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RESEARCH LETTER

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Interleukin-29 profiles in COVID-19 patients: Survival is associated with IL-29 levels

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COVID-19, interferon-lambda, interleukin-29, SARS-CoV-2

1 | INTRODUCTION

The 2019-novel coronavirus is the main reason for disaster in the recent century.[1] The innate immune system is the first line of antiviral defense. The host response and clearance of viral infections significantly rely on type I interferon (T1IFN) expression. It is guessed that SARS-CoV-2, similar to other coronaviruses, compromises the early defense against the virus because of delayed-T1IFN production and neutralizing T1IFN signaling through inhibition of STAT family transcription factor phosphorylation. Suppression of these innate immune mechanisms in infected cells permits coronaviruses without an impressive antiviral response to be proliferated. Also, an early burst of T1IFN leads to conservation against viral infections.[2] In this case, it has been demonstrated that the interferons type I and III are the essential components in defense against viruses. Shreds of evidence indicate that SARS-CoV-2 is sensitive to pretreatment with IFN-I/III in vitro. As a viral pathogenic mechanism, CoVs are using different tools for inhibition of induction and signaling of IFN-I.[3]

Interleukin-29 (IL-29) (interferon-λ1) is a newly discovered member of type III interferon.[4] It is mostly produced by maturing dendritic cells and macrophages and involved in many immunological responses and indicates antiviral activity similar to T1IFNs.[5] Pegylated form of IFN-λ1

decreased the disease severity and transmission of SARS-CoV-2 in preclinical investigations on different animal models.[6]

Due to limited research attending the role of IL-29 in COVID-19, it remains undecided whether IL-29 can affect the prognosis of COVID-19 or not. So, in the current study, we aimed to investigate whether IL-29 has a protective role in patients with COVID-19, and decreased levels of this cytokine could predict the severe condition in this disease or not.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

Fifty confirmed cases of COVID-19 from June 8, 2020 to July 14, 2020, in the Shahid Mostafa Khomeini hospital, Ilam, Iran, were included in this retrospective cross-sectional study. All serum samples were collected instantly after hospital admission and were further divided into the severe group (case) who died eventually ($n = 26$) and the nonsevere group (control) who recovered ($n = 24$).

SARS-CoV-2 was diagnosed in all patients according to the WHO interim guidelines. The inclusion criteria for patients were as follows: (1) All patients underwent PCR test for virus nucleic acid of SARS-CoV-2, (2) the ground-glass opacity view in CT scan, (3) adults (over 18 years) who had the informed consent for study participation, and (4) all healthy controls who had not any viral infections confirmed by serological tests. Exclusion criteria were as follows: (1) children and adolescents younger than 18 years old and pregnant women, (2) patients diagnosed with HIV, HBV, HCV, and IAV by serological tests, (3) patients whose cause of death was not justified by COVID-19. This study was approved by the local Ethics committee of the Ilam University of Medical Sciences (IR.MEDILAM.REC.1399.012).

2.2 | Data collection

All dataset around physical and radiological detections, laboratory tests, and demographic characteristics were rolled up from medical records. Also, the time from disease onset to hospitalization, duration of hospitalization, and hospital discharge time were determined for improved patients. The body temperature of all individuals was measured using an infrared thermometer. Venous blood samples were collected from very severe COVID-19 cases, who later died during the current investigation, improved patients. The blood samples were collected in tubes for the serum extraction and allowed to be clotted for 10–15 min at room temperature. Then tubes were centrifuged (at 2000–3000 revolutions per minute) for 10 min, then immediately aliquoted, and stored at −20°C until measurement by the enzyme-linked immunosorbent assay (ELISA).

2.3 | Analysis of IL-29 serum levels

Serum levels of IL-29 from these two study groups were determined by human IL-29 96-well plates ELISA Kit (ZellBio GmbH). After preparing reagents, samples, and the standards, ELISA was carried out according to the manufacturer's instructions. Briefly, undiluted serum samples from each participant were added to the appropriated wells of an anti-IL-29-coated plate in duplicate and incubated for 60 min at 37°C. After discarding the soluble and washing the wells, streptavidin-horseradish peroxidase was added to each well, incubated, washed, then substrate reagent, tetramethylbenzidine (Pishtazteb) was added and incubated for (10 min at 37°C) in the dark. The addition of H_2SO_4 stopped the reaction, and the optical density was read at 450 nm using 630 nm as the reference wavelength.

