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Expression profiling of inflammation-related genes including IFI-16, NOTCH2, CXCL8, THBS1 in COVID-19 patients

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ABSTRACT

The present study aimed to scrutinize the expression profile of inflammatory-related genes (IFI-16, NOTCH2, CXCL8, and THBS1) from acute to post-acute stage of this infectious epidemic. The current cross-sectional study consisted of 53 acute-phase COVID-19 patients and 53 healthy individuals between February and March 2021. The extraction of total RNA was performed from PBMC specimens and also expression level of selected genes (IFI-16, NOTCH2, CXCL8, and THBS1) was evaluated by real-time PCR. Subsequently, levels of these factors were re-measured six weeks after the acute phase to determine if the levels of chosen genes returned to normal after the acute phase of COVID-19. Receiver operating characteristic (ROC) curve was plotted to test potential of genes as a diagnostic biomarker. The expression levels of inflammatory-related genes were significantly different between healthy and COVID-19 subjects. Besides, a significant higher CXCL8 level was found in the acute-phase COVID-19 compared to post-acute-phase infection which may be able to be considered as a potential biomarker for distinguishing between the acute phases from the post-acute-phase status. Deregulation of the inflammatory-related genes in COVID-19 patients, especially CXCL-8, can be serving as potent biomarkers to manage the COVID-19 infection.

1. Introduction

A novel coronavirus, called SARS-CoV-2 or severe acute respiratory syndrome coronavirus 2, develops an acute respiratory disease known as coronavirus disease-2019 (COVID-19) that is a highly transmittable and pathogenic infection. In December of 2019, the outbreak of COVID-19 disease occurred in Wuhan, China. SARS-CoV-2 is the positive-strand RNA viruses (+ssRNA viruses) that is a member of the *beta Coronavirus*. Clinically, COVID-19 disease is classified into mild, moderate, and severe [1,2]. The COVID-19 symptoms include cough, loss of taste, loss of smell, respiratory problems, fever and abnormal immune responses and can lead to organ dysfunction, and also lung, kidney, and liver damage, as well as acute respiratory distress syndrome (ARDS), with a high rate of death [3].

By binding of this virus to angiotensin-converting enzyme 2 (ACE2) located on the various cell surfaces, it enters cell and after releasing its genome and replicating, the virus can infect the host cells and a large number of viruses are replicated and released by the respiratory tract, sputum, and nasal fluid. In the host body, viral infections confront immune responses. Pattern recognition receptors (PRRs) can detect viruses and also activate the inflammatory signaling pathway, as well as lead to the production of proinflammatory cytokines [3].

Inflammation is a defense response of the body that occurs following tissue damage, scratches, burns, and microbiological infections

Abbreviations: **SARS-CoV-2**, severe acute respiratory syndrome coronavirus 2; **COVID-19**, Coronavirus disease of 2019; **IFI16**, Interferon-γ-inducible protein 16; **NOTCH2**, Notch gene homolog 2; **CXCL8**, C-X-C motif ligand 8; **THBS1**, Thrombospondin 1.

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Table 1
Primers used in this research.

Real-time PCR using SYBR-Green I fluorescence	Direction	Name	Sequences	Size/bp
Real Time PCR for IFI16[a]	**Forward Primer**	**IFI16-F**	**F: 5′-GAAGTGCCAGCGTAACTCCTA-3′**	**80/bp**
	Reverse Primer	**IFI16-R**	**R: 5′-TACCTCAAACACCCCATTCAC-3′**	
Real Time PCR for NOTCH2[b]	**Forward Primer**	**NOTCH2-F**	**F: 5′-TCAACTGCCAAGCGGATGT-3′**	**191/bp**
	Reverse Primer	**NOTCH2-R**	**R: 5′-CTTGGCTGCTTCATAGCTCC-3′**	
Real Time PCR for THBS1[c]	**Forward Primer**	**THBS1-F**	**F: 5′-TTGTCTTTGGAACCACACCA-3′**	**187/bp**
	Reverse Primer	**THBS1-R**	**R: 5′-CTGGACAGCTCATCACAGG-3′**	
Real Time PCR for CXCL8[d]	**Forward Primer**	**CXCL8-F**	**F: 5'-ACTGAGAGTGATTGAGAGTGGAC-3'**	**112/bp**
	Reverse Primer	**CXCL8-R**	**R: 5'-AACCCTCTGCACCCAGTTTTC-3'**	
Real Time PCR for GAPDH[e]	**Forward Primer**	**GAPDH-F**	**F: 5′-CGACCACTTTGTCAAGCTCA-3'**	**163/bp**
	Reverse Primer	**GAPDH-R**	**F: 5′-CCCTGTTGCTGTAGCCAAAT-3'**	
Real Time PCR for β-actin	**Forward Primer**	**β-actin-F**	**5′- GTGGCCGAGGACTTTGATTG-3'**	**73/bp**
	Reverse Primer	**β-actin-R**	**5′- CCTGTAACAACGCATCTCATATT-3'**	

[a] . IFI16: Gamma-interferon-inducible protein 16.
[b] . NOTCH2: Neurogenic locus notch homolog protein 2.
[c] . THBS1: Thrombospondin 1.
[d] . CXCL8: C-X-C Motif Chemokine Ligand 8.
[e] . GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

