


Predicting roles of IL-27 and IL-32 in determining the severity and outcome of COVID-19

International Journal of
Immunopathology and Pharmacology
Volume 36: 1–10
© The Author(s) 2022
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/03946320221145827
journals.sagepub.com/home/iji


Batool Zamani¹, Maedeh Najafizadeh², Hossein Motedayyen¹  and Reza Arefnezhad³

Abstract

Objective: Immune changes play fundamental roles in the pathogenesis and severity of coronavirus disease 2019 (COVID-19). Previous studies have revealed alterations in immune responses of patients with non-severe and severe COVID-19. Therefore, this study investigated whether interleukin-27 (IL-27) and interleukin-32 (IL-32) levels may be considered as predicting factors for determining the severity and outcome of COVID-19.

Methods: The blood samples were collected from 50 non-severe and severe patients infected with COVID-19 and 25 healthy subjects. The serum samples were isolated from the whole blood. The levels of IL-27 and IL-32 were measured by enzyme-linked immunosorbent assay and percentages of some immune cells were studied by cell counter.

Results: The levels of IL-27 and IL-32 were significantly higher in COVID-19 patients than healthy subjects ($p < 0.0001$ – 0.01). IL-27 was significantly reduced in severe COVID-19 patients who needed to undergo ICU therapy ($p < 0.05$). Disease severity was significantly associated with IL-27 level in patients with COVID-19 ($p < 0.05$), unlike IL-32 level. There was a significant association between IL-27 and IL-32 in participants ($p < 0.0001$, odds ratio (OR) = 0.9873; 95% confidence interval (CI) = 0.9998 to 1.000; $p < 0.05$, OR = 0.4462; 95% CI = 0.08,579 to 0.7802; $p < 0.01$, OR = 0.6640, 95% CI = 0.3007–0.8590). IL-27 level was significantly higher in the recovered subjects than dead cases ($p < 0.0001$). IL-27 and IL-32 levels in patients who had fever were significantly higher than those who did not have ($p < 0.01$ – 0.05), unlike patients who suffered from cough ($p < 0.001$ – 0.01). The IL-27 level in patients with non-severe COVID-19 was directly correlated with CRP value ($p < 0.05$, OR = 0.5,722,357, 95% CI = 0.06,807,176–0.8,435,928). IL-27 and IL-32 levels in non-severe patients were positively associated with NLR ($p < 0.01$, OR = 0.7292; 95% CI = 0.2809 to 0.9163; $p < 0.01$, OR = 0.6537, 95% CI = 0.1425–0.8896). Patients with severe COVID-19 had a significant increase in NLR ($p < 0.0001$ – 0.05). NLR was significantly correlated with the disease severity ($p < 0.0001$ – 0.05). Survivors had a significant reduction in NLR compared with those who succumbed to COVID-19 ($p < 0.05$).

Conclusion: Change in IL-27 level along with the frequencies of some immune cells may serve as a predictor of the severity and outcome of COVID-19.

¹Autoimmune Diseases Research Center, Kashan University of Medical Sciences, Kashan, Iran

²Infectious Disease Research Center, Shahid Beheshti Hospital, Kashan University of Medical Sciences, Kashan, Iran

³Department of Anatomy, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Corresponding authors:

Hossein Motedayyen, Autoimmune Diseases Research Center, Shahid Beheshti Hospital, Kashan University of Medical Sciences, 5th Kilometer of Ravand Road, Kashan, Iran.

Email: hmotedayyen@gmail.com

Reza ArefNezhad, Department of Anatomy, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.

Email: reza.aref1374@gmail.com



Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (<https://creativecommons.org/licenses/by-nc/4.0/>) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (<https://us.sagepub.com/en-us/nam/open-access-at-sage>).

Keywords

COVID-19, immune response, disease outcome, disease severity, immune changes

Introduction

Coronavirus disease 2019 (COVID-19), as an acute infectious respiratory disorder, is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In December 2019, it was initially reported in Wuhan, Hubei Province, China, and quickly became a pandemic. In addition to pulmonary involvement, different organs can be influenced by SARS-CoV-2 infection, such as renal, cardiac, skin, endocrine, hepatic, gastrointestinal, and neurological systems, which have non-severe and severe phenotypes.^{1,2} Epidemiological studies have indicated that elder patients had greater susceptibility to severe COVID-19, while children tend to have the disease with non-severe symptoms.³ Patients with severe COVID-19 suffer from acute respiratory distress who require hospitalization and mechanical ventilation.⁴ The pathogenesis of the severe disease is largely related to a “cytokine storm,” characterized by a significant increase in pro-inflammatory cytokines levels over a short time period.⁵ These cytokines participate in alveolar exudation and lung damage.⁵ Several studies have indicated that immune changes can be a predictor to determine which patients will develop the severe form of SARS-CoV-2 infection.^{6,7} It is reported that changes in immune cells and different cytokines along with clinical and biochemical indexes can be served as predicting factors for clarifying disease severity and survival for patients with COVID-19.⁸ The reduced numbers of lymphocytes and elevated levels of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are the most frequent abnormalities associated with COVID-19 and contribute to acute lung injury.⁵

Until now, many cytokines and chemokines along with immune cells have been investigated to clarify their roles in COVID-19 pathogenesis and provide a targeted personalized approach for its management.^{8,9} Severe COVID-19 patients show the increased levels of IL-1, IL-8, IL-6, and tumor necrosis factor-alpha (TNF- α), and a decreased expression of IFN- γ .^{6,10,11}

