Health Science Reports

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Neuropilin-1 as a Neuroinflammatory Entry Factor for SARS-CoV-2 Is Attenuated in Vaccinated COVID-19 Patients: A Case–Control Study

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Received: 19 October 2024 | **Revised:** 5 March 2025 | **Accepted:** 24 March 2025

Funding: This work was supported by National Elite Foundation of Iran (Grant No. 1640/151). Also, the present study was supported by the Deputy of Research and Technology (Grant No. 724134176), Babol University of Medical Sciences, Babol, Iran.

Keywords: COVID-19 | cytokine storm | interleukin-6 | NRP1 | SARS-CoV-2

ABSTRACT

Background and Aim: COVID-19 is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) as a pandemic infectious disease. So far, it has been known that this virus uses several receptors to enter the host cell, one of which is neuropilin-1 (NRP1). Also, one of the main causes of clinical manifestations, severity of disease, and mortality of patients is cytokine storm syndrome, one of these cytokines being interleukin (IL)-6. Our aim was to study the level of expression of NRP1 and IL-6 genes in COVID-19 patients by using peripheral blood mononuclear cells (PBMCs).

Materials and Methods: Our study population included the test group (80 patients with COVID-19) and the control group (30 healthy individuals). Venous blood was taken from all subjects. After isolating PBMCs from blood using Ficoll, RNA was extracted. Then, cDNA synthesis, the expression level of NRP1 and IL-6 compared to GAPDH housekeeping gene was measured by real-time PCR.

Results: The level of NRP1 gene expression was increased significantly in COVID-19 different groups compared to the control group. Surprisingly, it was observed that the amount of NRP1 gene decreased in the vaccinated group compared to non-vaccinated groups. IL-6 gene expression was also significantly increased in all groups except vaccinated patients compared to the control group. Also, the results indicated that there was a positive and statistically considerable relationship between IL-6 expression level and NRP1 expression level ($p = 0.03$).

Conclusion: The significant increase in the expression of NRP1 and IL-6 genes in COVID-19 patients, especially in moderate and severe cases, indicates their potential involvement in the progression of the disease, which may serve as biomarkers of disease severity. Also, since these genes play an important role in causing severe inflammation, cytokine storm, and immunopathological complications of COVID-19, further investigations maybe needed to achieve therapeutic goals to control COVID-19 and similar diseases.

1 | Materials and Methods

1.1 | Sample Election and Data Gathering

Our study was performed on 110 subjects in five groups in 2020. Also, COVID-19 patients who contracted the disease despite injection with COVID-19 vaccines were enrolled in a separate group. Therefore, vaccinated cases were collected after COVID-19 vaccines were made available globally. Overall, this study included 20 ICU COVID-19 (severe), 20 hospitalized COVID-19 (moderate), 20 COVID-19 out-patients (mild), and 30 healthy persons as controls. Also, 20 vaccinated patients were later added to these samples. In this study, the eligibility criteria for healthy controls were no history of any critical hyperinflammatory disease, including diabetes, excessive weight (evaluated by body mass index [BMI] higher than 30), severe cardiovascular, kidney, cerebral-vascular, or autoimmune conditions. COVID-19 cases were enrolled without age restriction. Demographic data, COVID-19 clinical symptoms, and previous medical history were collected for patients. Supplementary laboratory data, namely CBC, Na, K electrolytes, C-reactive protein (CRP), ESR as well as essential symptoms and O_2 saturations were obtained for hospitalized and ICU COVID-19 patients. Diagnosis of COVID-19 patients was certified by IgM/IgG serological rapid test and (RT-PCR). Additionally, hospitalization or acceptance of cases to ICU was based on various clinical features, including CT-scan, pulse rate, SpO_2, and various blood parameters such as IL-6, CRP, LDH, CPK, ferritin, and troponin similar to criteria published by the National Guidelines of the Diagnosis and Treatment Committee for COVID-19 as well as National Institutes of Health (NIH) (covid19treatmentguidelines.nih.gov) [19]. Also, this work was approved by Ethics Committee of Babol University of Medical Sciences, Babol, Iran (IR.MUBABOL.REC.1401.038). The patients/participants prepared their written informed consent to participate.

1.2 | Preparation of PBMCs, RNA Isolation, and Synthesis of cDNA

Whole blood samples (5 cc) were collected in EDTA tubes. PBMCs were separated by lymphocyte separation media (Ficoll, Capricorn, Germany). Then, blood specimens were added to cover Ficoll. The biphasic mix was centrifuged at $800 \times g$ for 20 min. Washed PBMCs were used for RNA isolation using Total RNA Extraction Kit (Yekta tajhiz Azma, Tehran, Iran). Reverse transcription of RNA to cDNA was carried out using the Easy cDNA Reverse Transcription kit (ParsTous, Iran). After cDNA synthesis steps were performed, the reaction was halted by incubating at 85°C for 5 min. Synthesized cDNA was kept at −80°C [19].

1.3 | Expression Analysis of NRP1 and IL-6 by Real-Time PCR

The utilized sequences were oligonucleotide primers designed to detect NRP1 and IL-6 genes. Moreover, National Center for Biotechnology Information (NCBI) blast was performed to assess the primer by exploring the human genome. Glyceraldehyde 3- phosphate dehydrogenase (GAPDH) was chosen as the reference housekeeping gene [20]. The mentioned sequences were reassessed for suitability via Oligo7 software. To quantify relative mRNA expression, RT-PCR was performed. Following cDNA amplification by PCR using SYBR Green qPCR Master Mix 2X (ParsTous Biotechnology, Mashhad, Iran), RT-PCR assay was run by Applied Biosystems 7300 real-time thermocycler (Applied Biosystems, Massachusetts, USA). The presentation of NRP1 and IL-6 mRNA in severe, moderate, and mild COVID-19 cases, vaccinated patients, and control subjects was investigated by RT-PCR based on normalized expression ratios and reported as fold change. Also, primer sequences that were utilized in this study are displayed in Table 1. Additionally, protein–protein interaction (PPI) by STRING database was evaluated for major proteins corresponding to genes studied in the present research as well as key proteins in COVID-19. The STRING tool (https://string-db.org/) systematically gathers and interconnects PPIs—both physical interactions alongside function relationships. The information is derived from a handful of origins: automatic text mining of the scientific evidence, computational interaction forecasts from co-expression, conserved genetic background, databases of action and reaction tests, and identified complexes/signaling from curated references [21, 22].

