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Evaluation of the Genes and Molecular Pathways Involved in Skin Lesions in Patients with COVID-19: Systems Biology and Bioinformatics Analysis Approach

Motahareh Babazadeh[1], Reza Maddah[2], Sana Delavari[3], Mahya Razmi[4], Arsalan Jalili[5,6], Maryam Bahadorzadeh[7] and Masoumeh Rohaninasab[8*]

[1]Assistant Profesor, Department of Dermatology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2]Department of Bioprocess Engineering, Institute of Industrial and Environmental Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
[3]Ahvaz jundishapur University of Medical Science, Ahvaz, Iran
[4]Student Research Committee, Faculty of Paramedical Sciences, Mashhad University of Medical Sciences, Mashhad, Iran
[5]Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACER, Tehran, Iran.
[6]Parvaz Research Ideas Supporter Institute, Tehran, Iran
[7]Department of Internal Medicine, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[8]Department of Dermatology, Rasool Akram Medical Complex, Iran University of Medical Sciences, Sattarkhan St., Tehran, Iran

*** *Corresponding author:*** Masoumeh Rohaninasab, Department of Dermatology, Rasool Akram Medical Complex, Iran University of Medical Sciences, Tehran, Iran. masoomehrohani@yahoo.com.

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Abstract

Background: Coronavirus disease 2019 (COVID-19) was first identified in 2019 in Wuhan, China. Initially, although the number of COVID-19-infected individuals was very low, the infected cases increased as the virus spread worldwide. Skin manifestation is one of the symptoms observed in COVID-19 patients.

Objectives: This study investigated the critical genes and molecular pathways involved in skin manifestations in COVID-19 patients through a biological system approach.

Methods: In this study, the microarray dataset was downloaded from the Gene Expression Omnibus (GEO) database and analyzed for identifying differentially expressed genes (DEGs). The enrichment analysis of DEGs was evaluated using the DAVID database. Afterward, protein-protein interaction (PPI) networks were constructed via the STRING database and visualized using Cytoscape software. The hub genes were recognized using the cytoHubba. The interaction of the microRNA (miRNA)-hub genes, transcription factor (TF)-hub genes, and drug-hub genes was also evaluated in this study.

Results: After analysis, some genes with the highest degree of connectivity, which were involved in the pathogenesis of HELLP syndrome were identified, and they were known as hub genes. These genes are as follows: IFN-γ, CXCL1, CCL2, CCL3, TLR2, IL-1B, CXCL6, IL-6, CCL4, and CXCL2. has-mir-34a-5p, has-mir-20a-5p, and has-mir-27a-3p as miRNA, as well as RELA as TF had the most interaction with the hub genes.

Conclusion: Finally, IL-6 and CXCL10 that were compared to the other hub genes had the highest interaction with other genes; therefore, their role in Shamgir's pathogenesis is significant. Targeting the cited genes would be a strategy to prevent symptom manifestation and better patient management.

Keywords: Bioinformatics analysis, COVID-19, Molecular pathway, Skin lesion, System biology

1. Background

Coronavirus disease 2019 (COVID-19) was first identified in 2019 in Wuhan, China. Initially, the number of people infected with COVID-19 was very low; however, the number of infected individuals increased as the virus spread worldwide (1-3). At first, little information was available about the biology of the virus and its symptoms in patients; nonetheless, with the spread of the disease, the methods of virus identification and also obtainment of the necessary information about its symptoms are increasing (4, 5).

Clinical symptoms associated with the virus initially included cough, sneezing, runny nose, and headache (6, 7). However, depending on the patient's underlying disease and the strength of the immune system, some of the symptoms would be different in the patients (8, 9). Based on the studies and evidence, the stimulation of the immune system in COVID-19-infected patients can eventually lead to a cytokine storm (10). The inflammation caused by cytokine storms can damage the main organs in the patient's body (11). One of the damaged organs is the skin. According to previous documents, 80% of skin manifestations turn back to inflammatory reactions caused by COVID-19. These symptoms are mainly manifested in the form of erythema, pruritus, and vesicles in most of the patients (12-15).

