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ORIGINAL ARTICLE

A comparative study on proinflammatory cytokines interleukin-17A and interleukin-17F expressions in whole blood of patients with COVID-19

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ABSTRACT

Background and Objectives: Coronavirus disease 2019 (COVID-19), due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has expanded rapidly to all over the world. Interleukin-17 is one of the inflammatory cytokines which is highly expressed in the blood of individuals with COVID-19. Our aim in the present survey was to assess the mRNA expression levels of cytokine IL-17A, IL-17F and TNF-α in the blood of COVID-19 patients compared with healthy control individuals.

Materials and Methods: A total of 69 patients including 32 mild patients, 20 severe and 17 asymptomatic patients in comparison with 25 healthy controls were evaluated. To measure the expression profile of IL-17A and IL-17F in whole blood, quantitative PCR was used.

Results: Asymptomatic, mild, and severe SARS-CoV-2 infections were found to have significantly higher levels of IL-17A and IL-17F mRNAs than the healthy group (fold change IL-17A: 3.778; p= 0.002, 4.003; p= 0.001, 2.608; p= 0.0001 respectively, fold change IL-17F: 2.967; p= 0.003, 3.819; p= 0.001, 2.617; p= 0.0012 respectively). TNF-α mRNA expression was also measured, which shows an approximately similar increase compared to IL-17 (fold change: 2.726; p= 0.002, 2.383; P= 0.001, 2.631; p= 0.001, respectively).

Conclusion: SARS-CoV-2 infection severity was associated with blood levels of IL-17A and IL-17F mRNA.

Keywords: COVID-19; Gene expressionss; Cytokines; Interleukin-17; Tumor necrosis factor-alpha

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INTRODUCTION

A novel coronavirus known as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the cause of coronavirus disease 2019 (COVID-19). The virus was initially identified in Wuhan, China, in December 2019 in the respiratory systems of pneumonia patients. SARS-CoV-2 is an unsegmented RNA virus with a genome size of 29.9 kb (1-3). Spike protein is a transmembrane protein found on the virus's outer surface, which helps the viral coat attach to the cell by binding to the human angiotensin-converting enzyme 2 (hACE2) (4-6). The disease advancement is more likely to affect those who have underlying issues, such as diabetes, heart disease, and high blood pressure (2). Apart from the less frequent symptoms like hemoptysis, angina, diarrhea, nausea, vomiting, and chest pain, COVID-19 patients frequently have pyrexia, coughing, exhaustion, headaches, shortness of breath, sore muscles, and sputum production (1-3).

A SARS-CoV-2 infection leads to a number of dysregulated inflammatory reactions by continuously interfering with the immune system's normal functions. Lymphopenia is the primary feature of COVID-19 in patients, particularly those with severe illness. TCD4+, TCD8+, NK, and B lymphocytes are significantly reduced in these individuals (1, 7, 8). Another feature of severe COVID-19 can be considered as increased cytokine production; these patients experience a significant rise in inflammatory cytokines, including IL-6, IL-1β, TNF-α, IL-10, MCP-3, IP-10, GM-CSF, IL-17, and IL-1Ra, that is suggestive of a cytokine storm (1, 2, 9-12).

IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F are the cytokines that make up IL-17. The biological activities along with adaptation of IL-17F and IL-17A are among the most well-known of the cytokines (13). T helper 17 cells make up the majority of the IL-17A and IL-17F found in adaptive responses (14). Moreover, these cytokines are made by other cells, including natural killer cells, macrophages, and innate Th17 lymphocytes (15). In order to eradicate pathogens such *Citrobacter rodentium*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*, IL-17F and IL-17A should be considered necessary (16-18). Dysregulated secretion of IL-17F and IL-17A can possibly cause inflammation, tissue damage, and autoimmune illnesses including psoriasis, inflammatory bowel disease (13) rheumatoid arthritis (RA) and multiple sclerosis (MS) (19, 20).

GM-CSF, TNF-α, and IL-17 cytokine levels are elevated in patients with severe COVID-19, a condition known as the Th17-type cytokine storm, which may result to organ damage. Th17 cells stimulate Th17 responses by overexpressing TNF-α. In the context of viral diseases, IL-17 can be linked to IL-6, a predictor of COVID-19 severity. Indeed, elevated amounts of IL-6 have been shown to boost Th17 cell development in mouse viral models. IL-6 and IL-17 elevated secretion may protect infected cells from apoptosis, thereby increasing viral resistance (21). Furthermore, IL-17 excess levels can induce T-cell responses and expand the concentrations of inflammatory cytokines, including IL-1β, IL-6, and TNF-α (22). These factors have motivated researchers to explore the potential of IL-17 as a remedial option in the context of COVID-19, employing cytokine inhibitors (22-25).

To assess the mRNA quantities of IL-17A, IL-17F, and TNF-α in the whole blood of SARS-CoV-2 patients compared to a normal control category, we conducted this study, as cytokines, particularly IL-17, play critical roles in predicting the severity and pathogenesis of viral infections like COVID-19.