1.4 | Statistical Analysis

In our study, gene expression was analyzed using the 2-DDCT method for NRP1 and IL-6. Nature of each parameter, histogram, and Shapiro–Wilk test were used to evaluate normality of data. Gene expression was reported as fold change, which was calculated based on normalization using GAPDH. Spearman correlation test was employed to explore the relationship of NRP1 and IL-6 expression. We specified the use of the Mann–Whitney *U* test for nonparametric comparisons and calculated effect sizes by

TABLE 1 | Primers for genes of interest and housekeeping gene.

Gene	Primer sequence	Primer length
NRP1 forward	TTCAGGATCACACAGGAGATGG	22
NRP1 reverse	TAAACCACAGGGCTCACCAG	20
IL-6 forward	AATCATCACTGGTCTTTTGGAG	22
IL-6 reverse	GCATTTGTGGTTGGGTCA	18
GAPDH forward	ACAGTCAGCCGCATCTTC	18
GAPDH reverse	CTCCGACCTTCACCTTCC	18

bootstrapping independent samples for all key outcomes. All analyses were performed by Prism 9 (GraphPad Inc, USA).

2 | Results

2.1 | Comparison of NRP1 Gene Expression Changes Between Different Study Groups

The NRP1 gene showed significant changes in sick people with mild, moderate, and severe COVID-19 evaluated in the healthy group (Mann–Whitney U $p < 0.0171$, $p < 0.0001$, and $p < 0.0001$, respectively). This indicates that NRP1 possibly plays a role in the process of this disease. The fold change diagram shows the gradual increase in NRP1 gene expression from mild to severe cases of COVID-19 sicks, and the differences are greater in the last two groups (moderate and severe). Also, the difference in expression of this gene between patients with mild form compared to moderate and intensive form of the disease was significant (Mann–Whitney U $p < 0.0050$ and $p < 0.001$, respectively); Additionally, bootstrapping to determine the mean difference of fold change as the effect size showed a change in NRP1 expression when comparing mild with moderate and severe groups, respectively (95% confidence interval [CI] [−12.69, −2.40], two-tailed $p = 0.021$; 95% CI [−43.10, −7.05], two-tailed $p = 0.060$). Our observations show a potential relationship between NRP1 expression levels and disease severity in different groups of patients. Also, the expression of NRP1 increased significantly in vaccinated individuals compared to the control group (Mann–Whitney U $p < 0.0018$) (Figure 1).

2.2 | Comparison of IL-6 Gene Expression in Different Study Groups

Similar to NRP1, IL-6 gene showed significant changes in sick's with mild, moderate, and intensive COVID-19 evaluated to the healthy group (Mann–Whitney U $p = 0.0097$, $p < 0.0001$, and $p < 0.0001$, respectively) which indicates the potential involvement of this pro-inflammatory cytokine in this disease. Also, the difference in expression of this gene between patients with mild form compared to moderate and severe forms of the disease was significant (Mann–Whitney U $p = 0.0089$, $p = 0.0021$). Mean difference between mild and severe fold change values was significant (bootstrapped 95% CI [−48.51, −12.44], two-tailed $p = 0.016$). Fold change diagram shows a similar pattern of increased IL-6 gene expression from mild to moderate cases. These findings show that IL-6 plays an essential role in the development and severity of COVID-19. Also, a significant increase in IL-6 gene expression was observed in severe and moderate COVID-19 patients evaluated to vaccinated individuals ($p < 0.0001$), which indicates the main role of IL-6 in the severity of COVID-19 (Figure 2).

2.3 | Comparison of the Relationship Between NRP1 Gene Expression and Gender in Different Study Groups

The highest expression of NRP1 was related to the severe form of the sick in women, and the lowest expression of this gene was

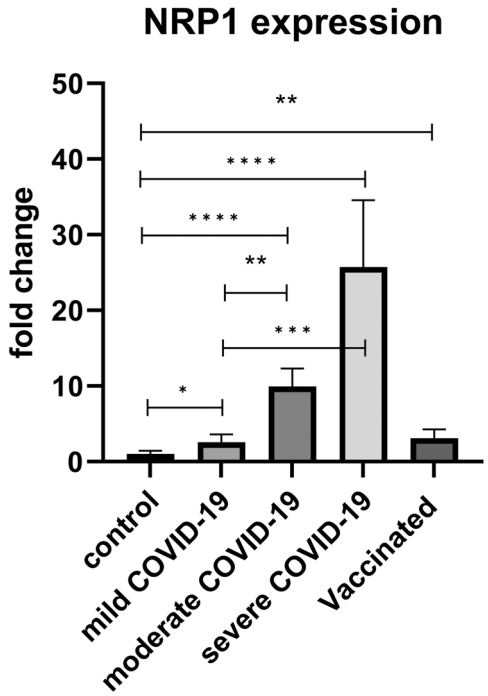

FIGURE 1 | Comparison of fold change of NRP1 gene expression between different studied groups. According to this graph, the expression of NRP1 gene in mild, moderate, severe groups, and vaccinated patients increased by 8.848 ± 25.73, 2.407 ± 9.924, 1.030 ± 2.561, and 1.129 ± 3.122 times, respectively, compared to the control group. Significance levels are shown as follows: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

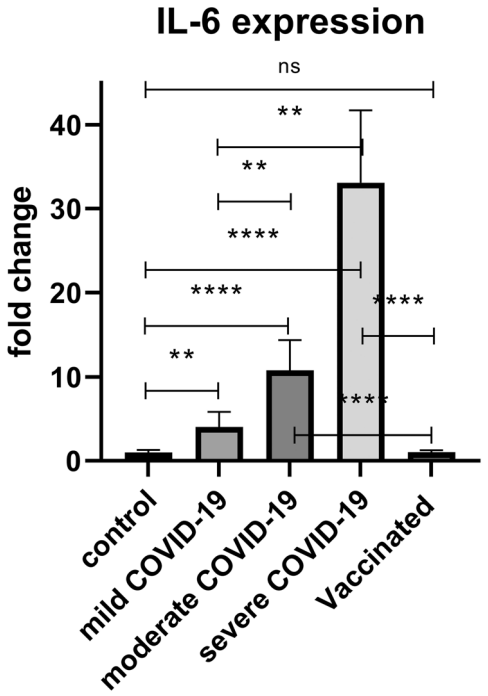

FIGURE 2 | Comparison of fold change of IL-6 gene expression between different studied groups. According to this diagram, IL-6 gene expression in mild, moderate, and severe groups increased by 1.831 ± 4.698, 3.544 ± 10.70, and 17.98 ± 48.65 times, respectively, compared to the control group. No significant difference in IL-6 gene expression was observed between vaccinated patients and healthy subjects (1.049 ± 0.1944).