Interleukin-27 (IL-27), a member of the IL-12 cytokine family, is a heterodimeric cytokine composed of p28 and Epstein-Barr virus-induced 3 (EBI3). This cytokine induces antiviral proteins in an IFN-independent way and can exert pro- and anti-inflammatory impacts.¹⁰ IL-27 inhibits differentiations of Th17, Th2 cells, and regulatory T cells (Tregs), while it participates in generations of Th1 and Tr1 cells.^{12,13} Several studies have reported that these impacts are dependent on the surrounding microenvironment of IL-27.^{12,14}

Interleukin 32 (IL-32) as a pro-inflammatory cytokine can stimulate different cell from the immune system through activating signaling pathways of nuclear factor (NF)- κ B and of

mitogen-activated protein kinases (MAPKs). IL-32 gene is transcribed as nine alternative splice variants.⁹ This cytokine is produced by epithelial and various immune cells such as NK cells, T cells, and monocytes. Although the mechanism (s) involved in exerting signaling properties of IL-32 is not well known yet⁵ it can induce immune cells to produce inflammatory cytokines and chemokines, including IL-6, TNF- α , IL-8, and MIP-2/CXCL2.¹⁵ Some reports have revealed that IL-32 plays a key role in pathogenesis of various inflammatory diseases through inducing important inflammatory pathways involved in the expressions of TNF- α , IL-6, and IL-1 β .⁵

Although a few studies have reported the levels of IL-27 and IL-32 in COVID-19,^{8,16,17} impacts of these cytokines on the disease severity and mechanisms involved in their roles in the disease development have not yet been identified. Therefore, this study was focused on determining whether the levels of IL-27 and IL-32 are associated with the severity and progression of COVID-19. We also determined how neutrophil-lymphocyte ratio (NLR) is related to COVID-19 disease severity and outcome. Furthermore, the relationships of IL-27 and IL-32 levels with some laboratory and clinical findings were evaluated.

Materials and methods

Study populations

This work is an analytical observational (case-control) study. A total of 50 patients with COVID-19 (25 cases with non-severe COVID-19 and 25 cases with severe COVID-19) were recruited among 290 individuals referred to the emergency ward of Shahid Beheshti hospital, Kashan, Iran, from March 2021 to December 2021 (Table 1). Disease diagnosis and severity were approved by the specialist according to clinical and laboratory criteria, including cough, fever, fatigue, headache, myalgias, diarrhea, dyspnea, blood oxygen saturation (SpO₂), ESR, CRP, complete blood count (CBC), SARS-CoV-2 RNA detection in respiratory secretions, and chest radiography. Based on the guidelines on the treatment and management of patients with COVID-19,^{18,19} severe illness was defined as dyspnea, a respiratory rate of 30 or more breaths per minute, a SpO₂ of \leq 93% on room air, a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen (Pao₂:Fio₂) of $<$ 300 mmHg, or infiltrates in more than 50% of the lung field. Severe patients needed hospitalization and mechanical ventilation. However, non-severe cases had SpO₂ more than 94% and mild clinical manifestations of SARS-CoV-2. Chest CT scan imaging was used to determine pulmonary involvement as described by previous studies.²⁰ Furthermore, real time-

Table 1. The demographic, laboratory, and clinical features of COVID-19 and healthy individuals.

	Healthy subjects	Non-severe COVID-19	Severe COVID-19	p Value
Age	60.14 ± 18.24	54.06 ± 17.54	60.23 ± 18.63	
Gender	Male: 13 Female: 12	Male: 11 Female: 14	Male: 12 Female: 13	
ESR	9.1 ± 5.1	33.4 ± 29	46.85 ± 33.81	<0.0001
CRP	Negative	47.06 ± 48	99.47 ± 73.85	<0.05
Lymphocyte count	40 ± 7.881	26.01 ± 10.66	19.64 ± 16.06	<0.0001
Neutrophil count	51.12 ± 8.6	68.74 ± 14.2	78.94 ± 10.7	<0.0001
Platelet count	218 ± 42.88	173 ± 61.45	190 ± 51.14	<0.05
RT-PCR	Negative: 25 (100%)	Positive: 25 (100%)	Positive: 25 (100%)	–
GGO	–	5 (20%)	16 (64%)	
Hemoglobin	14.25 ± 1.360	12.28 ± 2.641	12.94 ± 1.343	–
Background diseases	0 (0.0%)	Diabetes: 5 Hypertension: 3 IBD: 1 CVA: 1 ACS: 1	Diabetes: 7 Hypertension: 7 Lung disease (COPD, asthma): 3 Gastrointestinal problems: 1 IHD: 1 CVA: 1 HLP: 2 Kidney stone: 1 Other metabolic disorders: 1	
Anorexia	0 (0.0%)	3 (12%)	7 (24%)	–
Fever	0 (0.0%)	9 (36%)	17 (68%)	–
Smoking history	5 (20%)	0 (0.0%)	3 (12%)	–
Temperature	37.01 ± 0.1	37.74 ± 0.6777	38.98 ± 1.033	–
Headache	0 (0.0%)	2 (8%)	13 (52%)	–
Dyspnea	0 (0.0%)	3 (12%)	21 (84%)	–
Sore throat	0 (0.0%)	2 (8%)	7 (28%)	–
Diarrhea	0 (0.0%)	0 (0.0%)	2 (8%)	–
Vomiting	0 (0.0%)	4 (4%)	6 (24%)	–
Cough	0 (0.0%)	10 (40%)	16 (64%)	–
O2 saturation	98 ± 1.2	94.44 ± 3.464	90.73 ± 5.184	<0.05
Window period	–	6.33 ± 3.95	8.57 ± 5.82	–