Studies have shown that the occurrence of symptoms in patients is age dependent. It has been shown that Chilblains-like lesions occur in young patients and are mostly observed in feet. This lesion occurs by INF-I-mediated inflammatory response. The vascular lesions are more common in adults and usually is observed on the arms and trunks. Co-infection with Herpesviridae causes vascular lesions (16-19). In addition, it has been shown that the treatment of patients can also be associated with skin

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manifestation, the same as treatment with corticosteroids that can cause some symptoms, such as petechiae and purpura (16, 20, 21). Furthermore, the results showed that urticaria occurs mainly in adults and is seen in the trunk and face areas. The use of drugs to treat systemic disease can lead to urticaria. Acute generalized exanthematous pustulosis (AGEP) and erythema multiforme (EM) are also among the symptoms observed in COVID-19 patients treated with hydroxychloroquine (17, 22, 23).

System biology is an approach that has been used recently in many diseases. In this approach, interactions among genes, proteins, and molecular pathways are evaluated. On the other hand, their role as diagnostic and prognostic factors in the pathogenesis of diseases is investigated. In addition, System biology identifies Micro RNAs (miRs) and Transcription Factors (TF) involved in pathogenesis. Their interaction with the hub genes is also evaluated (24-27).

2. Objectives

Despite the report of skin manifestations in COVID-19 patients, the factors involved in their pathogenesis have not been well-investigated. Accordingly, this study aimed to investigate the genes and molecular pathways involved in skin manifestations in COVID-19 patients through a biological system approach.

3. Methods

3.1. Data selection and DEGs analysis

The GSE193068 microarray dataset was used in the current study. These pieces of data were selected from Gene Expression Omnibus (GEO) (http://www.ncbi nlm.nih.gov/geo/) for analysis, representing a GPL31171 Gene Expression nCounter® RCC-Human Immunology V2 panel. Since the studies concerning COVID-19 and skin lesions due to the unknown nature of this virus are few, the number of datasets in databases is limited. All available databases were explored for selecting the most appropriate dataset, and the dataset used in this study was fully compatible with the criteria. For example, merely datasets with a minimum of three biological replicates in the case and control groups were included and others were excluded, or just datasets with food quality samples were included and others were discarded. Finally, the studied groups included COVID-19 patients with skin lesions (n=10) and healthy donors (n=4). Differentially Expressed Genes (DEGs), including up- and down-regulated genes, were determined using the GEO2R online tool. A schematic view of the study process is shown in Figure 1. Genes with log fold change (FC)| > 1.0 and P-value < 0.05 were considered DEGs.

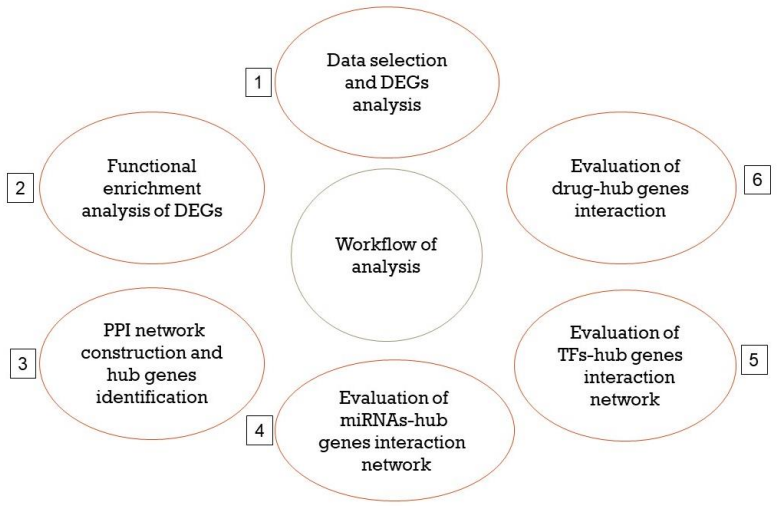

Figure 1. Workflow of analysis

3.2. Functional enrichment analysis of DEGs

COVID-19 patients and healthy donors were evaluated in terms of the signaling pathways and Gene Ontology (GO) via DAVID tools. The GO analysis was used for the evaluation of the molecular function (MF), biological process, and cellular component (CC) of the genes. The Kyoto Encyclopedia of Genes and Genomes (KEGG) and REACTOME pathways were also used for the evaluation of molecular pathways. The P-value <0.05 was considered statistically significant.