MATERIALS AND METHODS

Study design and sample collection. 32 mild patients (14 females and 18 males) along with 20 severe patients (5 females and 15 males) were admitted to Taleghani and Imam Hossein Hospitals for this survey. Additionally, 17 asymptomatic patients (8 females and 9 males) who were clinically diagnosed and real-time PCR SARS-CoV-2 positive throat swab samples were included, in comparison to 25 healthy subjects (7 females and 18 males). From March 25 to August 25, 2020, all sample groups were collected. The Research Institute's ethics committee accepted the protocols (IR.SBMU.RIGLD.REC.1399.008, Iran). Furthermore, all participants gave their informed consent.

Extraction and cDNA preparation. To extract total RNA from whole blood samples, the Hybrid-RTM blood RNA isolation reagent (GeneAll Biotechnology, South Korea) was utilized following the manufacturer's instructions. The extraction was performed from peripheral blood based on glass fiber membrane technology (26). cDNA was synthesized using the Thermo Scientific RevertAid Strand cDNA Syn-

thesis Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA) (27). IL-17A and IL-17F expression levels in the whole blood of patients and a normal control category was measured employing the SYBR Green (RealQ plus 2x Master Mix Green, Ampliqon, Denmark) technique. The reference gene was the stably expressed reference gene in whole blood, the ß2-Microglobulin. The quantitative PCR utilized pertinent forward and reverse primers (Table 1). The following steps were used to run the quantitative PCR: 15 minutes at 95°C, 40 cycles of 60°C for 60 seconds, and 15 seconds at 95°C. For calculating relative expression, the 2-ΔΔCT method was utilized.

Measurement of anti-SARS-CoV-2 IgG and IgM by ELISA assay. Anti-SARS-CoV-2 nucleocapsid IgM and IgG antibodies were counted in the plasma samples of mild and severe patients, using BioTek Elx800 microplate reader according to manufacturer protocol (Highland park, Winooski, Vermont, USA).

Statistical analysis. The statistical version of the Social Science Software Package 16 (SPSS Inc., Illinois, USA) was utilized to conduct the statistical analysis. The data was analyzed employing one-way ANOVA, Tukey's post-hoc analysis, or the Kruskal-Wallis test, followed by Dunn's post-hoc comparisons. The parametric variables were compared utilizing either the independent sample t-test or the Mann-Whitney U test. Non-parametric variables were analyzed applying the $\chi 2$. The potential use of blood IL-17A and IL-17F levels as a diagnostic marker was analyzed employing area under the curve (AUC) and receiver operating characteristic (ROC) curve analysis. The appropriate cut-off was determined by maximizing Youden's index, defined as max [sensitivity(c) + specificity(c) - 1], which provides an excellent diagnostic threshold for determining whether an individual is ill or healthy (28). A logistic regression test was conducted to remove the influence of age. Charts were plotted using GraphPad Prism 8.

RESULTS

Demographic and clinical characteristics. This study includes data from 17 asymptomatic, 32 mild, and 20 severe COVID-19 patients, as well as 25 healthy controls. Table 2 shows the demographic information for the examined subjects. Regression analysis was used to adjust for the potential confounding effect of age (Table 2). When comparing moderate and severe patients by age, there was no significant relationship between cytokine production and age (after adjustment to remove the confounding effect). Ten days after infection, samples from patients with moderate and severe infections were taken. Table 3 shows the laboratory results, whereas Table 4 describes the clinical characteristics. According to Table 3, those with severe diseases showed elevated amounts of D-dimer, CRP, and neutrophil count, as well as lower O2 saturation and lymphocyte count.

A study of the patients' medical history found that 55.8% of them had underlying medical disorders, such as chronic heart disease (19.23%), diabetes mellitus (21.15%), chronic kidney disease (9.61%0), hypertension (25%) or cancer (25%).

There was no significant relationship between the examined mRNA levels and the clinical features as well as comorbidities of moderate and severe COVID-19 individuals. The illness severity was estimated using the COVID-19 clinical therapy guidelines (29). As a result, asymptomatic patients are those who have caught SARS-CoV-2 nevertheless have not developed signs at all. Individuals with SARS-CoV-2 who need hospitalization and have unique pneumonia

Table 1. Primer sequences

Gene	Primer	Sequence
β2- microglobulin	Forward	TGCTGTCTCCATGTTTGATGTATCT
	Reverse	TCTCTGCTCCCCACCTCTAAGT
Interleukin-17A	Forward	TCCCACGAAATCCAGGATGC
	Reverse	GGATGTTCAGGTTGACCATCAC
Interleukin-17F	Forward	GCTGTCGATATTGGGGCTTG
	Reverse	GGAAACGCGCTGGTTTTCAT
TNF-α	Forward	CATCCACAAAGCCCTCATCGAC
	Reverse	GACTGAGGCTTGGAATCTGC

Table 2. Demographic data of the studied groups

	Healthy control	Asymptomatic patient	Mild patients	Severe patients	P-value
Number of subjects	25	17	32	20	
Age (mean ± SD)	36.96 ± 6.463	39.40 ± 9.318	56.03 ± 14.19	65.85 ± 15.72	<0.0001
Gender					0.408
Male, n (%)	18 (72%)	9 (52%)	18 (56.25%)	15 (75%)	
Female, n (%)	7 (28%)	8 (48%)	14 (43.75%)	5 (25%)	