HLP: Hyperkeratosis lenticularis perstans; IBD: inflammatory bowel disease; RT-PCR: real time polymerase chain reaction; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; ESRD: end stage renal disease; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; IHD: ischemic heart disease; CVA: cerebrovascular accident; ACS: acute coronary syndrome.

polymerase chain reaction (RT-PCR) assay was performed on nasopharyngeal swab samples collected from all participants and the SARS-CoV-2 RNA was detected at the time of admission. The patients were not on treatment with immunosuppressive agents prior to entering the study. All patients had clinical manifestations of COVID-19 at least 3–5 days before referring to Shahid Beheshti hospital to initiate disease treatments. Exclusion criteria included: (1) patients with autoimmunity, various infectious diseases, malignancy, and other disorders affecting immune responses; (2) individuals on treatment with immunosuppressive agents.

A total of 25 age- and sex-matched healthy volunteers without any history of health problems, autoimmunity, infectious diseases, and malignancy participated as a control group (Table 1). Experimental protocols were approved by the Ethics Committee of Kashan University of

Medical Sciences (IR.KAUMS.MEDNT.REC.1400.003), which were in accordance with the declaration of Helsinki. All participants and legally authorized representatives of dead cases gave the informed consent prior to study initiation. Based on the SD values reported in previous reports,^{6,7} sample sizes were calculated by the following

$$\text{statistical formula: } n = \frac{(Z\alpha + Z\beta)^2 \times (S1^2 + S2^2)}{(m1 - m2)^2}$$

$$\alpha \text{ (study accuracy)} = 95\%$$

$$\beta \text{ (study power)} = 80\%$$

$$\text{Mean difference between group 1 and 2 (m1-m2)} = 0.95$$

$$Z\alpha = 1.96$$

$$Z\beta = 0.83$$

$$S1 = 1.3$$

$$S2 = 1.2$$

Investigations of cytokines and inflammatory mediators. The serum samples were isolated from whole blood (5 mL) of participants and were quantified for IL-27 and IL-32 using an enzyme-linked immunosorbent assay (ELISA) kit (ZellBio GmbH, Ulm, Germany) according to the manufacturer's guidelines. The levels of CRP and ESR of participants were measured using an automated biochemistry analyzer (Mindray BS-800, China) and an ESR analyzer (Parsian Teb, Iran), respectively.

Cell counting

The white blood cells (WBCs) and red blood cells (RBCs) were counted using a BioRad TC20 Automated Cell Counter (Hercules, CA, USA) within several hours after collecting EDTA-treated blood samples (5 mL).

Statistical analysis

The results were analyzed by GraphPad Prism 6 (GraphPad Software, USA) and are represented as the mean \pm standard deviation (SD) and mean \pm standard error of the mean (SEM). The normal distributions of data were studied by the Kolmogorov–Smirnov test. The groups with the normal distributions were analyzed using one-way analysis of variance (ANOVA) and unpaired t-tests, while those with non-normal distributions were compared using Kruskal–Wallis and Mann–Whitney tests. p values less than or equal to 0.05 were considered statistically significant.

Results

Patient descriptions

Fifty subjects with COVID-19 and 25 healthy subjects participated in the study (Table 1). Lymphocyte numbers were significantly higher in healthy subjects than COVID-19 patients ($p < 0.0001$, Table 1). Of 25 patients with severe COVID-19, 10 cases died up to 10 days of hospitalization. The values of CRP in severe cases significantly differed from non-severe subjects ($p < 0.05$, Table 1). A change in ESR level was observed between severe and non-severe patients, although this difference was not statistically significant (Table 1). The demographic and other information of healthy and COVID-19 individuals are indicated in Table 1.

The relationships of IL-27 and IL-32 with COVID-19 severity

The levels of IL-27 and IL-32 in patient and healthy subjects were assessed to determine the correlations of these cytokines with disease severity. As shown in Figures 1(A) and

(B), patients had significant increases in IL-27 and IL-32 levels compared with healthy subjects ($p < 0.0001$ – 0.01). Other results revealed that disease severity was significantly associated with IL-27 level in COVID-19 patients ($p < 0.05$, Figure 1(A)), while this correlation was not observed between IL-32 level and disease severity (Figure 1(B)). In line with these findings, other results indicated that severe COVID-19 patients who required intensive care unit (ICU) therapy had a significant reduction in IL-27 level ($p < 0.05$, Figure 1(C)). However, there was no significant change in IL-32 value between severe patients who needed and did not need ICU therapy (Figure 1(D)).

In the next step, the correlation between IL-27 and IL-32 was evaluated. As shown from Figures 1(E) to (G), IL-27 levels were significantly associated with IL-32 levels in healthy and patient groups ($p < 0.0001$, OR = 0.9873; 95% CI = 0.9998 to 1.000; $p < 0.05$, OR = 0.4462; 95% CI = 0.08,579 to 0.7802; $p < 0.01$, OR = 0.6640, 95% CI = 0.3007–0.8590).

