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Prognostic value of interleukin-6 serum levels in hospitalized COVID-19 patients: A case—control study in Iran

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Abstract

Introduction: The coronavirus pandemic (COVID-19) is an infectious disease with a high mortality rate that is challenging to treat. Cytokine storm is a crucial factor leading to acute respiratory distress syndrome in COVID-19 patients. Identifying factors that predict the severity of the disease may be primarily prognostic to guide drug therapy. The objective of this study was to investigate the prognostic role of interleukin 6 (IL-6) in the hospitalized patients infected with COVID-19.

Methods: This case-control study was conducted from October 2019 to April 2020 at Shahid Faqihi hospital in Iran. Fifty hospitalized COVID-19 patients and 50 healthy individuals were included while controlling demographics and comorbidities. IL-6 serum levels were measured and compared based on demographic characteristics (age, sex) and comorbidities in the case and control groups. Spearman rank correlation coefficient was also used to analyze the correlations between IL-6 levels and lung involvement in COVID-19 patients. Moreover, some laboratory parameters were compared based on the percentage of lung involvement.

Results: The level of IL-6 in the case group was significantly higher than the control ($p < 0.001$). We observed a positive and significant correlation between the level of IL-6 and the severity of lung involvement ($r = 0.0.79$, $p < 0.01$). The median level of IL-6 in patients who showed more than 75% lung involvement was 573 (IQR = 320−850).

Conclusion: Available evidence suggests that high levels of IL-6 are associated with the severity of COVID-19. According to the results, it could be proposed that inhibition of IL-6 might be a target for therapeutic managements to reduce mortality in the patients with COVID-19.

KEYWORDS

COVID-19, cytokine, interleukin-6, SARS-CoV-2

1 | INTRODUCTION

The Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), emerged from Wuhan, China in December 2019 and spread rapidly throughout the world. In March 2020, the World Health Organization (WHO) declared it a pandemic and throughout the wave shift, the virus has mutated causing new variants to emerge. Five variants of concern (Alpha, Beta, Gamma, Delta, and Omicron) have been identified by WHO by then. Dysfunction of multiple organ and acute respiratory distress syndrome were among the main causes of death in the patients severely infected with COVID-19.[1,2]

Epidemiological studies demonstrated that COVID-19 disproportionately affects different population subgroups depending on their demographic and clinical profiles.[3,4] The mortality rates of inpatient and outpatient are very different and ranged from 14.8% to 30.8% and from 0.28% to 24.3%, respectively.[5] The incidence rate was estimated at 10–30% in nonhospitalized cases and 50–70% in hospitalized cases.[6]

Although vaccination against COVID-19 is being developed, the outbreak of COVID-19 has not been controlled, with an estimated incidence of 10–12% of vaccinated cases. Concerns about the safety and efficacy of current COVID-19 vaccines in preventing infection against new circulating variants could also affect their acceptance.[7] An estimated 10–15% of patients with SARS-CoV-2 may have post-COVID-19 conditions affecting their life quality. For symptom relief, supportive care remains the mainstay of treatment,[8] therefore, there is still a need to update the evidence on COVID-19 to improve the therapeutic approaches.

Cytokine storm, the uncontrolled release of proinflammatory mediators are major risk factors for COVID-19 severity. Among the cytokines, interleukin 6 (IL-6) is one of the key cytokines[9,10] and there are some evidence that blood IL-6 level is positively correlated to disease severity in COVID-19 patients with severe complications.[11,12] To guide the local health authorities and predict the future severity of the disease, the primary objective of this study was firstly to measure the levels of IL-6 in case and control groups as well as in relation to demographic factors, and secondly to determine the relationship between IL-6 levels with the percentage of lung involvement.

2 | MATERIALS AND METHODS

2.1 | Study design

This case-control study was conducted on 50 patients with positive findings confirming SARS-CoV-2 who were admitted to Shahid Faqihi Hospital in Shiraz, Iran, from October 2019, to April 2020 and 50 healthy individuals as control group who were referred to the hospital for medical examinations during the early phase of the COVID-19 pandemic. Before entering to the study, written consent was obtained from the case and control groups. The ethics committee of Shiraz University of Medical Sciences approved this study protocol. (Ethics Code: IR.SUMS.MED.REC.1402.050).