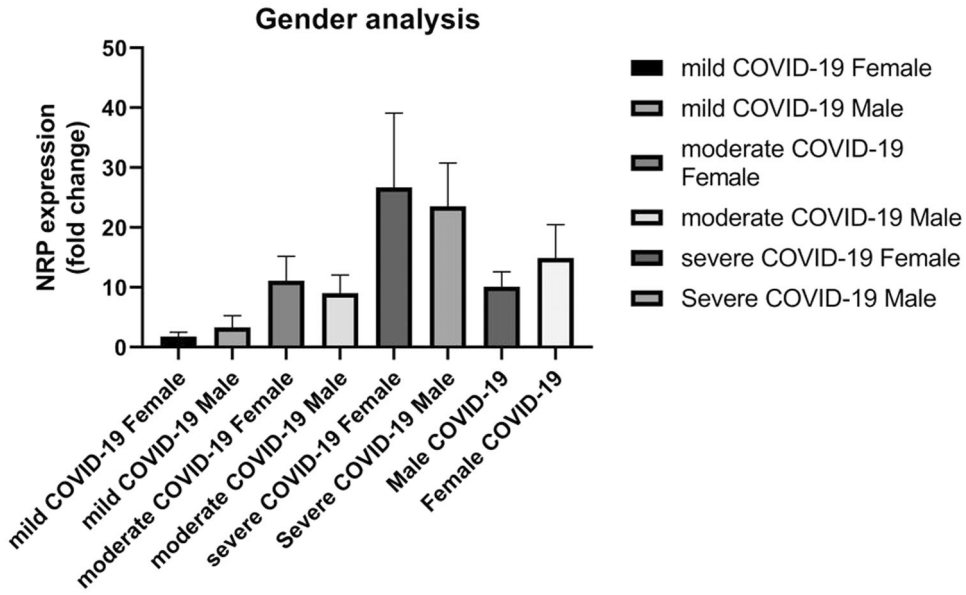

FIGURE 3 | Comparing the relationship between NRP1 gene expression and gender in the study groups. This graph shows that women in the severe COVID-19 group have the highest NRP1 expression.

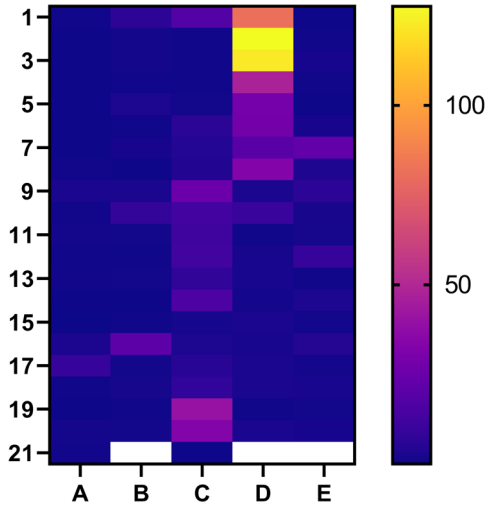

FIGURE 4 | Heatmap for the gene expression of NRP1 in various COVID-19 groups and healthy controls.

related to the mild form of the sick in women. In all groups (except the mild form), the expression of this gene was higher in women than in men, but this difference was not significant (Figure 3).

A correlation analysis was also performed to investigate the relationship between IL-6 and NRP1 expression levels in COVID-19 patients. Our findings in this project indicated that there is an affirmative and statistically remarkable correlation between IL-6 expression level and NRP1 expression level (p value = 0.03, $r = 0.2$, 95% CI [0.18-0.46]). This shows that the increase in the expression level of IL-6 and the enhancement in the expression level of NRP1 play a role in the function of each other in COVID-19 patients. Heatmap of gene expression is provided in Figure 4. PPI analysis of NRP1, NRP2, IL-6, and other major proteins involved in COVID-19 is provided in Figures 5 and 6.

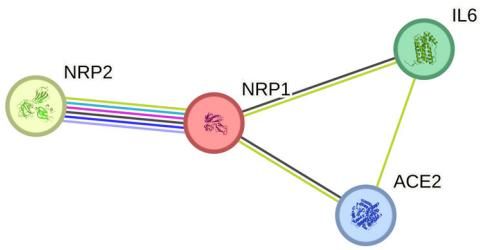

FIGURE 5 | Protein–protein interaction for NRP1 and major proteins involved in this study.

3 | Discussion

The 2019 coronavirus disease is an acute respiratory disease that was caused by SARS-CoV-2 in Wuhan, China, in December 2019. Therefore, the high mortality rate caused by this disease as well as its possible complications in the future prompted us to investigate the expression level of two important genes. In this study, we investigated the expression levels of two genes, NRP1 and IL-6, in different groups, including the control group, the group with mild, moderate, and severe forms, and vaccinated patients. The aim of this study was to investigate gene expression levels between different studied groups, to identify differences and the potential role of these genes in the immunopathogenesis of this disease and the effect of vaccination. NRP1 is one of the two homologous neuropilins that play an important role in physiological and pathological conditions in the body.