IL-27 and IL-32 levels in survivors and non-survivors

Comparisons of IL-27 and IL-32 levels in patients who survived and died during the disease recovery indicated that the IL-27 level was significantly higher in survivors than non-survivors ($p < 0.0001$, Figure 2(A)). The same trend was observed in IL-32 level of recovered patients, although the increased levels of IL-32 were not statistically significant (Figure 2(B)).

The associations of IL-27 and IL-32 with NLR, clinical, and laboratory findings

To evaluate the relationships of IL-27 and IL-32 levels with clinical and laboratory manifestations of COVID-19, the levels of these cytokines were assessed in patients with various clinical and laboratory features. Our data indicated that the levels of IL-27 and IL-32 were significantly higher in patients suffering from fever than those who did not have ($p < 0.01$ – 0.05 , Figures 3(A) and (B)). IL-27 and IL-32 levels were significantly lower in patients who suffered from cough than individuals who were negative for cough ($p < 0.001$ – 0.01 , Figures 3(C) and (D)). Other statistical analyses showed that IL-27 level in non-severe patients was directly correlated with CRP level (Figure 3(E), $p < 0.05$, odds ratio (OR) = 0.5,722,357, 95% confidence interval (CI) = 0.06,807,176–0.8,435,928). Furthermore, IL-27 and IL-32 levels in non-severe patients were positively correlated with NLR (Figures 3(F) and (G), $P < 0.01$, OR = 0.7292; 95% CI = 0.2809 to 0.9163; $p < 0.01$, OR = 0.6537, 95% CI = 0.1425–0.8896).

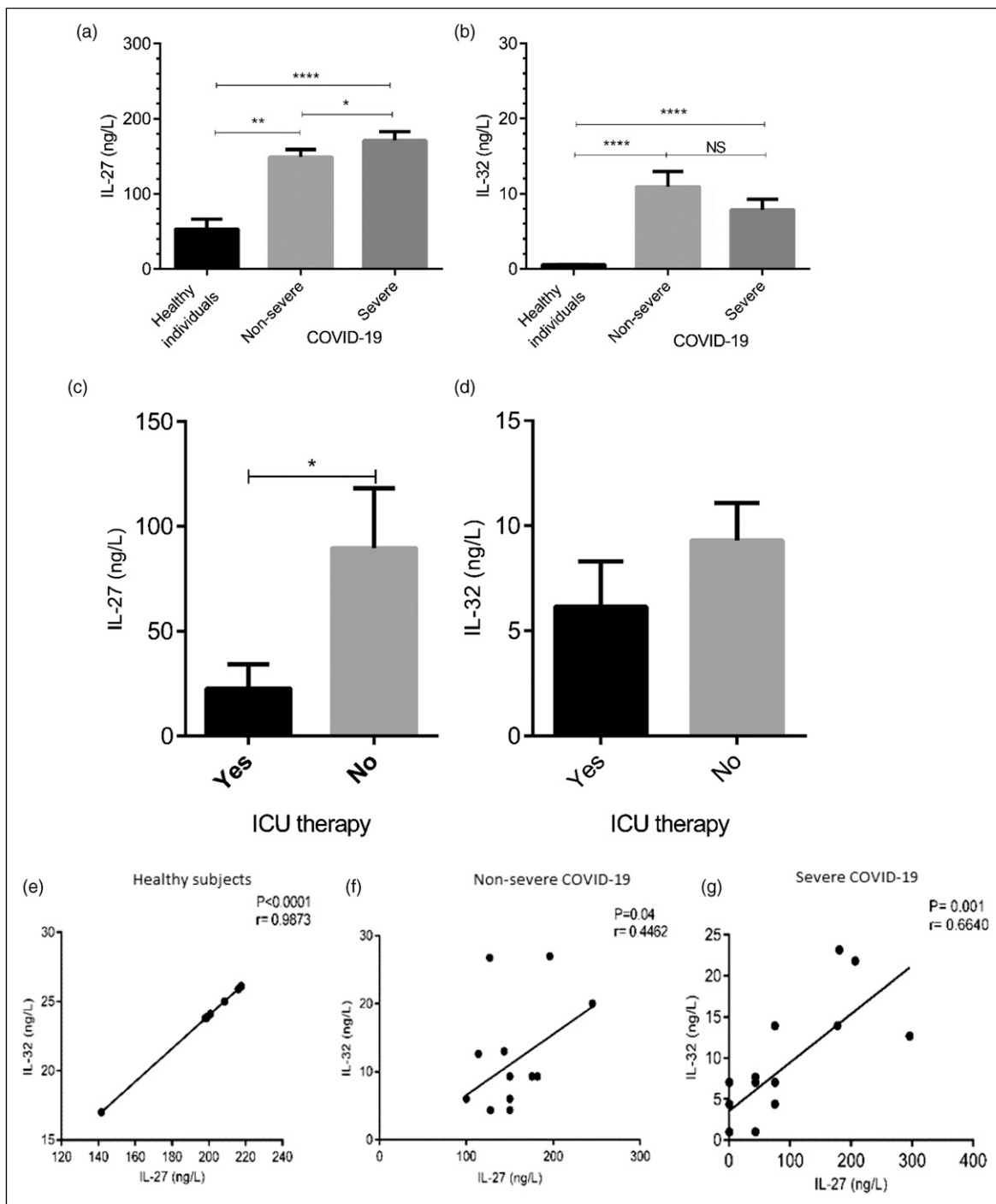


Figure 1. IL-27 and IL-32 values and their correlations in healthy subjects and COVID-19 patients (a–g). (a and b) IL-27 and IL-32 levels were studied by ELISA 19 (25 non-severe cases, 25 severe subjects, and 25 healthy individuals). (c and d) The depicted results are representative of 25 independent experiments for patients with severe COVID-19 (19 severe cases who did not need to ICU therapy and 6 severe subjects who needed to ICU therapy). (e, f, and g) the association between IL-27 and IL-32 was evaluated. IL-27 levels were significantly related to IL-32 levels in healthy and patient groups. All data show mean \pm SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