2.2 | Participants' characteristics

The case group was formed by hospitalized COVID-19 patients based on their initial clinical symptoms, chest CT scan and positive qRT-PCR test. The control group involved nonhospitalized patients with negative qRT-PCR, without disease symptoms and any inflammatory and infectious diseases. Demographic information such as age, gender and history of previous diseases and comorbidities were collected. Serum levels of IL-6 were measured by Human IL6 Kit (Karmania Pars Gene) using Luminescence Spectrophotometer Machine.

2.3 | CT quantification evaluation

The case group was subsequently divided into four subgroups according to the findings of the chest CT scan. CT images were analyzed based on the percentage of lung involvement of five lobes and were classified into: (0–25%), (25–50%), (50–75%), and (75–100%).[13] Two radiologists evaluated the images, who were blinded to the clinical data.

Patients who met the inclusion criteria were included if they did not have immunodeficiency and chronic inflammatory disease, did not use immunosuppressant drugs and had informed consent. The patients with doubtful outcomes and missing tests were excluded from the study.

2.4 | Statistical analysis

Descriptive statistics were used to summarize clinical symptoms of COVID-19 which were presented in frequency (percentage). Categorical variables were compared using the Chi-square test and were reported as counts and percentages. A comparison of IL-6 levels between case and control groups was performed using Mann–Whitney test. Spearman rank correlation coefficient was used to investigate the relationship between IL-6 concentration and the lung involvement percentage. A comparison of IL-6 levels and laboratory inflammatory test based on the severity of lung involvement on CT scan were performed with Kruskal–Wallis tests. Non parametric data are expressed as the median and interquartile range (IQR). All analyses were performed by SPSS (version 26) and $p < 0.05$ were considered as statistically significant.

3 | RESULTS

3.1 | Characteristics of hospitalized COVID-19 patients

A total of 100 participants (50 patients and 50 healthy individuals) were included in this study. Fifty patients with a confirmed COVID-19 infection were hospitalized. Patients were 24 men and 26 women with a median age of 36.5 years (IQR = 31.5–46.25).

The frequency of reported clinical finding in COVID-19-infected patients is shown in Figure 1. Oxygen saturation less than 90% was the

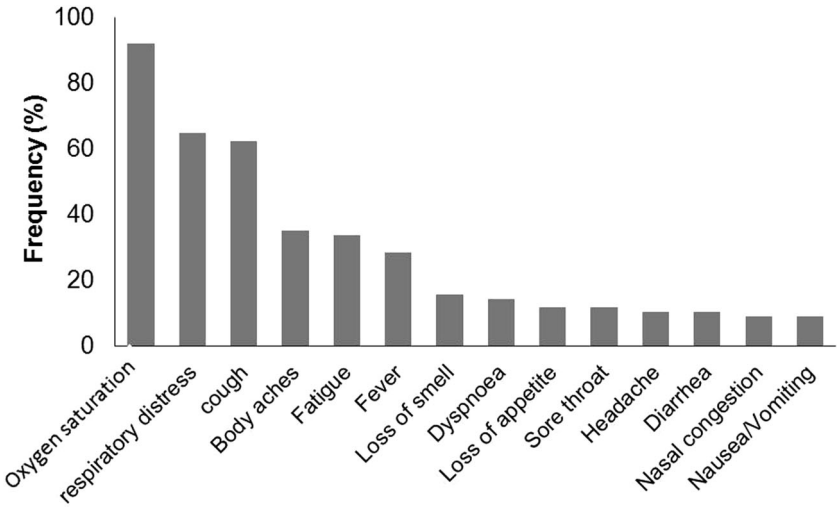

FIGURE 1 Percentage frequency of clinical findings of COVID-19-infected patients. COVID-19, coronavirus disease 2019.

TABLE 1 Distribution of cases and controls according to age, sex, and comorbidities.

Variable		Groups		*p* Value
		Control	COVID-19	
Age (No. of patients, %)	Median (IQR)	33(19)	36.5 (14.75)	0.40
	<50	34 (68)	40 (80)	0.17
	>50	16 (32)	10 (20)	
Sex (*n*, %)	Male	18 (36)	24 (48)	0.22
	Female	32 (64)	26 (52)	
Comorbidities (*n*, %)	Yes	7 (14)	9 (18)	0.59
	No	43 (86)	41 (82)	

most frequent sign (92.2%), followed by respiratory distress (64.8%), and cough (62.3%).