(bacteria, viruses, fungi) and causes swelling, loss of function, pain, warmth, and redness of the affected area [4]. Inflammation as a two-edged response is substantial for the defense system of host. It has also been elucidated that inflammatory products cause a spectrum of destructive effects [5]. In severe form of COVID-19 disease, inflammation-mediated damage and cytokine storm were observed, that is leading to multiorgan failure [6]. In the patients suffering from COVID-19 when the production of proinflammatory cytokines increased, cytokine storm occurred, and an abnormal increase in pathological neutrophils cause tissue damage [7]. The severity of the disease depends on the cytokine storm and increased levels of inflammatory mediators such as cytokines and inflammatory chemokines, which might be induced via TNF-α and IL-6, and IL-1β also regulate the NF-κB signaling pathway [8].

All the same, the interaction between inflammatory signaling pathways and viral infections has been emphasized in previous studies but is still shrouded in mystery. Examining the details of signal pathways related to virus-induced inflammation can be effective to discover new targets in detection of virus infections, prevention and/or treatment. The interferon-16 inducible protein (IFI16) stimulates interferon expression and can stimulate the inflammatory process. This protein is a leading actor during virus-induced inflammation [9]. Notch2 is a coding gene associated with innate immunity and inflammation. Notch2 has been shown to be active in inflammation, including rheumatoid arthritis, bacterial and viral infections [10]. Interleukin 8 (chemokine (C-X-C motif) ligand 8, CXCL8 or IL-8) is an inflammatory chemokine which secreted by a variety of cells such as blood monocytes, alveolar macrophages, fibroblasts, and epithelial cells in viral infections e.g., COVID-19. The CXCL8 expression is stimulated by various cytokines like CXCL12, TNF-α, IL-6 and IL-1 [11]. Thrombospondin 1 (THBS1) is a glycoprotein that is released and increased during the acute phase of inflammation, and plays a synergistic role in the inflammatory process; TSP-1 is known as a pro-inflammatory factor [12].

We assumed that COVID-19 would alter the expression pattern of genes involved in inflammation, especially IFI-16, NOTCH2, CXCL8 and THBS1, following acute or post-acute infectious stages. Likewise, maybe employed as diagnostic biomarkers and therapeutic for this infection. Accordingly, the of the current study aimed to profile the expression pattern of inflammation-related genes (IFI-16, NOTCH2, CXCL8, and THBS1) following acute or post-acute infectious stages in comparison with healthy subjects as control. Also, we investigated the correlation of expression levels of these genes with clinical characteristics, as well as nucleocapsid (N) gene and RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2.

2. Materials and methods

2.1. Study population

From February 2021 to May 2021, 53 COVID-19-infected individuals admitted to West health center in Tehran, Iran, were enrolled in the current cross-sectional work. These participants provided five ml of peripheral blood specimens two times (fist in acute phase and second in convalescence phase), and also from 53 healthy controls. It is noteworthy that none of the studied subjects had underlying medical conditions and any concurrent infection with mycobacterium tuberculosis, HIV, HCV, HBV, and CMV.

2.2. Ethical issues

The present research followed the ethical considerations approved by the committee of ethics (IR.IUMS.FMD.REC.1400.303) at Iran University of Medical Sciences (IUMS; Tehran; Iran), after obtaining informed written consent from research units.

2.3. Detection of SARS-CoV-2 genomic RNA

Viral RNA was isolated from oropharyngeal and nasopharyngeal swab samples of all study patients in two acute phases and recovery of COVID-19 disease using the Roche Diagnostics GmbH High-Pure Viral Nucleic Acid Kit (Mannheim, Germany) in accordance with the manufacturers' direction. To detect SARS-CoV-2 genomic RNA, COVID-19 (SARS-CoV-2) Nucleic Acid Test Kit (Sansure Biotech Medical laboratory company) was used in accordance with the Manufacturer's guidelines.

2.4. Separation of peripheral blood mononuclear cells

The separation of peripheral blood mononuclear cell (PBMC) samples was done based on the Ficoll Hypaque Density Gradient centrifugation (Lympholyte-H; CEDARLANE; HORNBY; Canada). This method is done in five stages including; 1) Peripheral blood sample (7 ml) was taken from each subject, 2) plasma of blood samples was separated by centrifugation; 3) PBMC of blood samples was isolated using Ficoll; 4) isolated PBMC specimens were rinsed thoroughly via phosphate-buffered saline (PBS, pH 7.2 to 7.4), and 5) separated PBMC samples were suspended again in 300 μl of RNALater (Ambion, Inc., Austin, TX) solution, as RNA preservative, and then maintained at a temperature of −80 °C for subsequent testing of RNA extraction.

Table 2
The demographic Parameters, clinical characteristics of research units.