2.4 | Statistical analysis

All the variables were shown as frequencies and percentages. To compare the continuous variables for data of different patient groups, Mann–Whitney test was used as appropriate. The frequencies of categorical variables were compared using the χ^2 and Fisher's exact test as appropriate. All statistical analyses and graphs were generated and plotted using GraphPad Prism version 9.00 software. The $p < 0.05$ was considered statistically significant.

3 | RESULTS

The clinical data of 26 severe patients, including 84.62% male and 15.38% female with a mean age of 66.38 ± 17.30 were evaluated. Furthermore, the second group included 24 nonsevere patients with a mean age of 52.92 ± 16.45 (75% were male and 25% were female). The most common symptoms of the illness in the recovered group did not show any significant differences in the recovered group in comparison with the patients of the death group. There were no significant differences between the two groups on clinical symptoms ($p > 0.05$). To determine the role of IL-29 in COVID-19 infection and its effect on the mortality of the patients, serum levels of IL-29 were examined in dead patients (before death) as well as recovered patients (Table 1). Using the Mann–Whitney test, the significant differences among the severe (deceased) group patients compared to recovered individuals have been found ($p = 0.007$) (Figure 1). The level of IL-29 cytokine was significantly higher in recovered (0.09217 ± 0.06037) compared to dead patients (0.05158 ± 0.03680). Further analysis of clinical characteristics, common initial symptoms, comorbidity, and laboratory findings of these two groups can be found in Table 1.

4 | DISCUSSION

Since December 2019, SARS-CoV-2 has rapidly infected millions of people from all over the world (JHU data June 5, 2020; https://coronavirus.jhu.edu/). In less than a quarter of cases that developed severe pneumonia, hospitalization, and oxygen therapy are needed. Also, admission to the intensive care unit is essential for patients

TABLE 1 Clinical characteristics, common initial symptoms, comorbidity, and laboratory findings of the admitted patients with coronavirus disease-19

Clinical characteristics and comorbidity findings				
Characteristics		**Recovered patients *N* = 24**	**Dead patients *N* = 26**	***p* Value**
Gender	Male	18 (75%)	22 (84.62%)	0.5
	Female	6 (25%)	4 (15.38%)	
Age (year)	30–44	8 (33.33%)	1 (3.85%)	0.05
	45–59	6 (25%)	11(42.30%)	
	≥60	10 (41.67%)	14 (53.85%)	
Weight (kg)	50–69	4 (16.67%)	9 (36%)	0.2
	70–89	16 (66.66%)	13 (52%)	
	≥90	4 (16.67%)	3 (12%)	
Blood group	A+	15 (65.22%)	8 (42.1%)	0.05
	B+	3 (13.04%)	3 (15.79%)	
	AB+	0 (0%)	1 (5.26%)	
	O+	5 (21.74%)	5 (26.32%)	
	O−	0 (0%)	2 (10.53%)	
Comorbidity	Yes	12 (50%)	17 (65.38%)	0.4
	No	12 (50%)	9 (34.62%)	
Hypertension	Yes	8 (30.77%)	8 (33.33%)	>0.99
	No	16 (69.23%)	18 (66.67%)	
Diabetes mellitus	Yes	6 (25.00%)	7 (26.92%)	>0.99
	No	18 (75.00%)	19 (73.08%)	
Common initial symptoms and laboratory findings				
Findings		**Recovered patients *N* = 24**	**Dead patients *N* = 26**	***p* Value**
Fever	Yes	15 (62.5%)	11 (42.31%)	0.2
	No	9 (37.5%)	15 (57.69%)	
Cough	Yes	19 (79.17%)	18 (69.23%)	0.53
	No	5 (20.83%)	8 (30.77%)	
Fatigue	Yes	9 (37.5%)	15 (57.69%)	0.2
	No	15 (62.5%)	11 (42.31%)	
Shortness of breath	Yes	23 (95.83%)	24 (92.31%)	>0.99
	No	1 (4.17%)	2 (7.69%)	
PCR	Positive	20 (83.33%)	20 (76.92%)	0.73
	Negative	4 (16.67%)	6 (23.08%)	
C-reactive protein	Negative	2 (9.09%)	3 (12.5%)	0.65
	1+	3 (13.64%)	5 (20.83%)	
	2+	5 (22.73%)	3 (12.5%)	
	3+	12 (54.54%)	13 (54.17%)	
Laboratory findings				
Findings		**Recovered patients *N* = 24, mean ± *SD***	**Dead patients *N* = 26, mean ± *SD***	***p* Value**
White blood cell count (cell/mm^3)		7874 ± 3849	11177 ± 3910	0.005
Absolute lymphocyte count		20.35 ± 10.66	12.62 ± 5.954	0.003
Absolute neutrophil count		78.04 ± 11.12	86.35 ± 6.331	0.002
Platelet count (cell/mm^3)		209,130 ± 99,358	192,000 ± 59,053	0.9
IL-29 (pg/ml)		0.09217 ± 0.06037	0.05158 ± 0.03680	0.02