Castelvetri et al. showed that SARS-CoV-2 binds to NRP1 by the S protein, and upon entering neurons, the expression of NRP1 and the two proteases furin and TMPRSS11A increases in virus-infected cells [23]. Also, a study by Davies et al. showed that NRP1 is expressed in the CNS and affects areas related to smell, indicating the potential role of NRP1 as another entry pathway for SARS-CoV2 [24]. In a study that aimed to evaluate the relationship between cytokine and NRP1 expression in COVID-

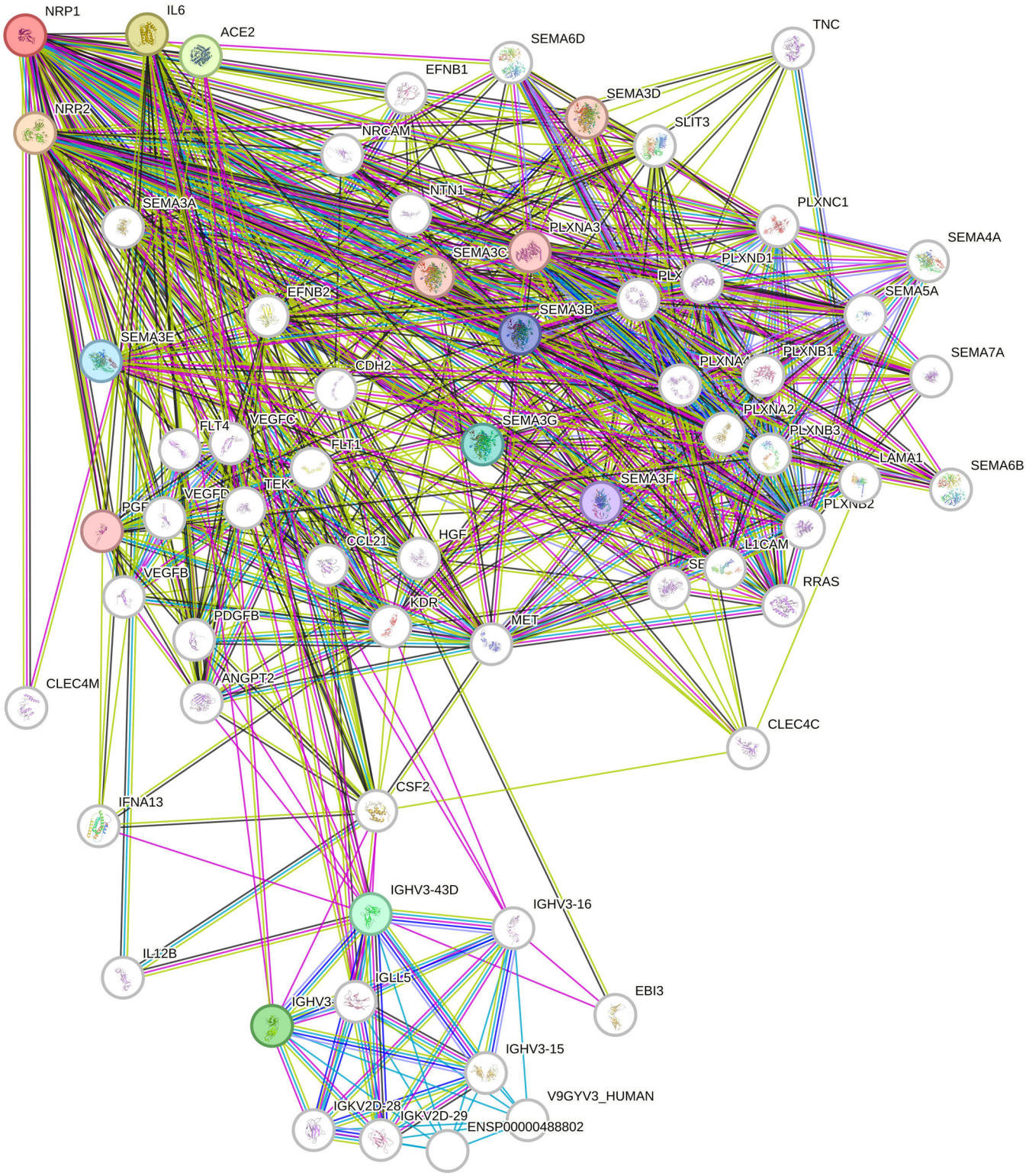

FIGURE 6 | Extended protein–protein interaction for NRP1 and major proteins involved in this study.

19 patients in Egypt, they observed that the expression level of NRP1 in patients was remarkably higher than in healthy individuals [25]. Hasan et al. observed that the serum level of NRP1 was higher in patients with the severe form of COVID-19 compared to the moderate form and control group. If confirmed in future studies, this may indicate that high concentration of NRP1 in the early stages of COVID-19 should be carefully controlled to prevent pulmonary fibrosis and damage to other organs as soon as possible [26]. We also observed in our study that the expression of the NRP1 gene increased in different groups of patients, which confirms the previous studies. Similar to the aforementioned studies, we also observed that the expression of NRP1 gene in patients with severe and moderate forms was upper evaluated to the healthy group ($p < 0.0001$), which shows that the increase in the expression of this gene is correlated to the intensity of the sick and mortality, which we

mentioned in our previous research [19]. The olfactory dysfunction after coronavirus infection in adults is caused by about 10%–15%. Recently, COVID-19 has become one of the causes of parosemia. Zamzami et al. introduced a 38-year-old healthy man who developed parosemia after the second dose of the AstraZeneca vaccine and 2 weeks after the injection [27]. In the study of Ohla et al., out of 12,313 patients with COVID-19, after infection and complete recovery, they reported loss of sense of smell and taste in 1468 individuals [28]. Increased expression of NRP1 may enhance virus entry into the CNS that collaborates to the development and progression of neurological symptoms and disease. In our previous study, which was conducted on the same patients, we saw a strong increase in IL-6 concentration and observed and reported myalgia signs in 30 sicks (45.45%) and neurological signs in 22 patients (33.33%) as the most clinical symptoms [19]. These results show the central role of IL-6 and NRP1 in the occurrence of neurological and muscular symptoms, as well as the prevalence and progression of neurological diseases after contracting COVID-19. However, to date, the potential role of NRP1 in neurological side effects after COVID-19 vaccination has not been elucidated. The COVID-19 pandemic may be a factor in the spread and progression of life-threatening diseases such as cancer [29]. In a study regarding the relationship between oral squamous cell carcinoma and COVID-19 infection, Norouzi et al. showed that the evaluation of NRP1 on tumor cells and its interaction with the membrane protein CMTM6 and PDL-1 (an inhibitor of immune checkpoints) caused resistance to treatment with antibody against PD-1. Also, the interaction of NRP1 with ACE2 causes resistance to the anticancer drug cisplatin and facilitates the entry of the virus, because hypoxia caused by the infection of COVID-19 leads to the expression of NRP1 [30]. Since in our project, the expression of NRP1 was enhanced in different groups of patients compared to healthy individuals and considering the role of this receptor in angiogenesis and increasing tumor proliferation mentioned in the above studies, increased NRP1 expression may make patients with COVID-19 prone to various tumors in the future or make individuals with cancer face a worse prognosis, which can be a big alarm for global health and increase the problems of cancer patients in the future. In the outbreak of this disease after public vaccination, we sampled additional cases. In this study, we showed that the NRP1 expression was greatly reduced in individuals who were reinfected with COVID-19 after vaccination. This shows that vaccination can reduce and control NRP1 gene expression.