Variables	Males	Females	Total	P. Value
Healthy controls individuals				
No%	28 (52.83%)	25 (47.17%)	53 (100.0%)	–
Age	36.8 ± 12.3 (23–65)	33.9 ± 9.6 (26–58)	35.4 ± 10.8 (23–65)	0.383 Student T Test
Covid-19-infected participants				
No%	30 (56.6%)	23 (43.4%)	53 (100.0%)	–
Age	39.5 ± 10.0 (28–55)	34.9 ± 13.1 (22–65)	37.2 ± 11.6 (22–65)	0.389 Student T Test
Clinical characteristics of Covid-19-infected participants				
Fever	18 (60.0%)	12 (52.17%)	30 (56.6%)	1.000 Fisher's Exact Test
Chills	15 (50.0%)	12 (52.17%)	27 (50.94%)	1.000 Fisher's Exact Test
Headache	15 (50.0%)	16 (69.56%)	31 (58.49%)	0.650 Fisher's Exact Test
Skeletal pain	21 (70.0%)	12 (52.17%)	33 (62.26%)	0.650 Fisher's Exact Test
Chest pain	3 (10.0%)	7 (30.43%)	10 (18.86%)	0.583 Fisher's Exact Test
Shortness of breath	6 (20.0%)	10 (43.47%)	16 (30.18%)	0.628 Fisher's Exact Test
Deceased smell	24 (80.0%)	18 (78.26%)	42 (79.24%)	1.000 Fisher's Exact Test
Deceased taste	24 (50.0%)	14 (60.86%)	38 (71.69%)	1.000 Fisher's Exact Test
Confusion	6 (20.0%)	7 (30.43%)	13 (24.52%)	1.000 Fisher's Exact Test
Dry cough	15 (50.0%)	10 (43.47%)	25 (47.16%)	1.000 Fisher's Exact Test
Sputum cough	3 (10.0%)	0 (00.0%)	3 (5.66%)	1.000 Fisher's Exact Test
Runny nose	6 (20.0%)	5 (21.7%)	9 (16.9%)	1.000 Fisher's Exact Test
Cape of nose	9 (30.0%)	7 (30.43%)	16 (30.1%)	1.000 Fisher's Exact Test
Bleeding stomach	0 (00.0%)	3 (13.04%)	3 (5.66%)	1.000 Fisher's Exact Test
Gastrointestinal symptom	18 (60.0%)	7 (30.43%)	25 (47.16%)	0.370 Fisher's Exact Test

2.5. Extraction of total RNA and analysis of gene expression

The miRNeasy Mini Kit (reference 217004, Qiagen, CA) was utilized to extract the total RNA from PBMC specimens in accordance with the kit instruction, followed by storage in the freezer at −20 °C until the test. The extracted RNA quantity and quality were determined by a Nano-Drop device (Thermo Fisher Scientific, Wilmington). To determine the level of IFI16، NOTCH2، CXCL8, THBS1, and also GAPDH (as normalization controls for relative quantification) genes expression, complementary DNA (cDNA) was synthesized, as mentioned earlier [13].

In a real-time polymerase chain reaction (RT-PCR), 20 μl of reaction mix contained 10 μl of SYBR® Premix Ex Taq™ (Tli Plus) Master Mix (TaKaRa Bio Inc. Shiga, Japan), 8 μl of nuclease-free water, 10 pmol of each primer for IFI16 [14], NOTCH2 [15], CXCL8 [16], THBS1 [17], GAPDH [18] and β-actin genes [19] (Table 1), and 1 μL of cDNA as template. The optimal RT-PCR method had the condition below: initial denaturing at 95 °C for 15 min, and subsequent 40-cycle amplification consisting of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 20 s. The results of this study were recorded at 72 °C.

2.6. Statistical methods

GraphPad Prism 6.0 and SPSS Version 16.0 (statistical Package for Social Sciences) software were used to carry out all statistical analyses. Fisher's test was used for the analysis of qualitative data. Independent *t*-test and Mann-Whitney tests was used to compare the expression profiles of selected mRNAs between COVID-19 groups in post-acute or acute phases and healthy individuals. Furthermore, expression level differences of selected mRNAs between the acute and post-acute phases of infection were analyzed using the paired *t*-test. The two-tailed P-value<0.05 was the significance level statistically. Further, we calculated the receiver-operator characteristic (ROC) curves for each mRNA to distinguishing three groups. Spearman correlation coefficients were calculated for determination of correlation between mRNA expression levels with demographic-clinical characteristics and SARS-CoV-2 genes in the acute-phase COVID-19 subjects. Also, false discovery rate was controlled applying Benjamini & Hochberg step-up procedure.

3. Results

3.1. Characteristics of the participants

The current cross-sectional study consisted of 53 acute-phase COVID-19 patients and 53 healthy individuals between February and May 2021. The mean age of patients was 37.2 ± 11.6 (between 22 and 65 years), and the mean age of the healthy controls was 35.4 ± 10.8 (between 23 and 65 years). Table 2 shows clinical and demographic profiles of research units.

3.2. The level of selected mRNAs among acute COVID-19 subjects is higher than that among healthy ones

In Fig. 1, the PBMC level of CXCL-8, NOTCH2, IFI16, and THBS-1 between the groups studied are presented. According to the results, CXCL-8 exhibited the largest expression difference among four mRNAs measured. The expression level of the CXCL-8 was significantly over-expressed in the acute COVID-19 (fold change (FC) = 4.03, $p < 0.0001$) when comparing to healthy subjects. In addition, the expression level of THBS1, NOTCH2 and IFI6 showed a significant elevation among acute COVID-19 subjects when comparing with healthy subjects (FC = 2.02, $p = 0.002$; FC = 2.28, $p = 0.002$; FC = 1.64, $p < 0.001$, respectively). In comparing the expression level of the mRNAs assayed between healthy and post-acute COVID-19 subjects, we found that the expression level of NOTCH2 and IFI16 was significantly overexpressed while no significant difference was seen in the expression level of CXCL-8 and THBS-1 in these two groups (P-value > 0.05, Fig. 1).