Table 3. Laboratory findings of mild and severe SARS-CoV-2 infected patients

Laboratory items	Normal range	Mild patients (n=32)	Severe patients (n= 20)	P-value
O2 saturation (SpO_2)	-	92.65	88.94	0.034
WBC× 10^9 (U/L)	4.5-10.5× 10^9	7.42	9.17	0.139
Lymphocyte× 10^9 (U/L)	1.32-3.57× 10^9	2.326	1.656	0.042
Neutrophil× 10^9 (U/L)	1.5-6 × 10^9	6.77	8.19	0.006
PLT× 10^9 (U/L)	150-400× 10^9	254.59	215.50	0.182
ALT (U/L)	0-41	38.84	109	0.171
AST (U/L)	0-40	41.53	89.45	0.113
Hb (g/ml)	13-17.5	10.89	11.34	0.582
LDH (U/L)	<248	512.40	765.75	0.056
ESR (mm/h)	0-15	29.60	51.17	0.10
D-dimer (mg/L)	0-500	1021.30	2121.92	0.022
CRP (mg/L)	>10	28.15	40.13	0.033

Table 4. Clinical features of mild and severe patients infected with SARS-CoV-2

Laboratory items	Mild patients (%) (n=32)	Severe patients (%) (n= 20)	P-value
Fever	37.5% (12 of 32)	40% (8 of 20)	0.857
Cough	40.62% (13 of 32)	50% (10 of 20)	0.508
Dyspnoea	43.75% (14 of 32)	60% (12 of 20)	0.254
Myalgia	28.12% (9 of 32)	20% (4 of 20)	0.510
Chest pain	12.5% (4 of 32)	25% (5 of 20)	0.246
Diarrhea	12.5% (4 of 32)	20% (4 of 20)	0.466

signs, including fever, cough, dyspnea, fast breathing, along with SpO2 values more than 90%, are classed as moderate. Patients with SARS-CoV-2, pneumonia symptoms, SpO2 <90%, organ failure requiring ICU admission, or death were considered to have a severe infection. Patients were classified as severe based on their history, which was verified by an infectious disease physician.

Characteristics of plasma IgM and IgG antibodies in patients. ELISA was used to detect anti-SARS-CoV-2 nucleocapsid (N) protein antibody tests for IgG as well as IgM antibodies in moderate and severe patients' blood samples. These findings demonstrated that the positive rates for IgM and IgG were 42.30% (22 of 52) and 63.46%, respectively (33 of 52).

Blood mRNA quantity of IL-17A, IL-17F, and TNF-α in COVID-19 patients and healthy controls. The expression rate of IL-17A, IL-17F and TNF-α remarkably elevated in asymptomatic, mild and severe patients in comparison to the normal category (Fold

change IL-17A: 3.778; p= 0.002, 4.003; p= 0.001, 2.608; p= 0.0001 respectively), (Fold change IL-17F: 2.967; p= 0.003, 3.819; p= 0.001, 2.617; p= 0.0012 respectively), (Fold change TNF-α: 2.726; p= 0.002, 2.383; p= 0.001, 2.631; p= 0.001, respectively), and mRNA levels in severe, mild and asymptomatic patients did not differ significantly (Fold change IL-17A: 0.2249; p= 0.9867, 1.171; p= 0.3845, 1.396; p= 0.1295, Fold change IL-17F: 0.8523; p= 0.5920, 0.3506; p= 0.9653, 1.203; p= 0.2487, fold change TNF-α: 0.3434; p= 0.5673, 0.0950; p= 0.8854, 0.2488; p= 0.6629, respectively), (Fig. 1A-C). The expression of IL-17F and IL-17A mRNAs is approximately equal to that of TNF-α as indicator of COVID-19 severity.

Diagnostic value of TNF-α, IL-17F, and IL-17A in SARS CoV-2 infection. Using the ROC curve, the diagnostic specificity and sensitivity of Interleukin 17A, 17F, and TNF-α were assessed. The AUC of IL-17A was 0.8662 (95% CI: 0.7850 to 0.9473, p-value <0.0001), with the optimal cut-off value estimated to be less than 0.0786 (Sensitivity, 76%; Specificity, 84.62%) (Fig. 2A). The AUC of IL-17F for SARS-CoV-2 diagnosis in compared with controls was 0.8681 (95% CI: 0.7881 to 0.9480, p-value <0.0001). The optimal cut-off was determined to be less than 0.0742 (Sensitivity, 72%; Specificity, 90.38%) (Fig. 2B). The optimal cut-off was established to be less than 0.1453 (Sensitivity, 92%; Specificity, 65.38%), as indicated by the AUC for TNF-α of 0.8238 (95% CI: 0.8238, P-value < 0.0001) (Fig. 2C).

DISCUSSION

The production and secretion of numerous proinflammatory cytokines, together with T cells triggering, including CD4+ and CD8+, are all examples of competent antiviral responses in innate and adaptive immunity. T cells has been considered indispensable for controlling of viral propagation, the restriction of viral dissemination, the elimination of infected cells, and the reduction of inflammation (30, 31). Cytokines are a class of signaling molecules which are responsible for modulating a variety of biological processes through cell surface receptors (32). They are primarily released by immune cells, such as lymphocytes, monocytes, and macrophages. Organ failure, tissue injury, and ultimately mortality may be the effect of too much inflammatory cytokines production (33). The primary functions of CD4+ T helper cells in the tissue injury are associated with autoimmune or inflammatory diseases as follows (34). Th-17 is classified as an inflammatory T-helper among the subsets of T-helper cells, as it generates IL-17. This leads to chronic inflammation in tissues and subsequent organ failure (14).