In addition, one of the cytokines produced during the cytokine storm of this disease is IL-6. IL-6 is released upon recognition of DAMPs or PAMPs by innate immune cells such as macrophages as part of the host's defense strategy to eliminate infected cells or damaged tissue [12, 13]. The researchers showed in their studies that the high concentration of IL-6 in the peripheral blood as well as the high level of expression of this gene can be one of the factors of the severity of COVID-19. IL-6 is correlated with worse sick outcomes and is critical for the progression of COVID-19 [25, 31–37]. In our study, we also showed that the significant increase in IL-6 gene expression in patients with severe moderate and COVID-19 evaluated to the healthy group can indicate the main role of IL-6 in the intensity of the sick, which confirms previous studies. These findings about the potential role IL-6 provide noteworthy insights into the immunopathogenesis of COVID-19. Several studies also found that baseline levels of IL-6 were significantly higher in those who required ICU compared to those who did not. They concluded that IL-6 can be considered as an early predictor of the exacerbation of COVID-19 [38–40]. In our study, a valuable increase in IL-6 gene expression was observed in intensive COVID-19 sick (ICU) evaluated in the healthy group ($p < 0.0001$), so our findings while confirming previous studies, show the potential role of IL-6 in the immunopathogenesis of COVID-19 and disease severity. In a cross-sectional study conducted by Setyo Nugroho et al. with the aim of investigating the severity of the disease of COVID-19 in dead patients in terms of IL-6 expression in lung tissue, they observed that the mean tissue IL-6 expression was 72.63 with the highest frequency of 47.4%. They concluded that increased expression of IL-6 in lung tissue indicates the severity of COVID-19. Therefore, they suggested more research to clarify the role of IL-6 in predicting the mortality of patients with COVID-19, especially in its severe form [41]. In our previous research, it was found that increased IL-6 serum concentration was associated with disease severity and mortality in patients with COVID-19 [19]. We also showed in the present study that the expression of this gene increased significantly, which confirms our previous research on the increase in its production in COVID-19 patients. Therefore, IL-6 can be one of the risk factors for determining the severity and mortality of COVID-19 patients and one of the therapeutic goals of this disease and similar diseases. In a study, Malekpour et al. identified the "cytokine storm" created by SARS-CoV-2 as another proposed mechanism of the autoimmune pathway that could possibly lead to Guillain–Barré syndrome associated with COVID-19 [42]. It was proven long ago that viruses are a main part of environmental factors that play a role in the production of autoantibodies and causing autoimmune diseases [43]. It seems that the possible mechanisms that may lead to the development of autoimmune disease in these patients include the creation of neutrophilic extracellular traps (NETosis) with an increase in peptidyl arginine deiminases, molecular similarity between antigens of host tissues and virus (molecular mimicry) such as the existence of some similar peptide sequences between the virus and host tissues and the ability of the virus to overstimulate the immune system and create chronic inflammation that leads to the stimulation of the expression of costimulatory molecules that are effective in the formation of autoimmune diseases. COVID-19 is correlated with leukocyte subsets and an extreme increment in the concentration of pro-inflammatory cytokines, which is known as cytokine storm syndrome [44]. There are many similar reports of the occurrence of autoimmune diseases as one of the complications after contracting COVID-19 [45–47]. IL-6 signaling in liver sinusoidal endothelial cells (LSECs) leads to epitheliopathy and liver damage in patients with COVID-19. According to this study, IL-6 signaling indicates a link between coagulopathy/epitheliopathy and liver failure associated with COVID-19 [48]. Therefore, in our study, the excess in the expression of NRP1 in different groups of patients, followed by the increase in the expression of IL-6, may be related to the increase in enzymes and liver damage.

Our study provides novel insights on the role of NRP1 in COVID-19 immunopathology and the effect of vaccinations; however, this study is limited by limited sample size, variations

in types and dosages of vaccination, and demographics variations even while probability sampling. The present work used robust statistical tests to analyze gene expression data, and bootstrapping technique was used to calculate the effect size. Although non-parametric analysis of our hypothesis and the calculated effect size confirmed a dysregulated expression of NRP1, in some instances, the bootstrapped significance was not reached which could be due to variability in data and sample size limitations. Further study with larger sample size and grouping by type and dosages of COVID-19 vaccinations is warranted.

4 | Introduction

COVID-19 is an acute respiratory disease with pneumonia symptoms that broke out in Wuhan, China, in December 2019. The cause of this disease was a new virus called severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). This virus enters the upper respiratory tract mainly through droplets in the air [1, 2].

It has been shown that SARS-CoV-2 uses NRP1 as an entry factor for exacerbating viral infection [3]. NRP1 is best known for its role in cell signaling and its function as a cell surface receptor [4]. The membrane form of NRP1 has a ligand-binding site that is naturally reserved for growth factors such as vascular endothelial growth factor, but it can also bind to viruses such as human T-cell lymphotropic virus (HTLV)-1, Ebstein-Barr virus (EBV), and SARS-CoV-2 [5–7]. Analysis of NRP1 gene expression in cells isolated from bronchoalveolar lavage of patients with severe COVID-19 showed increased NRP1 gene expression [3]. NRP1 may serve as an additional mediator of SARS-CoV-2 infection involved in the neurological manifestations of COVID-19. NRP1 is also expressed in the olfactory epithelium as well as olfactory neurons. Therefore, NRP1 may be a factor in the development and progression of olfactory and neurological disorders in COVID-19 [8]. Also, Sherafat et al. showed that activated microglia express Nrp1, which plays an important role in promoting the proliferation of oligodendrocyte progenitor cells in early postnatal white matter tracts [9].