The correlation test detected the correlation of expression levels of mRNAs (CXCL-8, NOTCH2, IFI16, and THBS-1) with demographic-clinical characteristics and SARS-CoV-2 genes (RdRP and N) among acute COVID-19 subjects (Table 3). According to the result, there was no significant correlation among the delta Ct of selected mRNAs and delta Ct of N genes and RdRP of the study virus. However, there was significant linear correlation between fold change of CXCL-8 with Fever ($r = 0.81$, $p < 0.0001$), between fold change of NOTCH2 and THBS with Dry cough ($r = 0.72$; $p < 0.001$; $r = 0.63$; $p = 0.002$; sequentially). In addition, fold change of IFI-16 was significant positive correlated with skeletal pain and headache ($r = 0.73$; $p = 0.0002$; $r = 0.63$; $p = 0.0024$; sequentially). Moreover, the fold change of NOTCH2 ($r = 0.65$, $p\ 0 = 0.001$) was positively correlated with chest pain. Further information is

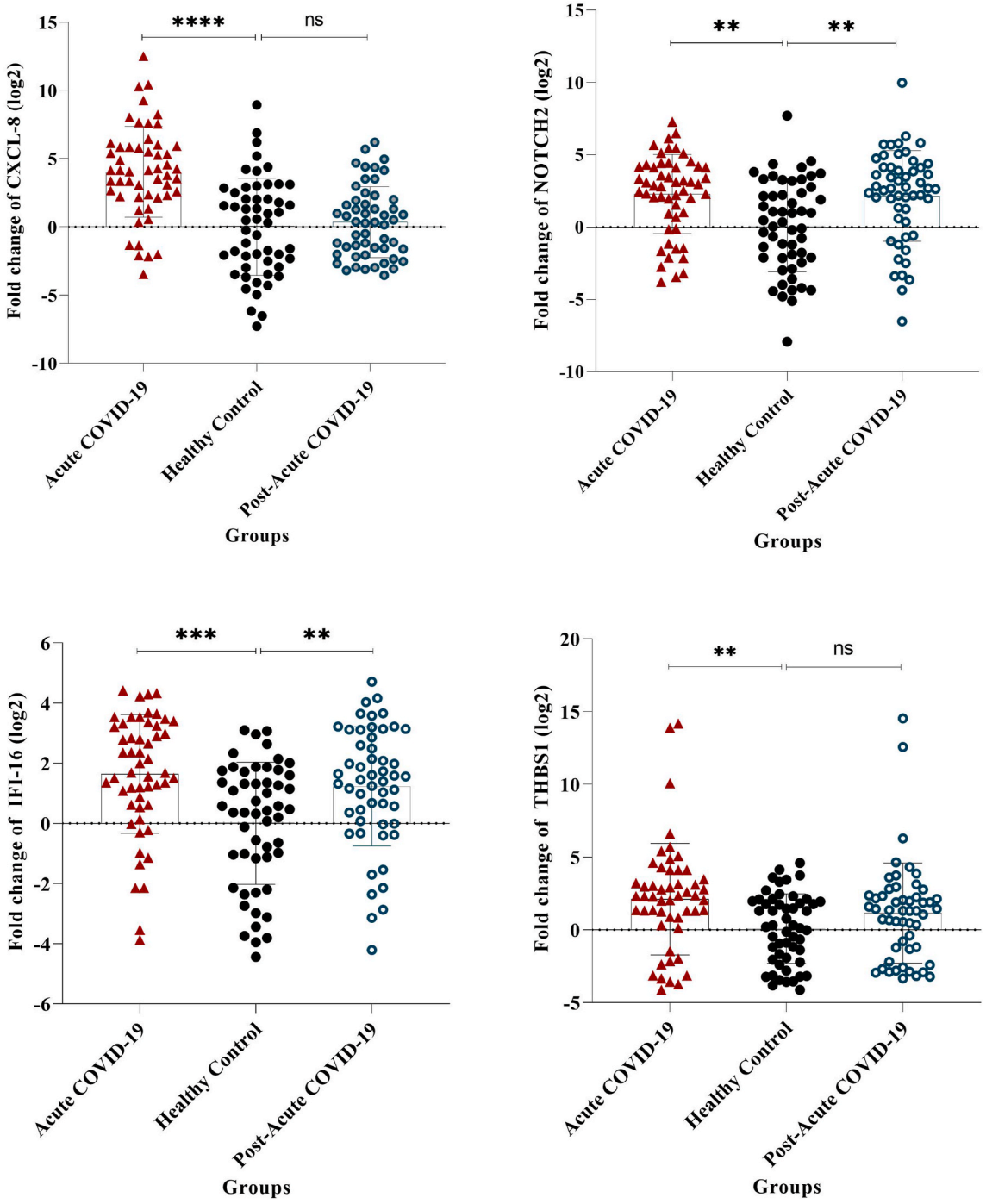

Fig. 1. Comparison of the expression level of CXCL8, NOTCH2, IFI16, and THBS1 between healthy control and COVID-19 subjects.

given in Table 3.