3.2 | Demographic factors and comorbidities description

The distribution of demographics and comorbidities are presented in Table 1. The median age in case group was higher than the control group (36.5 vs. 33), which were not significantly different ($p = 0.4$). Participants were divided based on their age into two groups: <50 and ≥50 years. Most of the participants were under 50 years old. No statistically significant differences were found between the two groups with regard to their age, so that 40 (80%) patients in the case group and 34 (68%) in the control group were under 50 years old ($p = 0.17$).

Women outnumbered in both groups, 26 (52%) in the case group compared to 32 (64%) in the control group, while the gender difference in the case group compared with the control group was not statistically significant ($p = 0.22$).

Comorbidities were rare and similar ($p = 0.59$) between control and case groups; only 9 (18%) patients in the case group and 7 (14%) in the control group.

3.3 | Comparison of IL-6 levels in serum samples and correlations with demographic factors and comorbidities

Differences in medians (Q1–Q3) of IL-6 level in case 42 (17.47–72.25) and control 5.69 (4.88–7.92) groups are shown in Figure 2. Using Mann–Whitney *U* test, it was demonstrated that the serum IL-6 levels were significantly higher in the case group than in control group ($p < 0.001$).

IL-6 levels in relation to demographic factors in the case and control groups are presented in Table 2. There were no significant differences between the IL-6 levels with regard to age, sex, and comorbidities in the case and control groups.

The linear regression showed a negative correlation between age as a risk factor and IL-6 levels in the case group ($r = -0.12$, $p = 0.18$). Also, IL-6 level was negatively correlated to the age in the control group ($r = -0.13$, $p = 0.19$). No significant correlation was observed between risk factors associated with IL-6 levels in the case and control groups.

3.4 | Association of serum levels of IL-6 with chest CT scan

The median value of lung involvement in patients was 47 (IQR = 32.75–65). CT scan showed that 6 patients (12%) had lung involvement less than 25%, 20 patients (40%) between 25 and 50, 19 patients (38%) between 50 and 75 and five patients (10%) higher than 75%. There was a significant positive correlation between IL-6 levels and lung involvement as indicated by chest CT (Figure 3; $r = 0.0.79$, $p < 0.01$).

The median IQR values of IL-6 for the group with lung involvement below 25% were 14 (IQR = 11.75–42), 21.5 (9.65–29.27) and 58 (44–85) for the group with lung involvement between 25 and 50% and 50–75%,

respectively and 573 (320–850) for the group with lung involvement above 75% (Figure 4). The values with the severity of lung involvement were statistically significant ($p < 0.001$).

The median and IQR values of Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein (CRP) and lymphocyte count in patients were 31 (22.25–42), 51 (34.75–71.5) and 10 (6.5–15.1), respectively. The mean values of the above factors according to lung involvement are presented in Table 3 which shows that there was no meaningful relationship between these factors with the severity of lung involvement.

4 | DISCUSSION

In this case-control study, it was demonstrated that serum levels of IL-6 were significantly increased in the hospitalized COVID-19 patients compared to the control groups. No significant differences were observed between the two groups in terms of the distribution

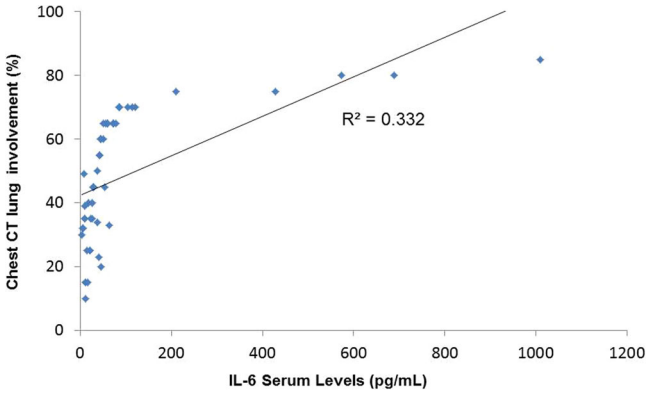

FIGURE 3 Correlation between percentage of lung involvement in chest CT scan and IL-6 levels in COVID-19 patients. Lung involvement values showed a linear trend with elevating rate of IL-6. COVID-19, coronavirus disease 2019; IL-6, interleukin 6.