On the other hand, most of the COVID-19 patients have mild to moderate symptoms. Moreover, some cases may experience a critical form of COVID-19 with symptoms such as sepsis, respiratory failure, and acute respiratory distress syndrome [10]. This set of features is reminiscent of a family of syndromes known as “cytokine storm syndromes,” in which severe inflammation and disease and multiorgan involvement occur through excessive cytokine release due to uncontrolled activation of the immune system [11]. An increase of TNF-α, IL-1, IL-6, IL-18, and IFN-γ levels in the serum is a typical feature of a cytokine storm. In particular, the high level of IL-6 is one of the hallmarks of cytokine storms. IL-6 is a prominent pro-inflammatory cytokine that is appeared upon recognition of pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) by innate immune cells such as macrophages as part of the host's defense strategy to eliminate infected cells or damaged tissue [12, 13]. As mentioned, the brain is susceptible to infection with SARS CoV-2. Dey and Bishayi showed that microgliosis causes the release of inflammatory cytokines such as IL-1β, IFN-γ, and IL-6, creating a cytokine storm that can increase the likelihood of cognitive and neurological disorders [14]. Many times, clinical trials have been performed to investigate the influence of tocilizumab on cytokine storms caused by SARS-CoV-2 infection, which showed that suppression of IL-6 signaling is possible beneficial in the cure of cytokine storms [15].

Evidence supports the possible importance of the novel entry factor of COVID-19 NRP1 through hyperinflammation and mediation of IL-6 secretion in COVID-19 patients, leading to severe complications and multiorgan damage in COVID-19 [16–18]. In the present research, we investigated the expression of NRP1 and IL-6, as well as the relationship between NRP1 and IL-6 in different severities of COVID-19 patients.

5 | Conclusions

The significant increase in the expression of NRP1 and IL-6 genes in COVID-19 patients, especially in moderate and severe cases, indicates their potential involvement in the progression of the disease, which may act as biomarkers of disease severity. Also, since these genes play an important role in causing severe inflammation, cytokine storm, and immunopathological complications of COVID-19, with more investigations, it is possible to achieve therapeutic goals to control this disease and similar diseases.

Author Contributions

Faezeh Hosseini: conceptualization, investigation, writing – original draft, writing – review and editing, visualization, methodology, software, formal analysis, data curation. **Abbas Azadmehr:** visualization, methodology, supervision, resources, project administration, funding acquisition, writing – review and editing, data curation, conceptualization, investigation. **Kiarash Saleki:** investigation, methodology, visualization, writing – review and editing, software, formal analysis, data curation. **Mohamadreza Ahmadifard:** methodology, investigation, visualization. **Morteza Oladnabi:** investigation, visualization, methodology. **Moein Shirzad:** methodology. **Mostafa Javanian:** visualization, investigation.

Acknowledgments

We acknowledged all contributions in this project. This work was supported by National Elite Foundation of Iran (Grant No. 1640/151). Also, the present study was supported by the Deputy of Research and Technology (Grant No. 724134176), Babol University of Medical Sciences, Babol, Iran.

Ethics Statement

This work was approved by Ethics Committee of Babol University of Medical Sciences, Babol, Iran (IR.MUBABOL.REC.1401.038). The patients/participants provided their written informed consent to participate in this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Result data are available from corresponding author of manuscript only on sensible request. “Moreover, all authors have study and confirmed

the final version of the manuscript had whole availability to all of the data in this study and takes full responsibility for the entirety of the data and the accuracy of the data analysis."

Transparency Statement

The lead author Abbas Azadmehr affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

References

1. P. Anfinrud, V. Stadnytskyi, C. E. Bax, and A. Bax, "Visualizing Speech-Generated Oral Fluid Droplets With Laser Light Scattering," *New England Journal of Medicine* 382, no. 21 (2020): 2061–2063, https://doi.org/10.1056/NEJMc2007800.

2. N. Van Doremalen, T. Bushmaker, D. H. Morris, et al., "Aerosol and Surface Stability of SARS-CoV-2 as Compared With SARS-CoV-1," *New England Journal of Medicine* 382, no. 16 (2020): 1564–1567, https://doi.org/10.1056/NEJMc2004973.

3. L. Cantuti-Castelvetri, R. Ojha, L. D. Pedro, et al., "Neuropilin-1 Facilitates SARS-CoV-2 Cell Entry and Infectivity," *Science* 370, no. 6518 (2020): 856–860, https://doi.org/10.1126/science.abd298.

4. H.-F. Guo and C. W. Vander Kooi, "Neuropilin Functions as an Essential Cell Surface Receptor," *Journal of Biological Chemistry* 290, no. 49 (2015): 29120–29126, https://doi.org/10.1074/jbc.R115.687327.

5. S. Lambert, M. Bouttier, R. Vassy, et al., "HTLV-1 Uses HSPG and Neuropilin-1 for Entry by Molecular Mimicry of VEGF165," *Blood* 113, no. 21 (2009): 5176–5185, https://doi.org/10.1182/blood-2008-04-150342.

6. F. Mota, C. Fotinou, R. R. Rana, et al., "Architecture and Hydration of the Arginine-Binding Site of Neuropilin-1," *FEBS Journal* 285, no. 7 (2018): 1290–1304, https://doi.org/10.1111/febs.14405.

7. H.-B. Wang, H. Zhang, J. P. Zhang, et al., "Neuropilin 1 Is an Entry Factor That Promotes EBV Infection of Nasopharyngeal Epithelial Cells," *Nature Communications* 6, no. 1 (2015): 6240, https://doi.org/10.1038/ncomms7240.

8. H. Zalpoor, A. Akbari, A. Samei, et al., "The Roles of Eph Receptors, Neuropilin-1, P2X7, and CD147 in COVID-19-Associated Neurodegenerative Diseases: Inflammasome and JaK Inhibitors as Potential Promising Therapies," *Cellular & Molecular Biology Letters* 27, no. 1 (2022): 10, https://doi.org/10.1186/s11658-022-00311-1.