3.3. Evaluation of protein-protein interaction and hub genes

STRING website (https://string-db.org/) was used to design the protein-protein interaction (PPI) network. Cytoscape software was also used to visualize the network between the proteins. According to the degree method and using the Cytohubba plugin in Cytoscape, 10 genes were selected as the hub genes.

3.4. Evaluation of the interactions between miRNAs and hub genes

The miRNet database was used to evaluate and detect microRNAs (miRNAs) targeting hub genes. The Cytoscape tool was also employed to view and design the interaction network between miRNAs and the hub genes.

3.5. Evaluation of the interactions between transcription factors and the hub genes

The transcription factors (TFs) that interact with the hub genes were studied. The Network analyst database was used to investigate the interactions between TFs and hub genes. JASPAR, ENCODE, and ChEA databases are three available resources for transcription factor profiles in the Network analyst. The ChEA database was also used to find related TFs to hub genes.

3.6. Evaluation of the interactions between drugs and hub genes

In this section, targeted hub genes by drugs were evaluated. Drugs were selected from the drug-gene interaction database (DGIdb). The selected drugs were approved by the Food and Drug Administration (FDA).

4. Results

4.1. Evaluation of DEGs

In this study, 117 DEGs were identified in COVID-19-associated skin lesions, compared to healthy individuals. The results showed that 62 genes were up- and 55 were down-regulated. All DEGs were visualized using a Volcano plot (Figure.2). The list of DEGs (up- and down-regulated genes) is inserted in Supplementary.1.

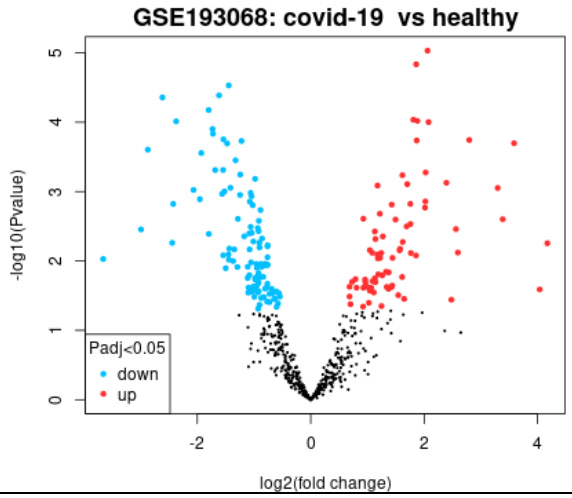

Figure 2. Volcano plot of DEGs: black dots indicate insignificant genes, red dots indicate up-regulated genes, and blue dots indicate down-regulated genes

4.2. Functional enrichment analysis

Functional enrichment analysis of DEGs was assessed using the DAVID database. According to the results of GO analysis, in BP terms (Figure.3), DEGs were mainly enriched in response to external stimulus (GO: 0009605), positive regulation of immune system process (GO: 0002684), and positive cellular response to cytokine stimulus (GO: 0071345). MF terms (Figure.3) were mostly enriched in CCR chemokine receptor binding (GO: 0048020), signaling receptor activity (GO: 0038023), and signal transducer activity (GO: 0004871). Furthermore, CC terms (Figure.3) were predominantly enriched in secretory granules (GO: 0030141), extracellular region part (GO: 0044421), and an intrinsic component of the plasma membrane (GO: 0031226). Finally, KEGG pathway analysis showed that the most involved pathway concerning DEGs is cytokine-cytokine receptor interaction. In addition, REACTOME pathway analysis displayed that the most involved pathway concerning DEGs is the immune system. Biological pathways are shown in Figure.4.

4.3. Evaluation of the PPI network and identification of the hub genes

The PPI network of DEGs was assessed by the STRING website, and it was visualized by Cytoscape software. This network consisted of 95 nodes and 327 edges. The top 10 genes were identified as the hub genes based on the degree connectively. These 10 hub genes included IFN-γ, CXCL1, CCL2, CCL3, TLR2, IL-1B, CXCL6, IL-6, CCL4, and CXCL2.