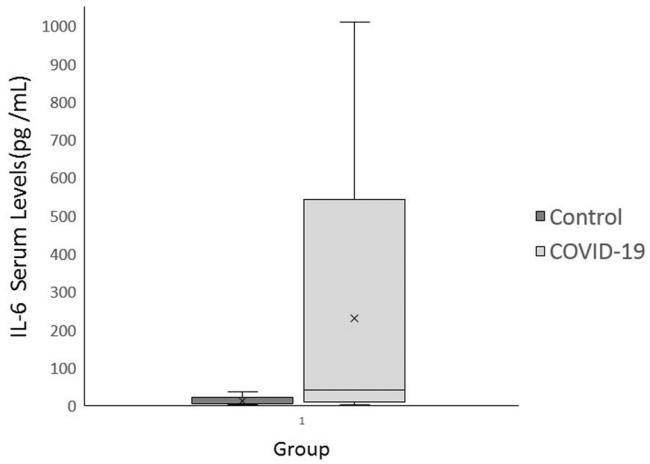

FIGURE 2 Comparison of IL-6 levels in control and case groups. Data were presented as mean ± SE *Present significant changes at $p < 0.05$. IL-6, interleukin 6.

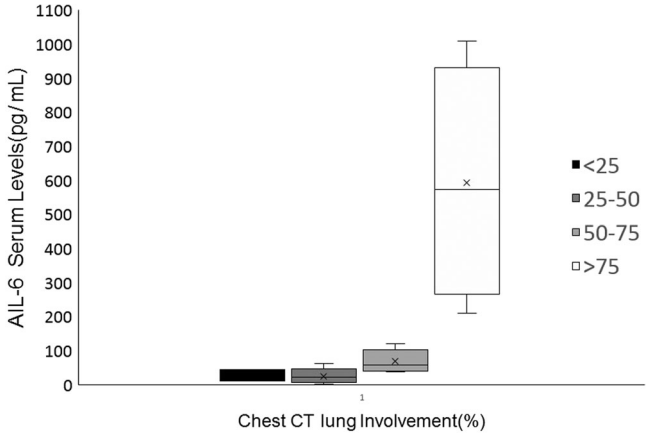

FIGURE 4 The difference in mean of IL-6 according to lung involvement on CT scan in the COVID-19 patients. The y-axis represents the classification of lung involvement. COVID-19, coronavirus disease 2019; IL-6, interleukin 6.

TABLE 2 The levels of IL-6 in relation to demographic variables in the case and control groups.

		Groups			
		Control		COVID-19	
Variable		IL-6	*p* Value	IL-6	*p* Value
Age (No. of patients, %)	<50	8.37 ± 1.24	0.15	107.06 ± 32.73	0.37
	>50	6.70 ± 1.23		47.37 ± 13.99	
Sex (*n*, %)	Male	9.45 ± 2.30	0.34	94.63 ± 38	0.99
	Female	6.99 ± 0.79		95.58 ± 37.50	
Comorbidities (*n*, %)	Yes	10.80 ± 4.37	0.82	57.58 ± 13.21	0.51
	No	7.40 ± 0.85		103.36 ± 32.06	

Abbreviation: IL-6, interleukin 6.

TABLE 3 difference between means of laboratory tests according to lung involvement on CT scan in the COVID-19 patients.

	Lung involvement (%)				
Biomarker	**<25%**	**25–50%**	**50–75%**	**>75%**	***p* Value**
ESR (mm/h)	31.2 ± 9.9	39.0 ± 5.9	32.6 ± 2.8	31.3 ± 12.9	0.75
CRP(mg/L)	46.4 ± 9.4	55.3 ± 6.2	53.2 ± 4.7	46.6 ± 21.5	0.80
lymphocyte (count)	12.7 ± 3.7	15.3 ± 2.3	11.5 ± 1.3	7.5 ± 2.9	0.39

Abbreviations: COVID-19, coronavirus disease 2019; CRP, C-Reactive Protein; ESR, Erythrocyte Sedimentation Rate.

of demographic variables (age and gender) and underlying diseases, and the confounding effect of the above factors on IL-6 level. Therefore, the observed differences in IL-6 concentration between the two groups might be attributed to COVID-19 infection.