The biological association of IL-17 produced by Th17 cells and their involvement in the pathogenesis of viral infections, including HBV, HCV, HIV, and Dengue virus, has been investigated. There has been a report of an elevation in IL-17 mRNA production in PBMC during HBV infection (35). Another study reported that IL-17 mRNA levels were substantially raised in HBV patients in comparison to healthy

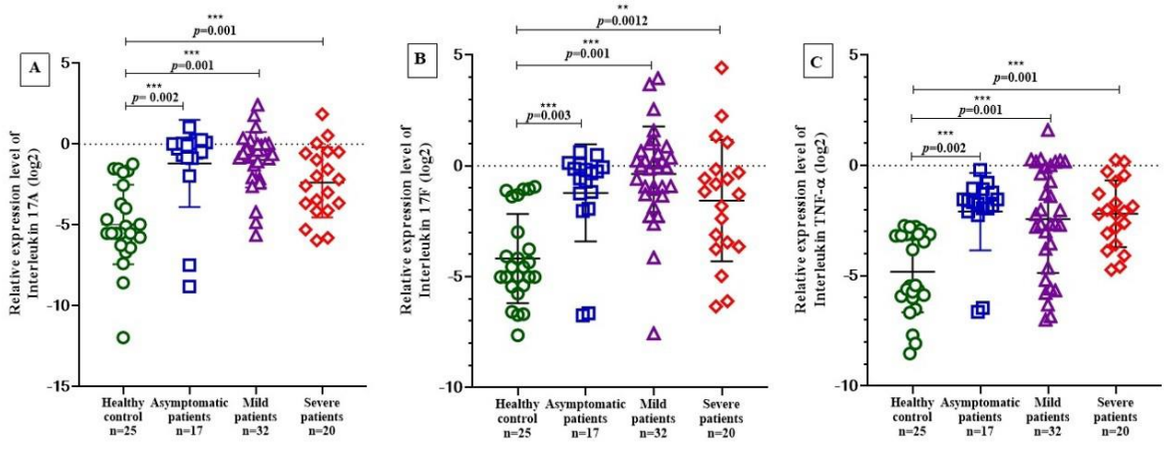

Fig. 1. The cytokine gene expression level in asymptomatic, mild, and severe COVID-19 patients and healthy controls. The gene expression analysis indicated significant increases in the expression level of IL-17A (A), IL-17F (B), and TNF-α (C) in asymptomatic, mild, and severe COVID-19 patients compared with normal subjects.

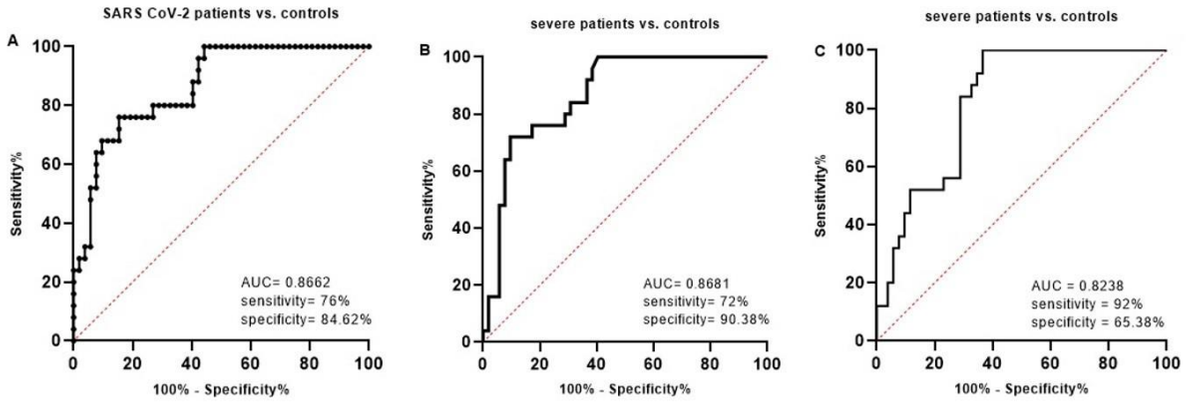

Fig. 2. ROC curve analysis of IL-17A (A), IL-17F (B), and TNF-α (C) for diagnosis of SARS-CoV-2

individuals (36, 37). Th17 cells are more abundant in the peripheral blood of persistently infected HCV patients during HCV infection (38, 39). Moreover, HIV infection is linked to a low percentage of Th17 cells, and IL-17 quantities are positively correlated to a Th17 cell percentage, and both IL-17 and Th17 are negatively correlated with the plasma viral load (40, 41). A high serum rate of IL-23, IL-22, IL-17F and IL-17A, which are marker cytokines primarily related to Th17 cells, is produced in Dengue virus infections compared with the healthy group (42). Additionally, patients with HCV had higher levels of IL-17 than healthy controls did (38, 39).