4.4. Evaluation of the interactions of candidate miRNAs with the hub genes

The miRNAs targeting the hub genes were predicted using miRNet software, and their connections were visualized by Cytoscape. Among evaluated miRNAs, the has-mir-34a-5p, has-mir-20a-5p, and has-mir-27a-3p had the most interaction with the hub genes (Figure.5).

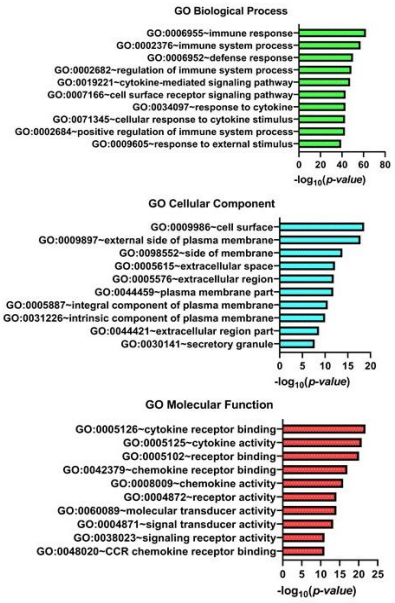

Figure 3. Gene ontology enrichment analysis of DEGs, including Biological Process, Cellular Component, and Molecular Function

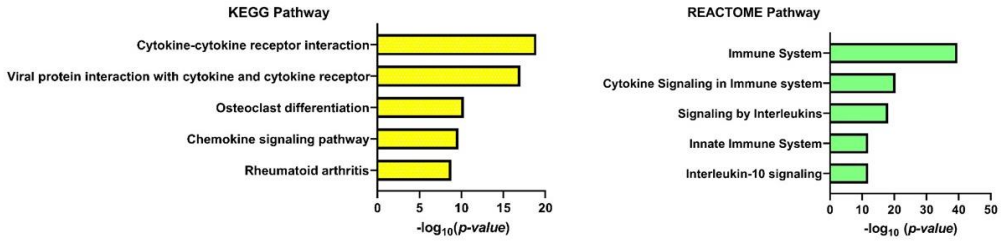

Figure 4. KEGG and REACTOME pathways enrichment analysis of DEGs

Figure 5. The network of miRNA-hub genes interaction. Red rectangles represent the hub genes while green rectangles represent miRNAs targeting the hub genes

4.5. Evaluation of the interactions of candidate TF with the hub genes

The evaluation revealed that some TFs were associated with the hub genes. Further investigation demonstrated that the RELA factor, which interacted with nine hub genes can be considered an essential TF (Figure.6).

4.6. Evaluation of the interaction between drugs and hub genes

Overall, 73 detected drugs by DGIdb were identified as potential drugs, which could have

therapeutic effects. Further investigation revealed that eight hub genes (CXCL10, IL-6, CXCL2, CCL3, CCL4, IFN-γ, CCL2, and IL-1B) could be targeted by FDA-approved drugs (Figure.7) (Supplementary.2).

Figure 6. The network of TF-hub genes interaction. Red rectangles represent the hub genes while green rectangles represent TFs targeting the hub genes

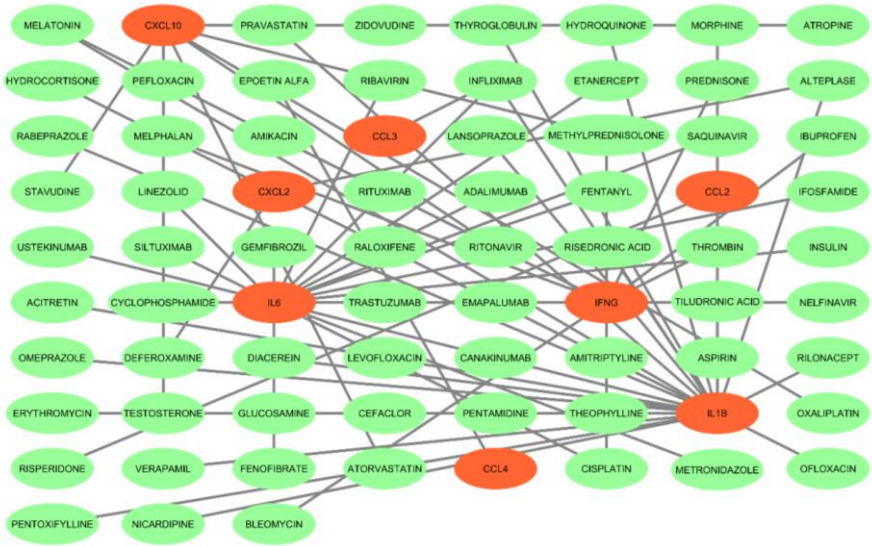

Figure 7. The network of drug-hub genes interaction. Red ovals represent the hub genes while the green ovals represent drugs targeting the hub genes