9. A. Sherafat, F. Pfeiffer, A. M. Reiss, W. M. Wood, and A. Nishiyama, "Microglial Neuropilin-1 Promotes Oligodendrocyte Expansion During Development and Remyelination by Trans-Activating Platelet-Derived Growth Factor Receptor," *Nature Communications* 12, no. 1 (2021): 2265, https://doi.org/10.1038/s41467-021-22532-2.

10. F. Zhou, T. Yu, R. Du, et al., "Clinical Course and Risk Factors for Mortality of Adult Inpatients With COVID-19 in Wuhan, China: A Retrospective Cohort Study," *Lancet* 395, no. 10229 (2020): 1054–1062, https://doi.org/10.1016/S0140-6736(20)30566-3.

11. L. A. Henderson, S. W. Canna, G. S. Schulert, et al., "On the Alert for Cytokine Storm: Immunopathology in COVID-19," *Arthritis & Rheumatology (Hoboken, N.J.)* 72, no. 7 (2020): 1059–1063, https://doi.org/10.1002/art.41285.

12. T. Tanaka, M. Narazaki, and T. Kishimoto, "Therapeutic Targeting of the Interleukin-6 Receptor," *Annual Review of Pharmacology and Toxicology* 52 (2012): 199–219, https://doi.org/10.1146/annurev-pharmtox-010611-134715.

13. T. Tanaka, M. Narazaki, and T. Kishimoto, "IL-6 in Inflammation, Immunity, and Disease," *Cold Spring Harbor Perspectives in Biology* 6, no. 10 (2014): a016295, https://doi.org/10.1101/cshperspect.a016295.

14. R. Dey and B. Bishayi, "Microglial Inflammatory Responses to SARS-CoV-2 Infection: A Comprehensive Review," *Cellular and Molecular Neurobiology* 44, no. 1 (2024): 2, https://doi.org/10.1155/2021/8874339.

15. S. Kang and T. Kishimoto, "Interplay Between Interleukin-6 Signaling and the Vascular Endothelium in Cytokine Storms," *Experimental & Molecular Medicine* 53, no. 7 (2021): 1116–1123, https://doi.org/10.1038/s12276-021-00649-0.

16. S. Seyfi, A. Azadmehr, K. Ezoji, et al., "Mortality in ICU COVID-19 Patients Is Associated With Neutrophil-to-Lymphocyte Ratio (NLR): Utility of NLR as a Promising Immunohematological Marker," *Interdisciplinary Perspectives on Infectious Diseases* 2023 (2023): 1–5, https://doi.org/10.1155/2023/9048749.

17. K. Saleki, M. Banazadeh, N. S. Miri, and A. Azadmehr, "Triangle of Cytokine Storm, Central Nervous System Involvement, and Viral Infection in COVID-19: The Role of sFasL and Neuropilin-1," *Reviews in the Neurosciences* 33, no. 2 (2022): 147–160, https://doi.org/10.1515/reneuro-2021-0047.

18. K. Saleki, P. Alijanizadeh, and A. Azadmehr, "Is neuropilin-1 the Neuroimmune Initiator of Multi-System Hyperinflammation in COVID-19?," *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie* 167 (2023): 115558, https://doi.org/10.1016/j.biopha.2023.115558.

19. K. Saleki, M. Shirzad, M. Javanian, et al., "Serum Soluble Fas Ligand Is a Severity and Mortality Prognostic Marker for COVID-19 Patients," *Frontiers in Immunology* 13 (2022): 947401, https://doi.org/10.3389/fimmu.2022.947401.

20. M. Oladnabi, A. Bagheri, M. Rezaei Kanavi, A. Azadmehr, and A. Kianmehr, "Extremely Low Frequency-Pulsed Electromagnetic Fields Affect Proangiogenic-Related Gene Expression in Retinal Pigment Epithelial Cells," *Iranian Journal of Basic Medical Sciences* 22, no. 2 (2019): 128–133, https://doi.org/10.22038/ijbms.2018.25023.6214.

21. D. Szklarczyk, R. Kirsch, M. Koutrouli, et al., "The STRING Database in 2023: Protein–Protein Association Networks and Functional Enrichment Analyses for Any Sequenced Genome of Interest," *Nucleic Acids Research* 51, no. D1 (2022): D638–D646, https://doi.org/10.1093/nar/gkac1000.

22. K. Saleki, C. Aram, P. Alijanizadeh, et al., "Matrix Metalloproteinase/Fas Ligand (MMP/FasL) Interaction Dynamics in COVID-19: An In Silico Study and Neuroimmune Perspective," *Heliyon* 10 (2024): e30898, https://doi.org/10.1016/j.heliyon.2024.e30898.

23. L. Cantuti-Castelvetri, R. Ojha, L. D. Pedro, et al., "Neuropilin-1 Facilitates SARS-CoV-2 Cell Entry and Provides a Possible Pathway Into the Central Nervous System," preprint, BioRxiv, 2020.

24. J. Davies, H. S. Randeva, K. Chatha, et al., "Neuropilin-1 as a New Potential SARS-CoV2 Infection Mediator Implicated in the Neurologic Features and Central Nervous System Involvement of COVID-19," *Molecular Medicine Reports* 22, no. 5 (2020): 4221–4226, https://doi.org/10.3892/mmr.2020.11510.

25. S. A. El Kazafy, Y. M. Fouad, A. F. Said, et al., "Correlations Between Cytokine Levels, Liver Function Markers, and Neuropilin-1 Expression in Patients With COVID-19," *Vaccines* 10, no. 10 (2022): 1636, https://doi.org/10.3390/vaccines10101636.

26. M. S. S. Hasan, H. A. Ali, R. A. Ali, et al., "Evaluation of Serum Neuropilin-1 Level as a Potential Marker of COVID-19 Severity in Iraqi Population," *HIV Nursing* 23, no. 3 (2023): 1311–1317, https://doi.org/1031838/hiv23.03.179.

27. O. S. Zamzami, A. F. Kabli, A. S. Alhothali, et al., "Post-COVID-19 Vaccine Parosmia: A Case Report," *Cureus* 13, no. 12 (2021): 20292, https://doi.org/10.7759/cureus.20292.

28. K. Ohla, M. G. Veldhuizen, T. Green, et al., "Increasing Incidence of Parosmia and Phantosmia in Patients Recovering From COVID-19 Smell Loss," preprint, MedRxiv, 2021, https://doi.org/10.1101/2021.08.28.21262763.