Two mechanisms generate the immune system malfunction in COVID-19 patients: the excessive pro-inflammatory cytokines production by monocytes and the aberrant generation of lymphocytes by CD4+ T cells (43, 44). Severe lung tissue damage has been linked to cytokine storms or cytokine release syndrome (CRS), which are marked by an over-inflammatory response and cytokines and chemokines (IL-8, IL-6, IL-17, TNF-α, IFN-γ and G-CSF) secretion into the lungs (45). It has been reported that the fundamental cause of ARDS (acute respiratory distress syndrome) can be the cytokine storm. Many coronavirus-infected people develop ARDS, which affects the liver, kidneys, and heart in addition to producing pulmonary edema (7).

Recent data suggests that the pathophysiology of COVID-19 involves host Th17 inflammatory responses. These responses include the diffusion of important cytokines like GM-CSF and IL-17 as well as other immune-boosting factors that help fight off viral infection by decreasing Treg cell counts, increasing neutrophil migration, and simultaneously triggering Th2 responses (46).

In patients with severe COVID-19, an excess of CCR4+/CCR6+ Th17 cells in the blood may have potent proinflammatory effects and encourage Th17-type cytokine storm (7). It has also been shown that patients with SARS-CoV, MERS-CoV, and other beta coronavirus members have elevated Th17 responses. According to recent research MERS-CoV and SARS-CoV infections increase host's expression of IL-17 (47-49).

A biological molecule in the blood or tissues that indicates a common or uncommon operation, circumstance, or illness is referred to as a biomarker, according to the National Cancer Institute. The body's reaction to a disease treatment may be evaluated using a biomarker (50). For instance, IL-17 may be a biomarker for liver transplant recipients who have liver injury (51, 52). The DNA methylation measure is generally suitable for use as a biomarker for diseases verification (53). Research has shown that suppressing the hypermethylated IL-17 promoter may stop CHB from developing and spreading. Furthermore, these results suggest that methylation of the IL-17 promoter might be a biomarker for HBV-HCC (54). Additionally, Zuñiga, Joaquín, et al. have shown the potential use of IL-17 as a biomarker for acute ZIKV infection (55).

Confirming the gene expression of T-helper 17 pathway-related elements, like IL-17F, IL-17A, and TNF-α, in normal controls and severe COVID-19 patients, moderate and asymptomatic individuals was the aim of this study. Furthermore, we evaluated TNF-α, IL-17A, and IL-17F's potential as diagnostic biomarkers for the infection. We found that the patients had considerably higher mean mRNA quanti-

ties of IL-17A, IL-17F, and TNF-α than the healthy category. Furthermore, whole blood quantities of TNF-α, IL-17A, and IL-17F may be circulating biomarkers for the infection, in line with our finding. It is important to note that the assessment of IL-17F and IL-17A mRNA quantities in the infection may be clinically complicated by other respiratory diseases, including rhinovirus, adenovirus, influenza viruses, and even bacterial lung infections. Our investigation also revealed that severe patients had greater levels of disease-related variables, such CRP and D-dimer, than mild patients. These findings are supported by the findings of previous studies (56). Additionally, our severe patients had significantly greater reduced O2 saturation and cell counts (57-59), which are indicators of infection severity, than our mild patients. According to earlier research, severe patients had higher neutrophil counts than mild patients, which is consistent with our findings (60, 61).

The current study showed a significant elevation in IL-17F, IL-17A, and TNF-α levels between severe patients and the healthy group, despite the fact that many studies have reported a significant difference in pro-inflammatory cytokines between asymptomatic, severe and mild patients (62, 63), However, according to the limitations of the research, there was no discernible difference between individuals who were asymptomatic and those who were severe. According to earlier research, inflammatory cytokines such IL-17 is increased and influential for resistance to the infection. In line with our findings, Sadeghi et al. reported that 40 COVID-19 patients had significantly over expressed plasma quantities of Th17-related cytokines like IL-23 in addition to IL-17, than the 40 healthy controls (64). According to a different research, moderate cases of SARS-CoV-2 may have higher blood concentrations of IL-17 than the severe and control groups (65). Furthermore, in 23 COVID-19 patients, De Biasi, Sara, et al. showed substantial increases in IL-17 and TNF-α plasma levels compared with 15 healthy individuals (66). Based on a research by Valizadeh et al., mRNA levels of TNF-α were enhanced compared to healthy patients (67), additionally, increased expression of TNF-α as an indicator of COVID-19 severity, has been supported by other studies (1, 68), which corroborates our data. In earlier investigations, the LDH, CRP, and D-dimer as inflammatory variables and age were identified to be connected with severity of COVID-19 (69-72). Tanacan, Atakan, et al. found a positive link between inflammatory variables such as CRP and IL17 expression levels (73), however, this link was not seen in the current investigation. Furthermore, comorbidities like diabetes and hypertension are linked to the probability of severity or mortality in the infected individuals (74, 75), however cytokine expression was not connected with these parameters in our investigation. Future research should include larger sample sizes that permit stronger subgroup analyses and prospective studies that carefully evaluate and document the presence and extent of underlying disease. The study's limitations included the limited number of samples, short sampling period, specific kind of therapy, and lack of tests for cytokine levels.