5. Discussion

The results of the present study demonstrated that the inflammatory reactions caused by a cytokine storm in COVID-19 patients can cause skin manifestations. For this purpose, it was found that the hub genes including IFN-γ, CXCL1, CCL2, CCL3, TLR2, IL-1B, CXCL6, IL-6, CCL4, and CXCL2 play an important role in the pathogenesis of skin manifestation. It was also found that has-mir-20a-5p, has-mir-27a-3p, and RELA transcription factors are related to the hub genes and affect disease exacerbation. Skin manifestations caused by COVID-19 infection have attracted much attention since differentiating skin manifestations caused by COVID-19 and drug treatments can be difficult due to overlapped symptoms (17, 28).

So far, most of the studies have investigated the prevalence of skin manifestations and also related drugs that cause similar symptoms. However, the study of gene expression changes that cause skin manifestation due to infection with COVID-19 has not been conducted (20, 29, 30). Investigating the expression/function of each gene related to skin manifestations would provide a better prognosis and therapy for drug targets and pharmaceutical discoveries. The results of the current study showed that the expression of some genes related to

inflammatory mediators, such as chemokines, interferons, Toll-Like Receptors (TLRs), and cytokines, increased in COVID-19 patients, which lead to skin lesions.

IL-1 is one of the inflammatory cytokines. Its receptor (IL-1R) together with TLRs produces inflammatory mediators in response to COVID-19. In addition, they activate macrophages, which have TLR2. The interaction of COVID-19 with TLR2 causes the production of IL-1β by macrophages (31) leading to IL-1β production and stimulation of immune cells, including phagocytes. The produced IL-6, IL-1β, and IL-6 lead to cytokine storm and skin manifestation in patients (32, 33).

Recent findings suggest that PI3-kinase/AKT is involved in cellular responses to IL-1 and subsequent activation of NF-κB and AP-1. It is also known that the kinase TAK1 (TGFβ-activated kinase) pathway is activated by IL-1 (34). TAK1 activates the NIK-IκB-NFκB pathway by phosphorylating the IKKα on the NFκB or MAP kinase cascade (35). Stimulation of the MAP kinase (MAPK) cascade is caused by the MKK4/7 to JNK/AP-1 activation and the MKK3/6 to p38 activation (36). Moreover, PI3-kinase/AKT can mediate the transactivation of the p65 (RelA) and p50 subunits of NFκB or activates the IKKαT23A/AP-1 pathway (37). Activation of NF-kB and AP-1 causes more production of IL-1 and IL-6, as well as other inflammatory mediators, which aggravate skin lesions in patients.

According to our result, has-mir-34a-5p, has-mir-20a-5p, and has-mir-27a-3p had the most interaction with skin manifestations, which are involved in chemokine secretions. Moreover, these molecules can affect the skin manifestations by impressing the downstream pathways, such as NF-kB. Wu et al. reported the pathological role of the mir-34 family in venous ulcers, the most common type of human chronic non-healing wounds, by targeting LGR4. This axis can further alter the activity of the NF-κB signaling pathway (38).

Decreased expression of hsa-miR-20a-5p in peripheral blood mononuclear cells was observed in patients with COVID-19 (39). mir20a alteration has been reported in association with different skin lesions. Higher expression of hsa-miR-20a-5p has been reported in peripheral blood mononuclear cells of progressive and stable non-segmental vitiligo patients, compared to the healthy controls (40). In another study by Chang et al., mir-20a-5p activates several pathways, which resulted in the down-regulated secretion of IL-17 via CD4+ T cells of patients with Vogt-Koyanagi-Harada disease (41). However, Valizadeh et al. reported down-regulation of mir20a, as one of the important TGF-b-associated miRNAs, in sulfur mustard-exposed skin lesions (42). has-mir-27a-3p role is not clear, but it might have important roles in vitiligo and cutaneous melanoma pathology (43, 44). Therefore, evaluation of the expression changes of has-mir-20a-5p and has-mir-27a-3p in COVID-19 patients can be effective in monitoring patients in order to prevent the progression of skin lesions.