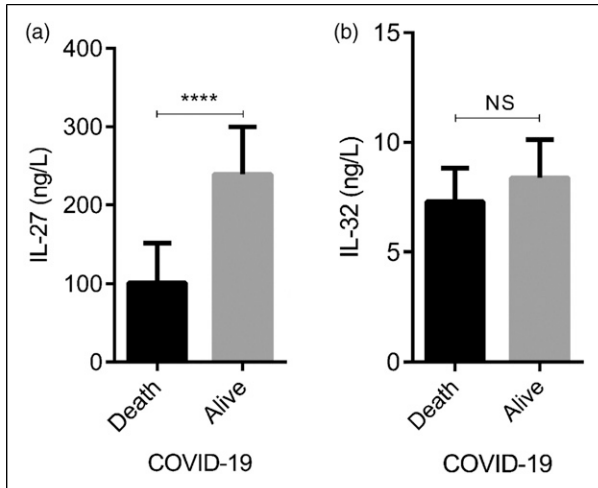


Figure 2. The serum levels of IL-27 and IL-32 in survivors and non-survivors. The levels of IL-27 and IL-32 were studied by ELISA (a and b). The depicted results are representative of 50 independent experiments for patients with COVID-19 (40 recovered subjects and 10 death cases). All data show mean \pm SD. **** $p < 0.0001$.

The correlations of NLR with COVID-19 disease severity and outcome

Other results of the present study demonstrated that severe COVID-19 patients had a significant increase in NLR compared with non-severe cases and healthy subjects ($p < 0.0001$ – 0.05 , Figure 4(A)). NLR was significantly associated with the disease severity ($p < 0.0001$ – 0.05 , Figure 4(A)).

In the next step, NLR was assessed in survivors and non-survivors. The results demonstrated that survivors had a significant reduction in NLR compared with death cases ($p < 0.05$, Figure 4(B)).

Discussion

COVID-19, as an invasive infectious disease, is largely related to defect(s) in immune system functions. There are several studies pointing to imbalance in the immune system which plays fundamental roles in pathogenesis, severity, and outcome of COVID-19.^{6,7} The present study was therefore focused on investigating how IL-27 and IL-32 levels were correlated with the severity and outcome of COVID-19.

Although there are some reports showing anti-inflammatory impacts of IL-27, other studies have mentioned that this cytokine can induce inflammation. These effects are largely dependent on cytokine microenvironment.^{12,14,21} In line with this notion, it is reported that IL-27 has positive impacts on the generations of Th1 and Tr1 cells, while it exerts inhibitory effects on the productions of Th2, Th17 cells, and Tregs.^{12,22,23} Regarding different effects on the immune

cells, its level was investigated in this study. Our data revealed that IL-27 was significantly higher in severe COVID-19 cases than non-severe COVID-19 individuals and healthy subjects. This result was consistent with previous studies pointing to circulating IL-27 positively correlated with more severe form of the disease and older age,⁹ although our data failed to show the association of IL-27 level with age. Another study has revealed that severe COVID-19 cases had an elevated IL-27 in late stages (4 weeks after onset of symptom) of the disease.¹⁶ These observations suggest that IL-27 is associated with disease severity. Furthermore, it is thought that the elevated level of IL-27 in severe COVID-19 is related to disease recovery, due perhaps to its impacts on inducing antiviral proteins and stimulations of some immune cells which play fundamental roles in viral infections.¹⁰ The results of this study were additional confirmation revealing the role of IL-27 in viral infections and determination of disease severity. In this regard, other data showed that IL-27 level in non-severe patients was directly correlated with CRP level, which plays a pivotal role in inducing immune responses against various infectious agents.²⁴ Furthermore, we also observed that severe patients who required ICU therapy had a significant reduction in IL-27 level. Another data of the present study indicated that IL-27 level was significantly higher in COVID-19 survivors than death cases due to COVID-19, which was in contrast with previous reports indicating higher IL-27 in COVID-19 non-survivors and an association between IL-27 and higher mortality in community-acquired pneumonia.^{25,26} This discrepancy observed in our results and other reports may be attributed to disease stage which patients were studied. However, this is a key question that must be illustrated in future studies.