CONCLUSION

SARS-CoV-2 patients had increased quantities of IL-17A, IL-17F, and TNF-α mRNAs. The survey reveals that IL-17F, IL-17A and TNF-α have appropriate sensitivity as well as specificity for evaluating the infected patients, and might work well as a viable biomarker for SARS-CoV-2 diagnosis. More study into interleukin-17 inhibitors is also suggested as a treatment option for lowering pulmonary inflammation and lung tissue damage.

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REFERENCES

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; 395: 497-506.
2. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, Chi-

na: a descriptive study. *Lancet* 2020; 395: 507-513.
3. Xu X-W, Wu X-X, Jiang X-G, Xu K-J, Ying L-J, Ma C-L, et al. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. *BMJ* 2020; 368: m606.
4. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *J Autoimmun* 2020; 109: 102433.
5. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. *J Virol* 2020; 94(7): e00127-20.
6. Blume C, Jackson CL, Spalluto CM, Legebeke J, Nazlamova L, Conforti F, et al. A novel ACE2 isoform is expressed in human respiratory epithelia and is upregulated in response to interferons and RNA respiratory virus infection. *Nat Genet* 2021; 53: 205-514.
7. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020; 8: 420-422.
8. Tan M, Liu Y, Zhou R, Deng X, Li F, Liang K, et al. Immunopathological characteristics of coronavirus disease 2019 cases in Guangzhou, China. *Immunology* 2020; 160: 261-268.
9. Liu Y, Zhang C, Huang F, Yang Y, Wang F, Yuan J, et al. Elevated plasma levels of selective cytokines in COVID-19 patients reflect viral load and lung injury. *Natl Sci Rev* 2020; 7: 1003-1011.
10. Liu J, Li S, Liu J, Liang B, Wang X, Wang H, et al. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. *EBioMedicine* 2020; 55: 102763.
11. Wang J, Jiang M, Chen X, Montaner LJ. Cytokine storm and leukocyte changes in mild versus severe SARS-CoV-2 infection: Review of 3939 COVID-19 patients in China and emerging pathogenesis and therapy concepts. *J Leukoc Biol* 2020; 108: 17-41.
12. Rotondo JC, Martini F, Maritati M, Mazziotta C, Di Mauro G, Lanzillotti C, et al. SARS-CoV-2 infection: new molecular, phylogenetic, and pathogenetic insights. Efficacy of current vaccines and the potential risk of variants. *Viruses* 2021; 13: 1687.
13. Jin W, Dong C. IL-17 cytokines in immunity and inflammation. *Emerg Microbes Infect* 2013; 2(9): e60.
14. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17–producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005; 6: 1123-1132.
15. Cua DJ, Tato CM. Innate IL-17-producing cells: the sentinels of the immune system. *Nat Rev Immunol* 2010; 10: 479-489.
16. Ishigame H, Kakuta S, Nagai T, Kadoki M, Nambu A, Komiyama Y, et al. Differential roles of interleukin-17A and-17F in host defense against mucoepithelial bacterial infection and allergic responses. *Immunity* 2009; 30: 108-119.
17. Cho JS, Pietras EM, Garcia NC, Ramos RI, Farzam DM, Monroe HR, et al. IL-17 is essential for host defense against cutaneous *Staphylococcus aureus* infection in mice. *J Clin Invest* 2010; 120: 1762-1773.
18. Aujla SJ, Chan YR, Zheng M, Fei M, Askew DJ, Pociask DA, et al. IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. *Nat Med* 2008; 14: 275-281.
19. Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang Y-H, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 2005; 6: 1133-1141.
20. Waite JC, Skokos D. Th17 response and inflammatory autoimmune diseases. *Int J Inflam* 2012; 2012: 819467.
21. Hou W, Jin Y-H, Kang HS, Kim BS. Interleukin-6 (IL-6) and IL-17 synergistically promote viral persistence by inhibiting cellular apoptosis and cytotoxic T cell function. *J Virol* 2014; 88: 8479-8489.
22. Pacha O, Sallman MA, Evans SE. COVID-19: a case for inhibiting IL-17? *Nat Rev Immunol* 2020; 20: 345-346.
23. Avdeev SN, Trushenko NV, Tsareva NA, Yaroshetskiy AI, Merzhoeva ZM, Nuralieva GS, et al. Anti-IL-17 monoclonal antibodies in hospitalized patients with severe COVID-19: A pilot study. *Cytokine* 2021; 146: 155627.
24. Casillo GM, Mansour AA, Raucci F, Saviano A, Mascolo N, Iqbal AJ, et al. Could IL-17 represent a new therapeutic target for the treatment and/or management of COVID-19-related respiratory syndrome? *Pharmacol Res* 2020; 156: 104791.
25. Megna M, Napolitano M, Fabbrocini G. May IL-17 have a role in COVID-19 infection? *Med Hypotheses* 2020; 140: 109749.
26. Ghafouri-Fard S, Oskooei VK, Omrani MD, Taheri M. Dysregulation of cytokine coding genes in peripheral blood of bipolar patients. *J Affect Disord* 2019; 256: 578-583.
27. Yang T, Ouyang Y, Gao Y, Liu D, Zang Y, Chen D. Enriched high-throughput reverse transcription-quantitative PCR template preparation without pre-amplification. *Mol Med Rep* 2020; 22: 3541-3548.
28. Yin J, Tian L. Joint confidence region estimation for area under ROC curve and Youden index. *Stat Med* 2014; 33: 985-1000.
29. World Health Organization (2020). Clinical management of COVID-19: interim guidance, 27 May 2020: World Health Organization. https://iris.who.int/handle/10665/332196