Many studies found a strong association between male gender and disease severity was observed.[14,15] Also, Epidemiological data throughout the world suggest that older age (~>50) and comorbidities are the major risk factors for a severe episode of COVID-19.[16,17] However, no significant difference with the mentioned factors in our study.

A retrospective study in Qatar showed that the majority of patients were men between 36 and 50 years during the first and second waves of the pandemic.[18] A cohort study in Iran reported that there was no significant association between gender and death rate.[19]

Our data also indicate that increased levels of IL-6 is significantly associated with the severity of lung involvement on CT scan in hospitalized COVID-19 patients, which is in the line with other studies reporting high levels of inflammatory cytokines, mainly IL-6 in COVID-19-affected patients with severe complications.[20]

Similar to our findings, a cross-sectional study in Romania showed a positive correlation ($r = 0.23$, $p = 0.04$) between IL-6 and lung damage on CT with median values of 8.66 and 46.49 with lung involvement below and above 50%, respectively, ($p < 0.001$).[21]

Most studies show that increased CRP levels are associated with severe COVID-19 symptoms, while our data indicated that increased levels of inflammatory markers (ESR and CRP) in patients were not significantly correlated with lung involvement. It is also interesting to note that among the investigated inflammatory markers, IL-6 level is a promising candidate for predicting disease severity.

A retrospective study of patients treated with monoclonal antibodies suggested that the IL6/lymphocyte count ratio is a valuable monitor to identify patients who need more treatment and support both in earlier and later stages of the disease.[22]

Moreover, many studies showed that in addition to risk factors such as old age and underlying comorbidities, several markers such as serum levels of CRP, D-dimers, ferritin, cardiac troponin and IL-6, are associated with mild and severe disease and may be used as prognostic markers. For example, IL-2, IL-6 IL-7, and IL-10 were significantly associated with disease severity. However, more studies with different patient origins and meta-analyses are needed to further support clinical decision-making.[23]

A prospective cohort study measure serum IL-6 level in Turkish patients with COVID-19 and reported relationship between the IL6 rs1800795 variant and IL6 level in asymptomatic, symptomatic, and intensive care unit patients.[24]

One possible mechanism for the rapid progression of disease and mortality is the cytokine storm. Therefore, determining the amount of IL-6 may be crucial to find an appropriate and effective treatment protocol in COVID-19 patients. The role of biological inhibitors of key cytokine pathways including siltuximab, sarilumab, and tocilizumab has been evaluated by several clinical trials as a treatment for COVID-19 and promising preliminary results have been reported.[25]

5 | CONCLUSION

The results obtained in our study confirmed that high levels IL-6 were correlated with severity of lung involvement based on chest CT scans and can be used to predict disease severity. This may be useful in clinics where CT evaluation is not available. Also, evaluation of IL-6 may be crucial to find an appropriate therapeutic protocol in COVID-19 patients. To understand geographic regional differences in the COVID-19 pandemic, this study was conducted on the IL-6 levels in COVID-19 patients for the first time in Iran. However, this study had several limitations including the small sample size, lack of follow-up and measurement of the IL-6 after treatment. Validation of these results requires future studies.

AUTHOR CONTRIBUTIONS

Asiyeh Shojaee: Methodology, writing—review and editing. reza rafiee: investigation, visualization, formal analysis. **Masoud Hosseinzadeh**: Supervision. **Mohamad Saboori**: Visualization, collected data. All authors have read and approved the final version of the manuscript. Corresponding authors 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.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

ETHICS STATEMENT

The ethics committee of Shiraz University of Medical Sciences approved this study protocol. (Ethics Code:IR.SUMS.MED.REC.1402.050). Before entering to the study, written consent was obtained from the case and control groups

TRANSPARENCY STATEMENT

The lead author Asiyeh Shojaee, Masoud Hosseinzadeh affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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