Regarding that IL-32 stimulates immune cells to produce pro-inflammatory cytokines associated with the pathogenesis of COVID-19,¹⁵ IL-32 level was investigated in severe and non-severe COVID-19 patients. IL-32 indicated a significant increase in patients with COVID-19. Other results revealed that severe COVID-19 patients had lower level of IL-32 than non-severe COVID-19 cases, although this reduction was not statistically significant. These findings were in contrast with a study showing IL-32 concentration was significantly higher in healthy subjects than COVID-19 patients.⁸ However, our data were consistent with reports revealing IL-32 level had an increase in patients with non-severe COVID-19 compared with those suffering from severe disease.⁸ Furthermore, it is speculated that IL-32 along with other cytokines may contribute to the increased occurrence of atherosclerosis observed in COVID-19 patients.¹⁷ Other studies on influenza A virus infection, a disease with similar pathogenesis to COVID-19, have revealed that IL-32 production was triggered by

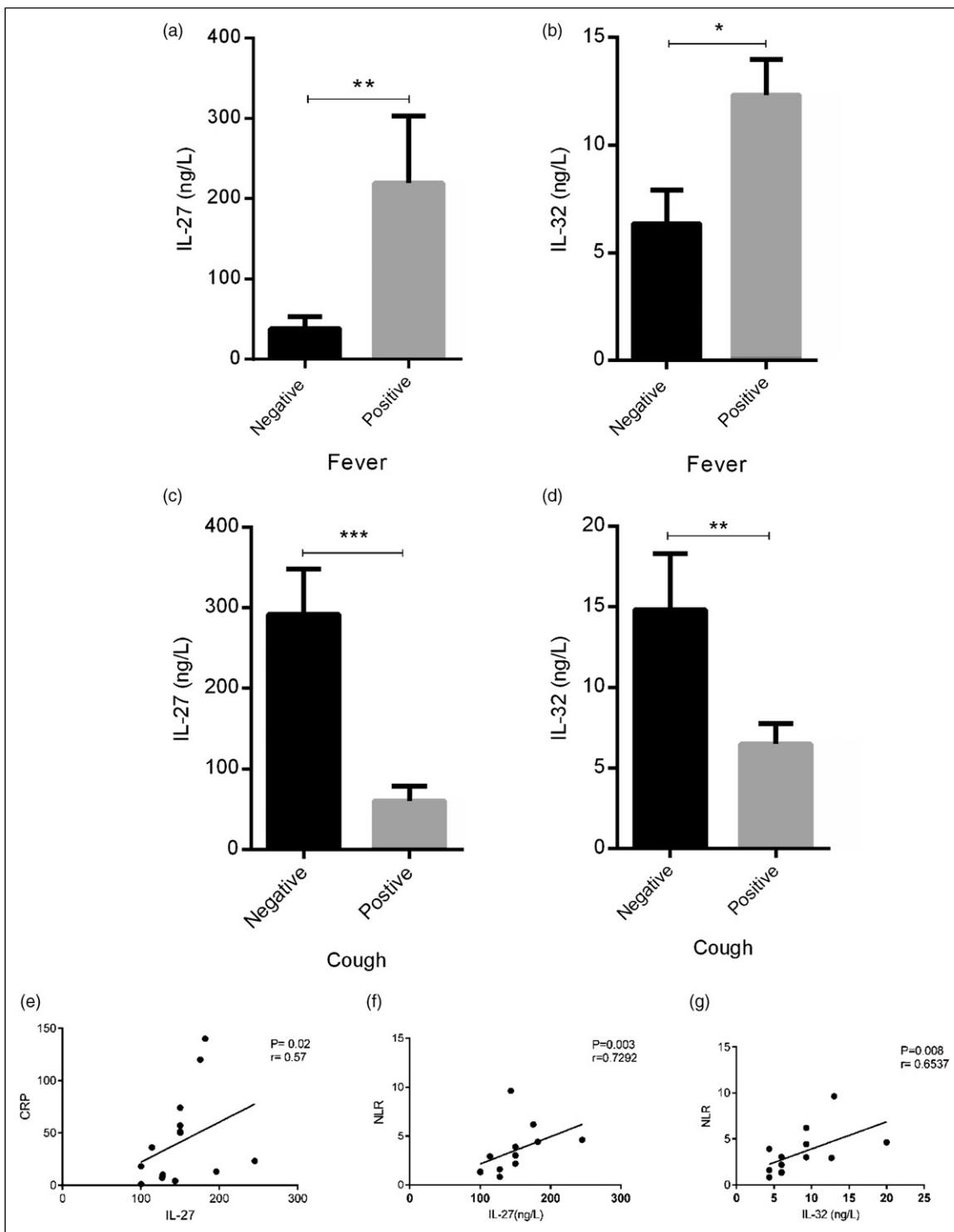


Figure 3. The serum levels of IL-27 and IL-32 in patients who suffered from clinical problems and their correlations with clinical and laboratory features. (a-d). The levels of IL-27 and IL-32 were measured by ELISA. (e) Results of Spearman test showed that IL-27 had a positive correlation with CRP values in non-severe patients ($p < 0.05$). (f and g) IL-27 and IL-32 had positive correlations with NLR in non-severe patients ($p < 0.01$).

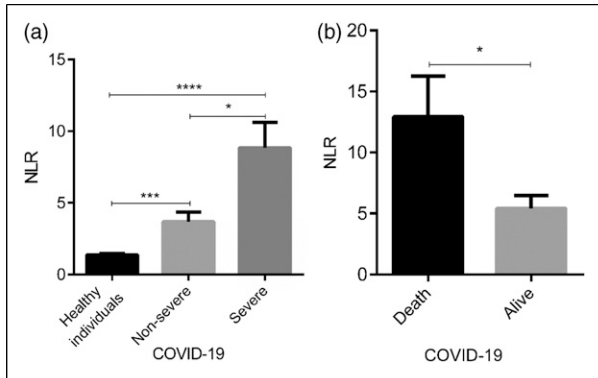


Figure 4. The NLR in severe and non-severe COVID-19 patients and those who recovered and died due to COVID-19. (a) Patients with COVID-19 (25 non-severe cases and 25 severe subjects) showed significant increases in the NLR compared with healthy subjects ($n = 25$). (b) Patients who recovered from COVID-19 had a significant reduction in the NLR compared with death cases. The results are representative of 50 independent experiments for COVID-19 patients who had recovered ($n = 40$) and died ($n = 10$) due to COVID-19. All data show mean \pm SD. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