3.3. The CXCL-8 expression level among acute-phase COVID-19 subjects is higher than that among post-acute-phase COVID-19 subjects

In order to identify useful biomarker to distinguish the COVID-19 prognosis, the PBMC level of study mRNAs was determined again five weeks following acute COVID-19. According to results (Fig. 2), a significant difference was not found in the expression pattern of NOTCH2, IFI-16 and THBS-1 between acute and post-acute COVID-19 phases ($p > 0.05$). However, a different CXCL-8 expression level was seen between acute and post-acute phases. The mean fold change of CXCL-8 mRNA expression in the acute phase was 4.06-fold more than the post-acute phase ($p < 0.0001$).

3.4. PBMC CXCL-8 levels as a promising biomarker for COVID-19

The ROC curve as a statistical tool was used to characterizing the discriminating power of CXCL-8, NOTCH2, IFI-16 and THBS-1 for COVID-19 disease diagnosis. The ROC curve findings are demonstrated in Fig. 3, which showed CXCL-8 (AUC = 0.8; 95% CI: 0.62 to 0.88; $p < 0.0001$), IFI-16 (AUC = 0.74; 95% CI: 0.59 to 0.89; p: 0.0016) and NOTCH2 (AUC = 0.73; 95% CI: 0.57 to 0.89; p: 0.0003) acted as a potential biomarker for distinguishing of between healthy control subjects and acute COVID-19 patients (Fig. 3A, ***C*** and ***3E***). Furthermore, according to ROC curve analyses, NOTCH2 (AUC:0.7; 95% CI: 0.53–0.86;

Table 3
Spearman correlation coefficients for expression levels of selected mRNAs compared with demographic/clinical characteristics and viral genes.

	CXCL-8	NOTCH2	IFI16	THBS
Age	0.08^{ns}	0.101 ^{ns}	0.03 ^{ns}	0.12 ^{ns}
Sex	0.24 ^{ns}	0.05^{ns}	0.17 ^{ns}	0.35^{ns}
Fever	0.81****	0.25 ^{ns}	−0.03 ^{ns}	0.24 ^{ns}
Headache	0.2 ^{ns}	−0.2 ^{ns}	0.63**	0.12 ^{ns}
Skeletal pain	0.36 ^{ns}	−0.36 ^{ns}	0.73***	0.32 ^{ns}
Chest pain	−0.4 ^{ns}	0.65 **	0.27 ^{ns}	0.5*
Dry cough	0.4*	0.72***	−0.35 ^{ns}	0.63**
Shortness of breath	0.06 ^{ns}	0.33 ^{ns}	0.05 ^{ns}	0.25^{ns}
Deceased taste	0.13 ^{ns}	0.11 ^{ns}	0.31^{ns}	0.08 ^{ns}
Deceased smell	0.16 ^{ns}	0.04 ^{ns}	0.47*	0.13 ^{ns}
Confusion	0.04 ^{ns}	0.06 ^{ns}	−0.09 ^{ns}	−0.14 ^{ns}
Chills	−0.2 ^{ns}	0.27 ^{ns}	−0.06 ^{ns}	−0.34 ^{ns}
Sputum cough	−0.28 ^{ns}	0.42 ^{ns}	−0.16 ^{ns}	0.21 ^{ns}
Runny nose	−0.16 ^{ns}	0.17^{ns}	0.4*	−0.22 ^{ns}
Cape of nose	−0.15 ^{ns}	0.19 ^{ns}	0.19 ^{ns}	−0.3 ^{ns}
Menstrual disorder	−0.07 ^{ns}	0.08 ^{ns}	0.19 ^{ns}	−0.19 ^{ns}
Bleeding stomach	−0.19 ^{ns}	0.02 ^{ns}	0.17 ^{ns}	−0.26 ^{ns}
Gastrointestinal symptom	−0.32 ^{ns}	0.03 ^{ns}	0.12 ^{ns}	−0.26^{ns}
SARS-CoV-2 RdRp gene	−0.27 ^{ns}	0.43 ^{ns}	−0.34 ^{ns}	−0.2 ^{ns}
SARS-CoV-2 N gene	−0.18 ^{ns}	0.42 ^{ns}	−0.13 ^{ns}	−0.15 ^{ns}

ns. P > 0.05; *. P ≤ 0.05; **. P ≤ 0.01; ***. P ≤ 0.001; ****. P ≤ 0.0001.

p: 0.0006) and IFI-16 (AUC:0.67; 95% CI: 0.51–0.85; *p*: 0.002) can probably discriminate between healthy and post-acute COVID-19 subjects (Fig. 3D and *F*). PBMC CXCL-8 obtained a highest AUC value 0.808, showing an acceptable amount in differentiating acute phase from post-acute phase of infection ($p < 0.0001$, Fig. 3B).

4. Discussion

In the present study, changes in the expression pattern of IFI-16, NOTCH2, CXCL8, and THBS1 genes in three groups of acute, post-acute COVID-19 patients, and healthy individuals were investigated and compared.

