *p < 0.007). Finally, a slight fall was seen in the onset of COVID-19 regarding AA-plus GA compared to the GG genotype (OR = 0.63, 95% CI = 0.44–0.90, *p = 0.011).

Interaction analysis revealed that AGGA combined genotype was more frequent in the control group compared to COVID-19 subjects and ruled as reference genotype. AAAA genotype decreased the risk of COVID-19 by (OR = 0.37, 95% CI = 0.14–1.00, *p = 0.044). Moreover, the AGAA genotype showed a protective role that decreased the risk of COVID-19 moderately (OR = 0.48, 95% CI = 0.24–0.94, *p = 0.031). In contrast, the risk of COVID-19 soared in regard to the GGGG genotype dramatically (OR = 2.87, 95% CI = 1.27–6.50, *p < 0.009) (Table 3).
3.3 | Genotype distribution, disease severity, and signs/symptoms

Table 4 depicts the disease severity, prognosis, and signs/symptoms of COVID-19 subjects, such as radiologic features, duration of hospitalizations, and risk of intubation or admission in the ICU ward, in different genotypes of studied variations. Statistical analysis showed no significant association concerning the evaluated parameters between different genotypes, except for TNFβ-252A/G, rs909253 A>G with the AA genotype, in that the CT scan pattern was different in comparison to cases in the AG genotype with \( p < 0.001 \).

In addition, in the severe cases of COVID-19, leukocyte and neutrophil count, and duration of ICU hospitalization in the death group significantly increased \(( p < 0.001 )\), and on the other hand, lymphocyte and platelet count, and SpO2 in the death group significantly decreased \(( p < 0.001 )\) when compared to the survival group (Table 5).

Computational analyses showed that a 20 nt flanking region containing TNFα-311A/G, rs1800629 G>A was introduced to the SpliceAid2 server to detect the impact of nucleotide substitution on the splicing factor sites of the TNF gene. The results of the SpliceAid2 server revealed that TNFα-311A/G, rs1800629 G>A variant is likely to change the pattern of splicing factor sites. The G allele of TNFα-311A/G, rs1800629 G>A makes a recognition site for SRp30c, while the A allele introduces a new recognition site for the heterogeneous nuclear ribonucleoproteins family of splicing factor (Figure 2). Furthermore, the conservation of TNFα-311A/G, rs1800629 G>A, and TNFβ-252A/G, rs909253 A>G SNPs was illustrated by the WebLogo tool, indicating relatively high-conserved regions across multiple mammalian species (Figure 3).

4 | DISCUSSION

Several case-control studies of assorted designs have recently elucidated the association of specific host genetic variants with clinical disease severity or susceptibility to SARS-CoV-2 infection and COVID-19 disease outcome.25 Devaux et al.27 suggested that functional polymorphisms in human ACE2, which affects ACE2 expression, might influence COVID-19 risk, severity and outcome. In another case-control experiment, Karst et al.25 suggested that C677T polymorphism located in the methylene tetrahydrofolate reductase gene influences the immune state of the COVID-19 patients and correlates with disease severity. Torre-Fuentes et al.29 proposed that TMPRSS2 rs75603675, rs61735792, and rs61735794 polymorphisms may be correlated with COVID-19 disease. Lastly, by performing a comprehensive analysis on multiple databases including 290 000 samples from more than 400 populations, Stawiski et al.30 showed that some ACE2 variations which could be mapped to the S-protein-interacting ACE2 surface (i.e., H37R, Q102P, T92I, N64K, T27A, E23K, S19P, I21V, and K26R) have a positive association with COVID-19 susceptibility. In contrast, some other mutations in this region, including Y50F, E35K, E37K, K31R, D509Y, D38V, D355N, Q388L, F27V, N33I, K68E, and so forth, protected the subjects from this infectious disease.30