influenza A virus in human peripheral blood mononuclear cells (PBMCs) from healthy volunteers and, in turn, reduced viral replication.²⁷ In line with antiviral impacts of IL-32, it is shown that IL-32 has anti-HIV-1 activity which can be inhibited by monocyte cloning stimulating factor (M-CSF). To support this notion, we observed that IL-32 level was higher in the recovered subjects than death cases. In addition, our data indicated that IL-27 and IL-32 levels were significantly associated with fever in patients with COVID-19. Further, the levels of IL-27 and IL-32 were inversely related to cough in patients. This finding could be associated with antiviral impacts of IL-32, which participate in improving pro-inflammatory cytokines through activating signaling pathways of MAPK and NF- κ B and thereby control viral infections.

Having considered that several reports have pointed that the NLR can be considered as a reliable indicator of disease severity, the numbers of lymphocytes and neutrophils were investigated in participants. Our results indicated that, along with CRP, ESR, and platelets values, patients had a significant increase in NLR compared with healthy subjects. This finding is consistent with numerous studies showing predictive value of the NLR for diagnosis and severity of the COVID-19.²⁸⁻³⁰ It is reported that elevated NLR was associated with higher COVID-19 mortality and can be considered as a biomarkers for identifying high-risk COVID-19 patients at an early stage.^{29,31,32} Shang et al. revealed that NLR is the best predictor of severe COVID-19 which along with CRP and platelets can effectively predict severe COVID-19.³³ In line with this notion, NLR was investigated in patients who survived or succumbed to COVID-19. Other data indicated that NLR was significantly

higher in survivors than death cases. Moreover, we observed that NLR had positive relationships with IL-27 and IL-32 levels. This finding was further confirmation to clarify predictive value of NLR in determining COVID-19 severity.

Conclusion

Taken together, the findings of the current study provide more evidence to indicate that immune changes can contribute to the severity and outcome of COVID-19 through affecting productions of pro-inflammatory mediators and different immune cells. Although patients with COVID-19 showed a significant increase in IL-32 level, its level was not statistically correlated with disease severity and outcome. It is likely that IL-27 along with CRP, ESR, platelets, and NLR play a key role in determining severity and outcome of COVID-19. Consequently, IL-27 may be considered as a biomarker in assessing severity and outcome of the disease. However, a limitation of the study was no investigation of cellular immune responses along with IL-27 and IL-32. This limitation must be considered to confirm these findings in future studies.

Acknowledgements

The authors would like to thank all subjects who participated in the study.

Authors' contributions

Batool Zamani and Maedeh Najafizadeh participated in the disease diagnosis and sample collections. Reza Arefnezhad carried out some of the experiments and statistical analysis of the data. Hossein Motedayyen participated in the study design, obtained funding for the work, and drafted the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

This study was financially supported by Kashan University of Medical Sciences (Grant No: 99254).

Ethics approval

Ethical approval for this study was obtained from the Ethics Committee of Kashan University of Medical Sciences (ethic code: IR.KAUMS.MEDNT.REC.1400.003).

Informed consent

Written informed consent was obtained from all participants and legally authorized representatives of dead patients gave the informed consent prior to study initiation