30. Li G, Fan Y, Lai Y, Han T, Li Z, Zhou P, et al. Coronavirus infections and immune responses. *J Med Virol* 2020; 92: 424-432.
31. Tufan A, Avanoğlu Güler A, Matucci-Cerinic M. COVID-19, immune system response, hyperinflammation and repurposing antirheumatic drugs. *Turk J Med Sci* 2020; 50: 620-632.
32. Bartee E, McFadden G. Cytokine synergy: an underappreciated contributor to innate anti-viral immunity. *Cytokine* 2013; 63: 237-240.
33. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* 2017; 9: 7204-7218.
34. Gaffen SL, Jain R, Garg AV, Cua DJ. IL-23-IL-17 immune axis: discovery, mechanistic understanding, and clinical testing. *Nat Rev Immunol* 2014; 14: 585-600.
35. Yang Y, Dai J, Yan M, Yue M, Wang XH, Min X, et al. Expression of interleukin-17 is associated with different immune phases in patients with chronic hepatitis B. *Eur J Inflamm* 2018; 16: 205873921879688.
36. Du W-J, Zhen J-H, Zeng Z-Q, Zheng Z-M, Xu Y, Qin L-Y, et al. Expression of interleukin-17 associated with disease progression and liver fibrosis with hepatitis B virus infection: IL-17 in HBV infection. *Diagn Pathol* 2013; 8: 40.
37. Wang L, Chen S, Xu K. IL-17 expression is correlated with hepatitis B-related liver diseases and fibrosis. *Int J Mol Med* 2011; 27: 385-392.
38. Chang Q, Wang YK, Zhao Q, Wang CZ, Hu YZ, Wu BY. Th17 cells are increased with severity of liver inflammation in patients with chronic hepatitis C. *J Gastroenterol Hepatol* 2012; 27: 273-278.
39. Fathy A, Ahmed AS, Metwally L, Hassan A. T helper type 1/T helper type 17-related cytokines in chronic hepatitis C patients before and after interferon and ribavirin therapy. *Med Princ Pract* 2011; 20: 345-349.
40. Alvarez Y, Tuen M, Nàdas A, Hioe CE. In vitro restoration of Th17 response during HIV infection with an antiretroviral drug and Th17 differentiation cytokines. *AIDS Res Hum Retroviruses* 2012; 28: 823-834.
41. Prendergast A, Prado JG, Kang Y-H, Chen F, Riddell LA, Luzzi G, et al. HIV-1 infection is characterized by profound depletion of CD161+ Th17 cells and gradual decline in regulatory T cells. *AIDS* 2010; 24: 491-502.
42. Sánchez-Vargas LA, Hernández-Flores KG, Thomas-Dupont P, Izaguirre-Hernández IY, Sánchez-Marce EE, Remes-Ruiz R, et al. Characterization of the IL-17 and CD4+ Th17 cells in the clinical course of dengue virus infections. *Viruses* 2020; 12: 1435.
43. Giamarellos-Bourboulis EJ, Netea MG, Rovina N, Akinosoglou K, Antoniadou A, Antonakos N, et al. Complex immune dysregulation in COVID-19 patients with severe respiratory failure. *Cell Host Microbe* 2020; 27: 992-1000.e3.
44. Mahmoudi S, Rezaei M, Mansouri N, Marjani M, Mansouri D. Immunologic features in coronavirus disease 2019: functional exhaustion of T cells and cytokine storm. *J Clin Immunol* 2020; 40: 974-976.
45. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 2020; 395: 1033-1034.
46. Hoe E, Anderson J, Nathanielsz J, Toh ZQ, Marimla R, Balloch A, et al. The contrasting roles of Th17 immunity in human health and disease. *Microbiol Immunol* 2017; 61: 49-56.
47. Faure E, Poissy J, Goffard A, Fournier C, Kipnis E, Titecat M, et al. Distinct immune response in two MERS-CoV-infected patients: can we go from bench to bedside? *PloS One* 2014; 9(2): e88716.
48. Josset L, Menachery VD, Gralinski LE, Agnihothram S, Sova P, Carter VS, et al. Cell host response to infection with novel human coronavirus EMC predicts potential antivirals and important differences with SARS coronavirus. *mBio* 2013; 4(3): e00165-13.
49. Mahallawi WH, Khabour OF, Zhang Q, Makhdoum HM, Suliman BA. MERS-CoV infection in humans is associated with a pro-inflammatory Th1 and Th17 cytokine profile. *Cytokine* 2018; 104: 8-13.
50. Pierce JD, McCabe S, White N, Clancy RL. Biomarkers: an important clinical assessment tool. *Am J Nurs* 2012; 112: 52-58.
51. Kim N, Yoon Y-I, Yoo HJ, Tak E, Ahn C-S, Song G-W, et al. Combined detection of serum IL-10, IL-17, and CXCL10 predicts acute rejection following adult liver transplantation. *Mol Cells* 2016; 39: 639-644.
52. Niu Y, Liu H, Yin D, Yi R, Chen T, Xue Ha, et al. The balance between intrahepatic IL-17+ T cells and Foxp3+ regulatory T cells plays an important role in HBV-related end-stage liver disease. *BMC Immunol* 2011; 12: 47.
53. Leygo C, Williams M, Jin HC, Chan MW, Chu WK, Grusch M, et al. DNA methylation as a noninvasive epigenetic biomarker for the detection of cancer. *Dis Markers* 2017; 2017: 3726595.
54. Tian C-H, Dai J, Zhang W, Liu Y, Yang Y. Expression of IL-17 and its gene promoter methylation status are associated with the progression of chronic hepatitis B virus infection. *Medicine (Baltimore)* 2019; 98(23): e15924.
55. Zuñiga J, Choreño-Parra JA, Jiménez-Alvarez L, Cruz-Lagunas A, Márquez-García JE, Ramírez-Martínez G, et al. A unique immune signature of serum cytokine and chemokine dynamics in patients with Zika virus infection from a tropical region in Southern Mexico. *Int J Infect Dis* 2020; 94: 4-11.
56. Wang L. C-reactive protein levels in the early stage of