Despite limited evidence on exact immunopathology of COVID-19, findings have recently demonstrated that the infection induced excessive inflammatory response and impaired immune system by disrupting host immune responses [20].

Reportedly, the expression levels of some proinflammatory cytokines (such as IL-10, IL-6, and TNF-α) and chemokines (such as CXCL10/IP-10, CCL2/MCP-1, and CCL3/MIP-1α) in severe COVID-19 cases were often higher than those mild cases of COVID-19. Therefore, altered inflammatory cytokine and chemokines expression is important in the progression of SARS-CoV-2. Therefore, altered inflammatory cytokine and chemokines expression are key actors in the progression of this infection [21] and it can maybe the potential prognostic factor for the spectrum of SARS-CoV-2 Infection. Chemokines are low molecular weight proteins with a key role in the immune cell absorption during inflammation. The IL-8 is a chemokine secreted by monocytes/macrophages implicated in the inflammation, especially in virus-mediated infections [22]. Based on in vivo and in vitro findings, the expression of CXCL8 chemokine shows a significant elevation following the infection with MERS-CoV and SARS-CoV [23]. Park et al. revealed that CXCL8 release from myeloid cells in group with severe COVID-19 was significantly greater than that in the mild status [24]. Recently, a phase 2 clinical trial (NCT04347226) is investigating the inhibitory effect of CXCL8 suppressor on COVID-19 patients which indicates the critical function of this factor in COVID-19 management. Moreover, Xu et al. analyzed the chemokines in the plasma of patients suffering from mild-to-severe infection [25]. They found that in all early-stage COVID-19 patients, the expression level of CXCL8 increased to similar levels, while two weeks following the COVID-19 diagnosis, the level of this factor remains steady in mild/severe forms of COVID-19 but the CXCL8 level is significantly increased further in the fatal form of COVID-19. In our work, similar to earlier investigations, the CXCL8 expression level among acute COVID-19 subjects indicated a statistically significant elevation than that among post-acute group (FC:4.03; $p < 0.0001$; Fig. 1; Table 3). No significant difference in CXCL8 expression level was also reported between the healthy and post-acute COVID-19 subjects. However, the CXCL8 level significantly was greater in the post-acute phase than that in acute phase. Also, according to the results of this study, CXCL-8 can be acting as a potential biomarker for distinguishing the healthy control subjects from the acute-phase COVID-19 patients as well as the acute-phase COVID-19 from the post-acute-phase COVID-19. Altogether, CXCL8 has been considered a prognostic biomarker and treatment target for this infection. It should be mentioned that more studies are required in order to clear the interaction of CXCL8 with SARS-CoV-2.

Up to now, proteomic and bioinformatics-based methods have been used to identify the major protein targets and molecular mechanisms of COVID-19 infection. In this regard, Azodi et al., by bioinformatics evaluation of plasma proteome in patients with severe COVID-19, showed that six proteins including four species of fibrinogen, Tallin-1 and THBS1 play important roles in the response to COVID-19 disease [26,27]. Additionally, Liu et al. evaluated the interaction between host proteins and COVID-19 using Cytoscape software and STRING database. In this study, it was predicted that six proteins (DBH, C1RL, SHGB, ICAM2, TF and THBS1) were significantly regulated while two others (ORM1 and APCS) were downregulated significantly [28]. However, no studies have been performed on the expression level of THBS1 in COVID-19 and its performance in the illness severity. In our work, we observed for the first time that the PBMC level of THBS1 was increased among COVID-19 patients when comparing with healthy subjects (a change of FC = 2.02-fold, P-value = 0.002).

Notch signalling plays an important role in the effective functioning of adaptive and innate immunity. Moreover, it is involved in regulation of inflammatory cytokines during viral infection in alveolar macrophages [29]. It has been reported that Notch signalling activates in response to the infection of respiratory syndrome virus (HP-PRRSV). Then HP-PRRSV indirectly reduces the induction of IL-1β and TNF-α by suppression of the Notch signalling pathway and as a result, escapes from the host's immune system [30]. Ying et al. Mendelian Randomization Analysis shows the association of Notch2 expression with a greater SARS-CoV2 risk. Moreover, Notch signalling induces SARS-CoV2 entry into the cell through host protein upregulation [31]. According to their suggestion, the Notch signalling, especially Notch2, may be a potential target to control and manage COVID-19 and may cause COVID-19. The Notch signalling pathway is essential for the control of cellular differentiation, cell survival, angiogenesis, invasion, migration, etc [32,33]. Accumulating evidence has suggested that some viruses manipulate Notch pathway to advance their targets in the host cell [32]. A recent study by Vandelli et al., using functional annotation analysis with GeneMania showed that 5′ of SARS-CoV RNA are associated with the Notch2 receptor pathway [34]. In the present study, it was observed that the Notch2 expression level among the COVID-19 subjects was significantly greater than that among the controls. However, a significant difference was not reported in the NOTCH2 expression level between acute and post-acute phases. Also, a positive correlation was significantly seen between fold change of NOTCH2 and Dry cough ($r = 0.72$, $p < 0.001$). Taking these findings together, it reinforces the possibility that SARS-CoV-2 is interfering with the NOTCH2 pathway, is central player in the pathogenesis of this virus, and can possibly be considered a therapeutic target. However, more experimental studies are needed.