The current study showed that CXCL1, CCL2, CCL3, CXCL6, CCL4, and CXCL2 chemokines play important roles in the occurrence of skin manifestation. In the study by Zhang et al., it was found that increased production of CCL3 and CCL4 in COVID-19 patients caused chronic urticarial (45). Moreover, in the study of Rybkina et al., CCL3 and CCL4 produced by cytokine storms cause the progression of skin lesions (46).

CC and CXC chemokines are involved in the skin manifestations of SARS-CoV-2, and they are secreted by epithelial cells and innate immune cells (47). Some chemokines are involved in the pulmonary pathogenesis of Coronaviruses. These chemokines are generally expressed in response to different cytokines. Cytokines, such as IL-1 and TNF-α, can induce the expression of chemokines, including CCL3 and CCL4, in response to viral infections. CCL3 and CCL4 promote NK cells to kill virally-infected cells and release IFN-γ. NK cell role is still under investigation; however, it has been assumed that in the early stage of virus invasion, they may play a role in inflammatory response induction.

In addition, CXCL1, CXCL2, and CXCL10 largely contribute to COVID-19 pathogenesis. CXCL10 or IFN-γ-inducible protein 10 is one of the players in the anti-viral responses, especially respiratory tract infections. It elevates the plasma and bronchial alveolar lavage fluid of patients with severe conditions (48, 49). In the study of Utami et al., it was shown that increased production of CXCL1, CXCL2, and CXCL10 in COVID-19 patients can lead to skin lesions and exacerbation of patients' clinical symptoms (50). Additionally, in the study of Carnevale et al., the production of mentioned chemokines can stimulate immune cells and cause skin symptoms in patients (51). Resident macrophages and/or epithelial cells of the lungs produce and secrete CXCL1 and CXCL2 into the bloodstream. These chemokines activate and recruit neutrophils to the infection site (52). Excessive recruitment of neutrophils to the lung prompts the formation of neutrophil extracellular traps (NETs), which is associated with disease severity (53, 54).

The local mediator, histamine, is associated with allergic reactions to COVID-19. However, the role of histamine receptor subtypes still needs to be further investigated. A handful of studies have suggested the role of Histamine 2 Receptor (H2R) in SARS-CoV-2 infection (55). H2R is expressed by different cells including epithelial, endothelial, and immune cells. H2R loss affects the invariant natural killer T (iNKT) cells in a murine lung inflammation model aggravating local inflammation (56). Moreover, H2R can inhibit the stimulation of IL-10 by affecting

different cytokines and chemokines, such as CXCL10, IL-12, and TNF-α. It may lead to Th2 polarization and virus invasions or activation (57). H2R antagonists could be helpful as therapeutic agents in COVID-19 management, as they are known to have immunomodulatory activities (55).

According to our result, H4R may also play a critical role in cytokine storms. TNF-α, IL-6, IL-10, and IL-13 regulate the expression of H4R. H4R inhibits cAMP accumulation, which increases the production of pro-inflammatory factors and decreases anti-inflammatory factors in immune cells (58). Furthermore, H4R activates the MAPK cascade. Activation of H4R results in the accumulation of inflammatory cells, such as mast cells and eosinophils, as well as subsequent inflammatory conditions. H4R antagonists have been proposed as a potential target for COVID-19. This therapeutic approach may prevent lung fibrosis and inflammatory responses caused by TNF-α and IL-6 (59).

5.1. Limitation

In order to confirm the results of our study, it would be better to conduct laboratory studies. Moreover, examining the response of patients to treatment based on each drug and their relationship with the occurrence of symptoms needs to be investigated in future studies.

6. Conclusion

Finally, it seems that IL-6 and CXCL10 had the highest interaction with other genes, compared to other hub genes. Therefore, their role in Shamgir's pathogenesis is significant, and targeting them can play an important role in preventing symptoms and better patient management.

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Footnotes

Conflicts of Interest: The authors declare that they have no conflict of interest.
Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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