The rs1800629 polymorphism is the most studied TNFα variation, which is a G/A substitution and is located in the promoter region at position −308.25 It has been established that the presence of the GG genotype for this SNP confers strong in vivo and in vitro transcriptional activity.32,33 Previous analyses of this polymorphism in different populations gave inconclusive results. Zhang et al.34 discovered that TNFα rs1800629 is associated with enhanced risk of sepsis, a systemic inflammatory response to infection, under allelic A versus G, GA versus GG, and GA + AA versus GG inheritance models. Tharwat et al.35 reported that the AA genotype of this variant confers susceptibility to hepatitis C virus infection in Egyptian patients. In connection with respiratory diseases, Yang et al.36 proposed that TNFα rs1800629 is a risk factor for asthma. This can be explained by the role of TNFα in the pathophysiology of respiratory diseases.37 Ding et al.38 showed that allele A of the TNFα rs1800629 polymorphism is associated with risk of ARDS in a Chinese population, whereas the GG genotype was linked to lower mortality. It has also been shown that the G allele of this variation was over-represented in patients with influenza A/H1N1 and correlated with disease severity in a Mexican population.39 In contrast to these findings, Wang et al.40 study showed no difference between the genotype distribution of TNFα SNPs, including -308G/A, -1031T/C, -863C/A, -572A/C, and -238G/A between cases with the SARS and healthy subjects. In our study, we found that the A allele and AA and GA + GG genotypes decrease the risk of SARS-CoV-2 infection. On the other hand, our study indicated that in TNFβ-252A/G, rs909253 A>G with the AA genotype, that the CT scan pattern was different...
### Table 4: Disease severity, prognosis, and symptoms in different genotypes of the studied COVID-19 cases

| Parameter evaluated | Case genotypes of TNFβ rs909253 A>G | Within-group significant | Case genotypes of TNF-α rs1800629 G>A | Within-group significant |
|---------------------|--------------------------------------|--------------------------|----------------------------------------|--------------------------|
|                     | AA N = 61                             | AG N = 141               | GG N = 73                               |                           |
| Severe              | 41 (67.2)                             | 85 (60.3)                | 53 (72.6)                               |                           |
| Nonsevere           | 20 (32.8)                             | 56 (39.7)                | 20 (27.4)                               |                           |
| Survive             | 53 (86.9)                             | 130 (92.2)               | 66 (90.4)                               |                           |
| Death               | 8 (13.1)                              | 11 (7.8)                 | 7 (9.6)                                 |                           |
| No lesion           | 1 (1.6)                               | 4 (2.8)                  | 1 (1.4)                                 |                           |
| Lesion in CT        | 60 (98.4)                             | 137 (97.2)               | 72 (98.6)                               |                           |
| Infectious ward     | 52 (85.2)                             | 129 (91.5)               | 66 (90.4)                               |                           |
| ICU ward            | 9 (14.8)                              | 12 (8.5)                 | 7 (9.6)                                 |                           |
| Nonintubation       | 53 (86.9)                             | 129 (91.5)               | 67 (91.8)                               |                           |
| Intubation          | 8 (13.1)                              | 12 (8.5)                 | 6 (8.2)                                 |                           |
| Hospitalization     | 8.45 ± 6.4                            | 7.49 ± 5.                | 7.5 ± 5.7                               |                           |

Note: TNFβ, p1: AA versus AG, p2: AG versus GG, p3: AA versus GG and TNF-α, p1: GG versus GA, p2: GA versus AA, p3: GG versus AA.

Abbreviations: COVID-19, coronavirus disease 2019; CT, computed tomography; ICU, intensive care unit; TNF, tumor necrosis factor.

*p < 0.05 was considered statistically significant.
compared to cases in the AG genotype. This can be explained by the role of TNF-β in the pathophysiology of ARDS of COVID-19.

TNFα resides approximately 252 base pairs downstream of the transcription start site for the gene coding TNFβ (also known as lymphotoxin alpha). As an inflammatory mediator, TNF-α affects the production of other cytokines. However, the interaction between cytokines might result in antagonistic (TNF-α and TNF-β, for instance) or synergistic (e.g., TNF-α with IL-1 interactions) effects. TNFs also regulate receptor expression of other cytokines or stabilize cytokine messages by another, and, therefore, play pivotal roles in signal transduction. It has been hypothesized that serum concentration of TNF-α is elevated in subjects with COVID-19; thus, these patients have a greater probability of developing ARDS and death. Karki et al. findings revealed that synergistic interaction of TNF-α and IFN-γ triggers severe inflammation, organ damage, and death during SARS-CoV-2 infection.