COVID-19. *Med Mal Infect* 2020; 50: 332-334.
57. Nabavi S, Javidarabshahi Z, Allahyari A, Ramezani M, Seddigh-Shamsi M, Ravanshad S, et al. Clinical features and disease severity in an Iranian population of inpatients with COVID-19. *Sci Rep* 2021; 11: 8731.
58. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest* 2020; 130: 2620-2629.
59. Wang C, Deng R, Gou L, Fu Z, Zhang X, Shao F, et al. Preliminary study to identify severe from moderate cases of COVID-19 using combined hematology parameters. *Ann Transl Med* 2020; 8: 593.
60. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. *Clin Infect Dis* 2020; 71: 762-768.
61. Tahtasakal CA, Oncul A, Sevgi DY, Celik E, Ocal M, Turkkan HM, et al. Could we predict the prognosis of the COVID-19 disease? *J Med Virol* 2021; 93: 2420-2430.
62. Kwon J-S, Kim JY, Kim M-C, Park SY, Kim B-N, Bae S, et al. Factors of severity in patients with COVID-19: cytokine/chemokine concentrations, viral load, and antibody responses. *Am J Trop Med Hyg* 2020; 103: 2412-2418.
63. Tjan LH, Furukawa K, Nagano T, Kiriu T, Nishimura M, Arii J, et al. Early differences in cytokine production by severity of coronavirus disease 2019. *J Infec Dis* 2021; 223: 1145-1149.
64. Sadeghi A, Tahmasebi S, Mahmood A, Kuznetsova M, Valizadeh H, Taghizadieh A, et al. Th17 and Treg cells function in SARS-CoV2 patients compared with healthy controls. *J Cell Physiol* 2021; 236: 2829-2839.
65. Ghazavi A, Ganji A, Keshavarzian N, Rabiemajd S, Mosayebi G. Cytokine profile and disease severity in patients with COVID-19. *Cytokine* 2021; 137: 155323.
66. De Biasi S, Meschiari M, Gibellini L, Bellinazzi C, Borella R, Fidanza L, et al. Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia. *Nat Commun* 2020; 11: 3434.
67. Valizadeh H, Abdolmohammadi-Vahid S, Danshina S, Gencer MZ, Ammari A, Sadeghi A, et al. Nano-curcumin therapy, a promising method in modulating inflammatory cytokines in COVID-19 patients. *Int Immunopharmacol* 2020; 89: 107088.
68. Leija-Martínez JJ, Huang F, Del-Río-Navarro BE, Sanchéz-Muñoz F, Muñoz-Hernández O, Giacoman-Martínez A, et al. IL-17A and TNF-α as potential biomarkers for acute respiratory distress syndrome and mortality in patients with obesity and COVID-19. *Med Hypotheses* 2020; 144: 109935.
69. Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. *J Allergy Clin Immunol* 2020; 146: 110-118.
70. Zhang JJ, Dong X, Cao YY, Yuan YD, Yang YB, Yan YQ, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy* 2020; 75: 1730-1741.
71. Wu C, Chen X, Cai Y, Zhou X, Xu S, Huang H, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Intern Med* 2020; 180: 934-943.
72. Angioni R, Sánchez-Rodríguez R, Munari F, Bertoldi N, Arcidiacono D, Cavinato S, et al. Age-severity matched cytokine profiling reveals specific signatures in Covid-19 patients. *Cell Death Dis* 2020; 11: 957.
73. Tanacan A, Yazihan N, Erol SA, Anuk AT, Yetiskin FDY, Biriken D, et al. The impact of COVID-19 infection on the cytokine profile of pregnant women: A prospective case-control study. *Cytokine* 2021; 140: 155431.
74. de Lucena TMC, da Silva Santos AF, de Lima BR, de Albuquerque Borborema ME, de Azevêdo Silva J. Mechanism of inflammatory response in associated comorbidities in COVID-19. *Diabetes Metab Syndr* 2020; 14: 597-600.
75. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. *JAMA* 2020; 323: 1239-1242.