ORCID iD

Hossein Motedayyen  <https://orcid.org/0000-0002-9152-981X>

References

- Villapol S (2020) Gastrointestinal symptoms associated with COVID-19: impact on the gut microbiome. *Translational Research*.
- Gavriatopoulou M, Korompoki E, Fotiou D, et al. (2020) Organ-specific manifestations of COVID-19 infection. *Clinical and experimental medicine*: 1–14.
- Yuki K, Fujiogi M and Koutsogiannaki S (2020) COVID-19 pathophysiology: A review. *Clinical immunology* 215: 108427.
- Kim J-Y and Kim H-N (2021) Changes in Inflammatory Cytokines in Saliva after Non-Surgical Periodontal Therapy: A Systematic Review and Meta-Analysis. *International Journal of Environmental Research and Public Health* 18(1): 194.
- Li H, Liu S-M, Yu X-H, Tang S-L and Tang C-K (2020) Coronavirus disease 2019 (COVID-19): current status and future perspectives. *International journal of antimicrobial agents* 55(5): 105951–57.
- Sami R, Fathi F, Eskandari N, et al. (2021) Characterizing the immune responses of those who survived or succumbed to COVID-19: Can immunological signatures predict outcome? *Cytokine* 140: 155439.
- Fathi F, Sami R, Mozafarpour S, et al. (2020) Immune system changes during COVID-19 recovery play key role in determining disease severity. *International journal of immunopathology and pharmacology* 34: 2058738420966497.
- Bergantini L, d'Alessandro M, Cameli P, et al. (2021) *Cytokine Profiles in the Detection of Severe Lung Involvement in Hospitalized Patients with COVID-19: The IL-8–IL-32 axis*. *Cytokine*, 151, 155804.
- Angioni R, Sánchez-Rodríguez R, Munari F, et al. (2020) Age-severity matched cytokine profiling reveals specific signatures in Covid-19 patients. *Cell death & disease* 11(11): 1–12.
- Alexandraki K, Piperi C, Kalofoutis C, et al. (2006) Inflammatory process in type 2 diabetes: The role of cytokines. *Annals of the New York Academy of Sciences* 1084(1): 89–117.
- Talwar D, Kumar S, Acharya S, et al. (2022) Interleukin 6 and Its Correlation with COVID-19 in Terms of Outcomes in an Intensive Care Unit of a Rural Hospital: A Cross-sectional Study. *Indian Journal of Critical Care Medicine* 26(1): 40.
- Meka RR, Venkatesha SH, Dudics S, et al. (2015) IL-27-induced modulation of autoimmunity and its therapeutic potential. *Autoimmunity reviews* 14(12): 1131–1141.
- Yendo TM, Sato MN, Branco ACCC, et al. (2021) Impact of Inflammatory Immune Dysfunction in Psoriasis Patients at Risk for COVID-19. *Vaccines* 9(5): 478.
- Yoshida H and Hunter CA (2015) The immunobiology of interleukin-27. *Annual review of immunology* 33: 417–443.
- Kim S-H, Han S-Y, Azam T, et al. (2005) Interleukin-32: a cytokine and inducer of TNF α . *Immunity* 22(1): 131–142.
- Zhao Y, Qin L, Zhang P, et al. (2020) Longitudinal COVID-19 profiling associates IL-1RA and IL-10 with disease severity and RANTES with mild disease. *JCI insight* 5(13).
- Law CC, Puranik R, Fan J, et al. (2021) Clinical Implications of IL-32, IL-34 and IL-37 in Atherosclerosis: Speculative Role in Cardiovascular Manifestations of COVID-19. *Frontiers in Cardiovascular Medicine*: 882.
- Bhimraj A, Morgan RL, Shumaker AH, et al. (2020) *Infectious Diseases Society of America Guidelines on the Treatment and Management of Patients with Coronavirus Disease 2019 (COVID-19)*. *Clinical Infectious Diseases*, 32, 1–10.
- Berlin DA, Gulick RM and Martinez FJ (2020) Severe covid-19. *New England Journal of Medicine* 383(25): 2451–2460.
- Simpson S, Kay FU, Abbara S, et al. (2020) Radiological society of north America expert consensus document on reporting chest CT findings related to COVID-19: endorsed by the society of thoracic Radiology, the American college of Radiology, and RSNA. *Radiology: Cardiothoracic Imaging* 2(2): e200152.
- Yoshimoto T, Chiba Y, Furusawa JI, et al. (2015) Potential clinical application of interleukin-27 as an antitumor agent. *Cancer science* 106(9): 1103–1110.
- Iwasaki Y, Fujio K, Okamura T, et al. (2015) Interleukin-27 in T cell immunity. *International journal of molecular sciences* 16(2): 2851–2863.
- Aparicio-Siegmund S and Garbers C (2015) The biology of interleukin-27 reveals unique pro-and anti-inflammatory functions in immunity. *Cytokine & growth factor reviews* 26(5): 579–586.
- Li G, Fan Y, Lai Y, et al. (2020) Coronavirus infections and immune responses. *Journal of medical virology* 92(4): 424–432.
- Ozger HS, Karakus R, Kuscu EN, Bagriacik UE, Oruklu N, Yaman M, et al. (2021) Serial measurement of cytokines strongly predict COVID-19 outcome. *PloS one* 16(12): e0260623.
- Xu Z, Wang X-M, Cao P, et al. (2022) Serum IL-27 predicts the severity and prognosis in patients with community-acquired pneumonia: a prospective cohort study. *International journal of medical sciences* 19(1): 74.
- Li W, Sun W, Liu L, Yang F, Li Y, Chen Y, et al. (2010) IL-32: a host proinflammatory factor against influenza viral replication is upregulated by aberrant epigenetic modifications during influenza A virus infection. *The Journal of Immunology* 185(9): 5056–5065.
- Ma Y, Shi N, Fan Y, Wang J, Zhao C, Li G, et al. (2020) *Predictive Value of the Neutrophil-To-Lymphocyte Ratio (NLR) for Diagnosis and Worse Clinical Course of the*

- COVID-19: Findings from Ten Provinces in China*. The Lancet, 1–43.
29. Moradi EV, Teimouri A, Rezaee R, Morovatdar N, Foroughian M, Layegh P, et al. (2021) Increased age, neutrophil-to-lymphocyte ratio (NLR) and white blood cells count are associated with higher COVID-19 mortality. *The American Journal of Emergency Medicine* 40: 11–14.
 30. Nalbant A, Kaya T, Varim C, Yaylaci S, Tamer A and Cinemre H (2020) Can the neutrophil/lymphocyte ratio (NLR) have a role in the diagnosis of coronavirus 2019 disease (COVID-19)? *Revista da Associação Médica Brasileira* 66: 746–751.
 31. Yang A-P, Liu J-P, Tao W-Q and Li H-M (2020) The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. *International immunopharmacology* 84: 106504.
 32. Feng X, Li S, Sun Q, Zhu J, Chen B, Xiong M, et al. (2020) Immune-inflammatory parameters in COVID-19 cases: a systematic review and meta-analysis. *Frontiers in medicine* 7: 301.
 33. Shang W, Dong J, Ren Y, Tian M, Li W, Hu J, et al. (2020) The value of clinical parameters in predicting the severity of COVID-19. *Journal of medical virology* 92(10): 2188–2192.