Growing evidence suggests that macrophages present SARS-CoV-2 spike antigens to T cells during an immune response, resulting in the release of TNF-β and other chemokines and cytokines (i.e., IL-1, IL-6, IL-8, IL-21, and monocyte chemoattractant protein-1) and causing the cytokine storm. Clinical data have shown that induction of a cytokine storm caused acute inflammatory lung injury and is associated with the severity of SARS-CoV-2 infection. Furthermore, the increased release of TNF-β is linked to hypercoagulation that causes impairment in the clinical
condition of COVID-19 patients.\textsuperscript{45,47} Martinez Mesa et al.\textsuperscript{48} reported that serum TNF-β levels were higher in COVID-19 patients with worse evolution.

The 252A>G polymorphism is located in intron 1 of the TNFβ encoding gene.\textsuperscript{49} Few studies have established a relationship between TNFβ rs909253 A>G, also known as LTA +252, and risk of viral infections, such as influenza A/H1N1 infection.\textsuperscript{50} In contrast, Reséndiz-Hernandez et al.\textsuperscript{51} demonstrated that this variation is not associated with the risk of chronic obstructive pulmonary disease secondary to tobacco smoking in Mexicans. Similarly, Solé-Violán et al.\textsuperscript{52} did not identify any association between TNFβ-252A/G, rs909253 A>G and disease severity and outcome in patients with pneumonia. The result of Puthothu et al.\textsuperscript{53} studies indicated no relationship between this variant and the risk of bronchial asthma and severe respiratory syncytial virus infection. We found a negative association between TNFβ-252A/G, rs909253 A>G and COVID-19 susceptibility under codominant GG and recessive AA versus GA + GG models, which was not consistent with the findings of the above-mentioned studies.

Accumulating evidence shows that genetic background influences the outcome and severity of COVID-19. Herein, for the first time, we reported an association between either TNFα or TNFβ polymorphisms and COVID-19 risk and outcome. However, there are some shortcomings to the current study. First, our small sample size was relatively small. Second, confounding factors such as health sector expenditure and the number of nurses and physicians for 1000 patients were not considered. Moreover, depending on their interactions with other risk factors and COVID-19 candidate SNPs, allelic variants of TNFα and TNFβ genes might exhibit different impacts in other races. Further studies on a larger population and different ethnicities are needed to confirm our results.

5 | CONCLUSION

Previous studies have shown that induction of a cytokine storm might substantially cause death among COVID-19 cases. Results of our analysis seem to suggest that both studied variations might substantially affect COVID-19 susceptibility. The allelic variants of the studied genes might show different impacts in other races depending on their interactions with other risk factors. Replicated studies on different ethnicities and larger populations are needed to validate our results.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

Saman Sargazi: Conceptualization. Saman Sargazi, Mohsen Rokni, Mohammad Sarhadi, Milad H. Nia, Shekoufeh Mirinejad, Maryam Kargar, and Sara Rahdar: Writing – original draft preparation. Saman Sargazi, Shekoufeh Mirinejad, Mohammad Sarhadi, Mohsen Rokni, Maryam Kargar, and Ramin Saravani: Writing – review and editing. Saman Sargazi: Supervision. All authors have read and agreed to the published version of the manuscript.

ETHICS STATEMENT

All procedures performed in studies involving human participants were following the Ethical Standards of the Institutional and/or National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study protocol was approved by the Ethics Committee of Zahedan University of Medical Sciences (IR.ZAUMS.REC.1399.122). Written consent was obtained from the patients or their guardians.

DATA AVAILABILITY STATEMENT

The data presented in this manuscript will be available by the corresponding author upon reasonable request.
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SUPPORTING INFORMATION
Additional supporting information may be found in the online version of the article at the publisher’s website.

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