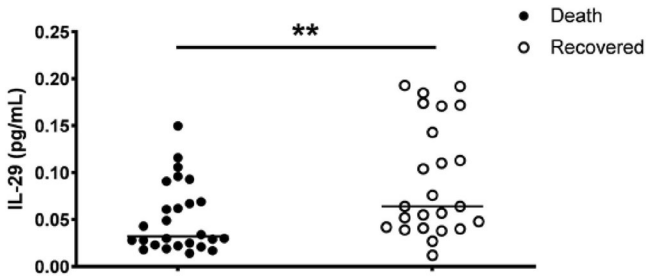

FIGURE 1 The levels of interleukin-29 (IL-29) in COVID-19 patients (recovered and dead). There was a significant difference among the dead patients compared to recovered patients. The serum concentration of IL-29 was demonstrated as mean ± *SD*

suffering from acute respiratory distress syndrome, a complication that probably is the leading cause of death in patients with COVID-19.[7,8]

In the present study, the levels of IL-29 were significantly higher in recovered patients than patients with acute respiratory syndrome who died at the end of the study. This data suggests the protective role of IL-29 in patients with COVID-19 and decreased levels of this cytokine could predict the severe condition in the COVID-19. As we have seen in our study, people who have improved, showed increased levels of IL-29 that may help improve the condition of people with SARS-CoV-2. IL-29 appears to have immune-regulating functions because IL-29 stimulates human cell-derived DCs that proliferate FOXP3-expressing suppressor T cells. Also, previous studies have examined the role of IFN-λ at epithelial levels and reported that IFN-λ and IL-22 have similar roles in epithelial tissue against viral and bacterial infections, respectively.[10] IFN-λ appear to have antiviral activity, tissue-protective and anti-inflammatory properties.[11] The defensive role of interferon type III at the epithelial levels and their importance in the primary line of defense have been shown. Type III IFNs function broadly studied at the anatomic barrier sites and had unique effects on hematopoietic cells, most strikingly neutrophils.[12] However, the important point is how SARS-CoV-2 escapes from the immune system; not much information is available yet.

5 | LIMITATIONS

The limited number of participants can be considered as a limiting factor of the study.

6 | CONCLUSION

Through our survey on the host cytokine effects on the COVID-19 complications, our data suggest the protective role of IL-29 in patients with COVID-19 and decreased levels of this cytokine could predict the severe condition in the COVID-19. Also, high levels of IL-29 might have a correlation with leukocyte, lymphocyte, and neutrophil count in the peripheral blood, which needs more investigation. To achieve better interpretation, evaluating the levels of other cytokines, especially T1IFNs, is helpful. All together higher profiles of IL-29 in patients' immune systems can be accompanied by a better prognosis.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Zahra Fallah Vastani, Alireza Ahmadi, and Sanaz Mami. *Formal analysis*: Motahareh Rouhi Ardeshiri and Abdollah Davodian. *Investigation*: Zahra Fallah Vastani, Alireza Ahmadi, Sanaz Mami, Sajad Mami, Iraj Ahmadi, Mohammadreza Kaffashian, Azra Kenarkoohi, and Shahab Falahi. *Writing – original draft preparation*: Zahra Fallah Vastani, Alireza Ahmadi, Sanaz Mami, Mahdi Abounoori, and Elham Masoumi. *Writing – review and editing*: Zahra Fallah Vastani, Mahdi Abounoori, and Elham Masoumi. All authors have read and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The corresponding author, Sanaz Mami had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

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