29. S. Hu, Z. Hu, J. Qin, C. Lin, and X. Jiang, "In Silico Analysis Identifies Neuropilin-1 as a Potential Therapeutic Target for SARS-Cov-2 Infected Lung Cancer Patients," *Aging* 13, no. 12 (2021): 15770–15784, https://doi.org/10.18632/aging.203159.

30. A. Norouzi, M. Liaghat, M. Bakhtiyari, et al., "The Potential Role of COVID-19 in Progression, Chemo-Resistance, and Tumor Recurrence of Oral Squamous Cell Carcinoma (OSCC)," *Oral Oncology* 144 (2023): 106483, https://doi.org/10.1016/j.oraloncology.2023.106483.

31. Y. Chen, Q. Liu, and D. Guo, "Emerging Coronaviruses: Genome Structure, Replication, and Pathogenesis," *Journal of Medical Virology* 92, no. 4 (2020): 418–423, https://doi.org/10.1002/jmv.25681.

32. M. Heidarvand, R. Hosseini, M. Kazemi, et al., "Differentially Expressed Inflammatory Cell Death-Related Genes and the Serum Levels of IL-6 Are Determinants for Severity of Coronaviruses Diseases-2019 (COVID-19)," *Advanced Biomedical Research* 12, no. 1 (2023): 102, https://doi.org/10.4103/abr.abr_232_22.

33. A. H. Lashin, et al., "Value of Interleukin 6 in Assessment of the Disease Severity in Patients With COVID-19 Infection Without Pre-existing Comorbidities," *Benha Medical Journal* 40: 74–86, https://doi.org/10.21608/bmfj.2023.185224.1738.

34. T. Liu, J. Zhang, Y. Yang, et al., "The Role of Interleukin-6 in Monitoring Severe Case of Coronavirus Disease 2019," *EMBO Molecular Medicine* 12, no. 7 (2020): e12421, https://doi.org/10.15252/emmm.202012421.

35. A. K. Pradhan, et al., "Correlation Between Inflammatory Markers With Disease Severity of COVID 19 Infection," *International Journal of Pharmaceutical and Clinical Research* 15, no. 7 (2023): 653–660.

36. R. Prasad, M. Awasthi, S. Kag, and S. Sethia, "Interleukin-6 Evaluation as a Biomarker for Disease Severity and Mortality in Covid 19 Patients," *Trends in Clinical and Medical Sciences* 3 (2023): 50–55.

37. S. Urazov, A. Chernov, O. Popov, et al., "Secretory Phospholipase A2 and Interleukin-6 Levels as Predictive Markers of the Severity and Outcome of Patients With COVID-19 Infections," *International Journal of Molecular Sciences* 24, no. 6 (2023): 5540, https://doi.org/10.3390/ijms24065540.

38. Y. Chang, M. Bai, and Q. You, "Associations Between Serum Interleukins (IL-1β, IL-2, IL-4, IL-6, IL-8, and IL-10) and Disease Severity of COVID-19: A Systematic Review and Meta-Analysis," *BioMed Research International* 2022 (2022): 2755246, https://doi.org/10.1155/2022/2755246.

39. B. Diao, C. Wang, Y. Tan, et al., "Reduction and Functional Exhaustion of T Cells in Patients With Coronavirus Disease 2019 (COVID-19)," *Frontiers in Immunology* 11 (2020): 827, https://doi.org/10.3389/fimmu.2020.00827.

40. N. Keshar, S. Kaddah, S. Selim, et al., "Study of Serum Level of Interleukin-6 in COVID 19 Patients: Prognostic Value and Its Relation to Disease Progression," *European Chemical Bulletin* 12 (2023): 3722–3731.

41. G. M. Setyo Nugroho, I. A. Marhana, E. H. Kusumastuti, et al., "Interleukin-6 (IL-6) Expression of Lung Tissue in COVID-19 Patient Severity Through Core Biopsy Post Mortem," *Annals of Medicine and Surgery* 82 (2022): 104648, https://doi.org/10.1016/j.amsu.2022.104648.

42. M. Malekpour, S. Khanmohammadi, M. Meybodi, et al., "COVID-19 as a Trigger of Guillain–Barré Syndrome: A Review of the Molecular Mechanism," *Immunity, Inflammation and Disease* 11, no. 5 (2023): e875, https://doi.org/10.1002/iid3.875.

43. A. Dotan, S. Muller, D. Kanduc, P. David, G. Halpert, and Y. Shoenfeld, "The SARS-CoV-2 as an Instrumental Trigger of Autoimmunity," *Autoimmunity Reviews* 20, no. 4 (2021): 102792, https://doi.org/10.1016/j.autrev.2021.102792.

44. Q. Ruan, K. Yang, W. Wang, L. Jiang, and J. Song, "Clinical Predictors of Mortality due to COVID-19 Based on an Analysis of Data of 150 Patients From Wuhan, China," *Intensive Care Medicine* 46, no. 5 (2020): 846–848, https://doi.org/10.1007/s00134-020-05991-x.

45. G. Toscano, F. Palmerini, S. Ravaglia, et al., "Guillain–Barré Syndrome Associated With Sars-Cov-2," *New England Journal of Medicine* 382, no. 26 (2020): 2574–2576, https://doi.org/10.1056/NEJMc2009191.

46. M. Mateu-Salat, E. Urgell, and A. Chico, "SARS-COV-2 as a Trigger for Autoimmune Disease: Report of Two Cases of Graves' Disease After COVID-19," *Journal of Endocrinological Investigation* 43 (2020): 1527–1528, https://doi.org/10.1007/s40618-020-01366-7.

47. C. Gutiérrez-Ortiz, A. Méndez-Guerrero, S. Rodrigo-Rey, et al., "Miller Fisher Syndrome and Polyneuritis Cranialis in COVID-19," *Neurology* 95, no. 5 (2020): e601–e605, https://doi.org/10.1212/WNL.0000000000009619.

48. M. J. McConnell, N. Kawaguchi, R. Kondo, et al., "Liver Injury in COVID-19 and IL-6 Trans-Signaling-Induced Endotheliopathy," *Journal of Hepatology* 75, no. 3 (2021): 647–658, https://doi.org/10.1016/j.jhep.2021.04.050.