IFN-I signalling as a host's first line of defensce against viral infections plays a key role in disease control and severity. Likewise, the expression levels of involved proinflammatory genes in the IFN-I signalling pathway (e.g., STAT, IFITM1, IFIT2, IFI35, IFIH1, IRF7, and IFI16) are altered in viral-infected patients. The homologous interferon-inducible protein 16 (IFI16) gene, belonging to family PYHIN (containing HIN and pyrin), can encode proteins sharing a 200-amino acid

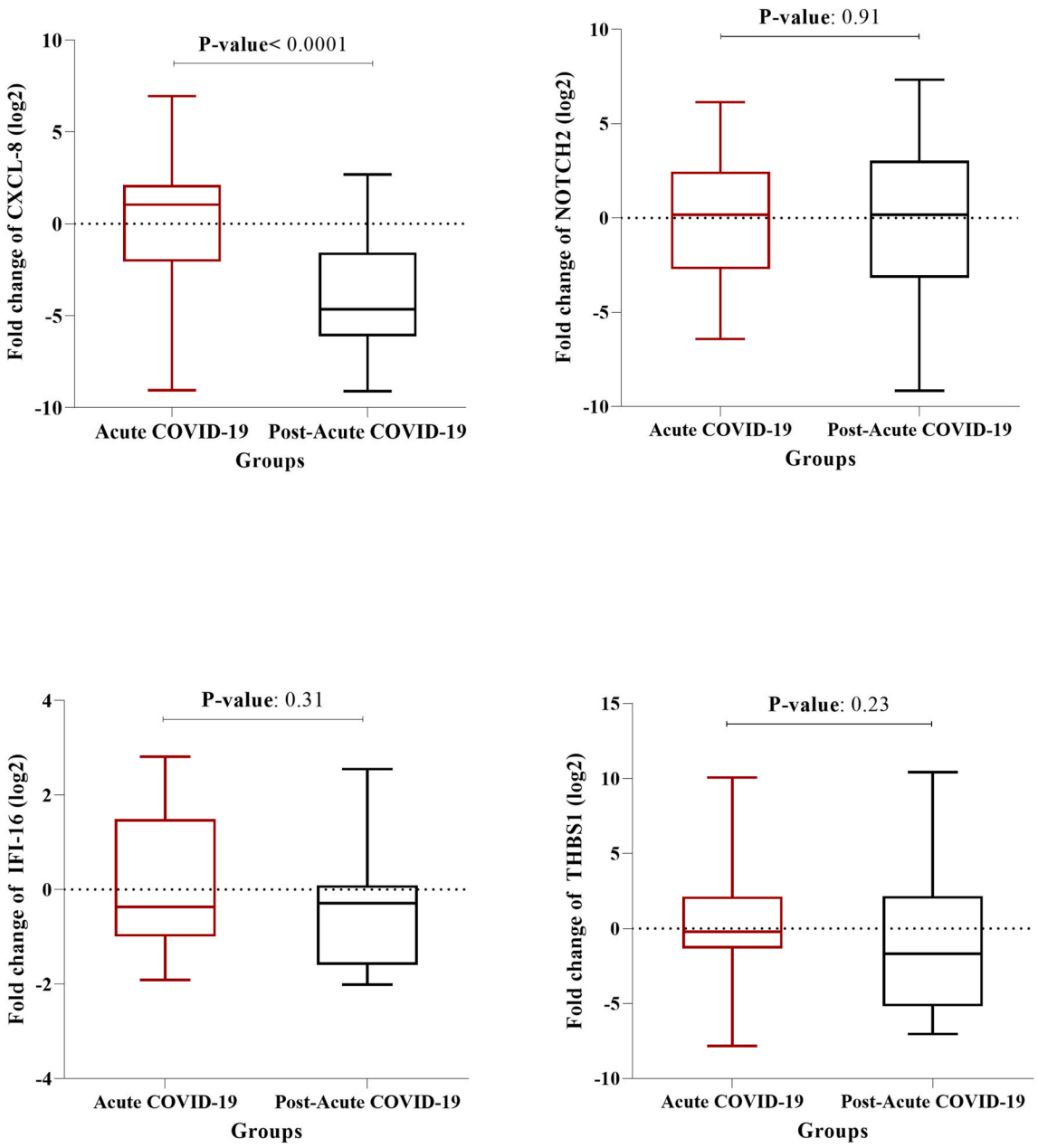

Fig. 2. Comparing expression profile of selected mRNAs (CXCL8, NOTCH2, IFI16, and THBS1) between acute and post-acute phase of COVID-19.

signature motif (HIN) [35]. IFI16 directly senses single-stranded RNA viruses such as coronaviruses, flaviviruses, rhabdoviruses, and Influenza viruses, and not only enhances antiviral responses through induction of RIG-I transcription but also induces IFN type I production, and antiviral cytokine expression [36–39]. In the current study, the IFI16 expression level showed a significant increase in COVID-19 groups compared to the healthy subjects as well as there was a positive correlation between the IFI-16 expression levels and the skeletal pain and headache, in line with recommended possible roles for IFI16. As shown in Fig. 3, according to the ROC curve findings and the admirable specificity and sensitivity for IFI16, it may serve as good biomarker for discriminating healthy status from acute and post-acute phases. The main limitation in our work was the small sample size.

To conclude from our findings, proinflammatory mediators' expression including CXCL8, IFI-16, NOTCH2, and THBS1 were significantly different among healthy subjects with patients suffering from COVID-19. Besides, the CXCL8 expression level exhibited a significant elevation in the post-acute phase of this infection when comparing with acute phase which may be consider as a potential biomarker for distinguishing between the acute phase from the post-acute phase. Knowing any change in the levels of inflammatory and proinflammatory mediators in different stages of the infection can be helpful in predicting the prognosis of the inflammation process, prevention and even treatment of infection. The small sample size was our study limitation; thus, we suggest investigations with larger sample size to confirm the diagnostic power of such inflammatory factors for COVID-19 infection.

Ethical approval

The present research followed the ethical considerations approved by the committee of ethics (IR.IUMS.FMD.REC.1400.303) at Iran University of Medical Sciences (IUMS; Tehran; Iran).

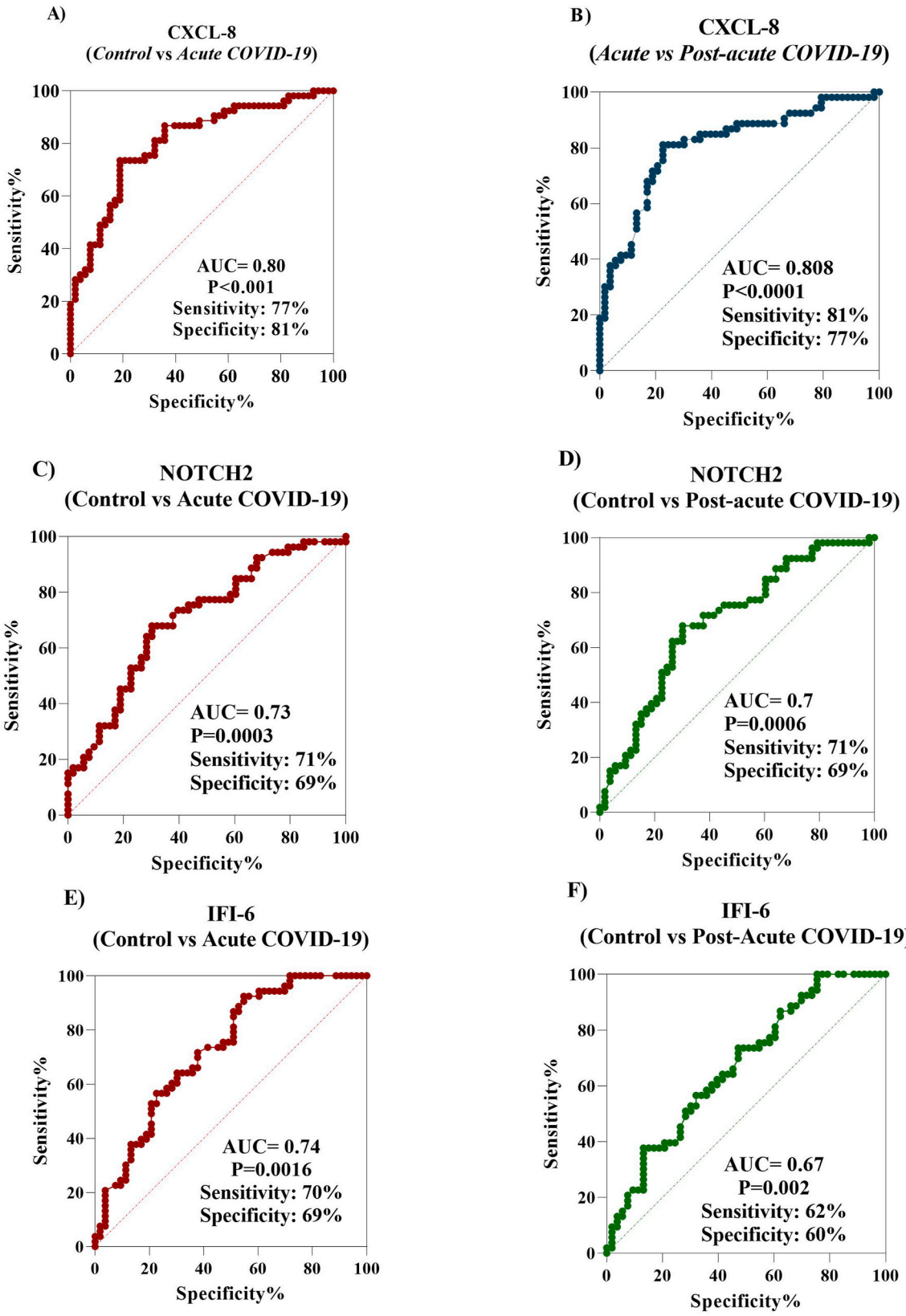

Fig. 3. Analysis of ROC curve based on PBMC IFI-16, NOTCH2, CXCL8, THBS1 to distinguish acute COVID-19 group from post-acute COVID-19 group and healthy group.

Informed consent

None.

Consent to participate

All authors have confirmed their participation in this study.

Consent to publish

All authors have agreed to publish this manuscript.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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