

Diagnosis of Coronavirus disease by measuring serum concentrations of IL-6 and blood Ferritin

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Research

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Abstract

Objectives: Coronavirus disease 2019 (COVID-19) associated by infection and high death rate. The unresolved questions about the fatality rate of COVID-19 is most probably related to cytokine storm syndrome. There is currently no specific medication. Understanding the pathogenic pathway of this disease will lead to production of treatment and decreases of death rate. The aim of this study is to investigate changes of peripheral blood parameters (Interleukin-6 and Ferritin) in COVID-19 patients, which may be beneficial in the management of patients.

Methods: In this case-control study, we collected data of 270 subjects in two groups including 133 patients with severe type COVID-19 (case) and 137 patients with nonsevere (control) between March 20 and May 21, 2020, and the clinical symptoms and inflammatory indications of patients diagnosed by laboratory test in Shahid Jalil hospital of Yasuj University of medical Sciences were collected to explore potential markers for disease monitoring. The data were analyzed by SPSS software version 20. Descriptive statistics, **T-test and bivariate correlation tests** were used to analyze.

Results: The enrolled COVID-19 patients consisted of 53.4% males and 46.6% females with the medium age of 45.56 ± 18.55 years and there were 50.04% males and 49.6% females with the medium age of 45.59 ± 17.0 years for non COVID-19 patients. There was no significant difference in the age and sex ratio between two population under study.

The proportion interstitial abnormalities evidenced by CT imaging in COVID-19 patients was 91.0%, while, 4.4% abnormalities was found in non COVID-19 patients. The frequency of positive RT-PCR test for case and control groups were 88.0% and 3.6%, respectively.

The mean IL-6 and Ferritin levels and hematological parameters in two groups of patients with COVID-19 and non- COVID-19, were significantly different across all comparisons.

There was a direct positively correlated between serum level of IL-6, Ferritin levels and hematological parameters including WBC, Lymphocytes, Neutrophils and Hb, except for platelets (negatively correlate), with COVID-19.

Conclusions: In conclusion, inflammatory markers specifically IL-6 and Ferritin and hematological parameters (WBC, Lymphocytes, Neutrophils, Platelet and Hb) were correlated with the severity of COVID-19. Measurement of IL-6, Ferritin and hematological indices might be workable tests to diagnosis and prognosis of patients with COVID-19.

Introduction

Coronavirus disease 2019 (COVID-19) or the severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), it was first reported in December 2019 in Wuhan City, China(1). COVID-19 is an infectious disease by typical symptoms include dry cough, muscle aches or fatigue, high fever and shortness of breath with severe acute respiratory syndrome (ARDS) on CT scans (2–4).

Coronavirus binds to the angiotensin-converting enzyme2 (ACE2) which is present on no immune cells, such as respiratory and endothelial cells and immune cells such as alveolar monocytes/macrophages(5), and can induce endogenous stress signals or mutations in the host genome and recruitment of inflammatory cells(6). In the defensive line, innate immunity senses microorganisms and release of cytokines, Chemokines and inflammatory mediators by immune cells, epithelial cells and etc. in pathological conditions(7). The increase in specific cytokine production can be linked to an activation cascade and uncontrolled cytokine release; the cytokine storm(8). Although cytokines expression is strictly controlled by transcriptional and posttranscriptional mechanisms, but high-level concentrations of cytokines for a long time leads to chronic inflammatory diseases and widespread tissue damage including ARDS(9, 10).

During a virus infection, the plasma concentration Interleukin (IL)-6, IL-1 and IL-10 were increased(11), especially, IL-6, which promptly stimulate the production of acute phase proteins in response to infections and tissue injuries (7). IL-6 alterations, reflect the presence and severity of inflammation, and they have long been used as a clinical Guideline for diagnosis and management of diseases. (11, 12).

Also, high levels of lactate dehydrogenase (LDH), and ferritin are suggestive due to the cytokine storm in Laboratory findings. Also, elevated Ferritin and Lactate dehydrogenase (LDH) levels due to cytokine storm syndrome have been reported in severe COVID-19 patients (13). In hospitalized patients, serum ferritin test- which is widely available and affordable-is a good qualified screening tool for informing physicians of cytokine storm syndrome for COVID-19 patients(14).

In general, COVID-19 in infected people of all ages, can be implications on different organs as lungs, brain and nervous system and eventually, may lead to death (15). Therefore, diagnose and treatment of cytokine storm and infection by measuring serum concentrations of IL-6 and blood ferritin has become an important part of rescuing severe patients.

Material And Methods

Samples Data collection

In this case-control study, we collected data of 270 subjects in two groups including 133 patients with severe type COVID-19 (case) and 137 patients with non-severe (control), between March 20 and May 21, 2020, and the clinical characteristics and inflammatory indications of patients which diagnosed by laboratory test in Shahid Jalil hospital of Yasuj University of medical Sciences, were collected to explore potential markers for disease monitoring.

In this study, General and specific biosafety guidelines for diagnosis of COVID-19 was accordance with the WHO guidance. A confirmed positive case for COVID-19, was assessed by real-time reverse transcriptase polymerase chain reaction (RT-PCR) test from the nasopharyngeal or oropharyngeal swabs specimens. Only laboratory confirmed cases were contained in this study, while disease diagnosed based on clinical presentation and CT imaging findings were excluded.

In this study, demographic data, clinical symptoms, imaging examinations, previous nucleic acid test results, and other laboratory findings, CT Scanning, whole blood cell count and blood chemistry of patients were collected from electronic medical records using data collection forms. In addition, ferritin, lymphocyte subset and cytokine tests was analyzed in severe patients by Flow cytometry and ELISA detection.

ELISA detection and Blood Count Test

In this study, Complete Blood Count (CBC) Test, were measured using a Sysmex KX21 Hematology Analyzer (Block Scientific, Bohemia, NY, USA).

Serum concentrations of IL-6 were also measured in in all COVID-19 and non COVID-19 samples. IL-6 levels were measured by the enzyme-linked immunosorbent assay (ELISA) kit (Gen-Probe, Diaclone France) as per the manufacturer's instructions.

Human serum ferritin concentrations were determined by using a diagnostic kit from Abbott Laboratories (Abbott Park, IL). All tests were performed according to the product manual.

Statistical analysis

All data were analyzed by descriptive statistics include: Chi-squared for categorical variables and averages and standard deviations for continuous data. Continuous variables were compared using the Student T-test between groups. The Pearson's correlation coefficient (r) was used to understand the correlations between various laboratory findings. Data were carried out by SPSS software version 20. For all statistical analysis, p-value less than 0.05 (typically ≤ 0.05) is statistically significant.

Ethical considerations

Since, there was no threat for participants and its results were only used to improve diagnosis planning of covid19 ; so we obtained informed consent. It should also be noted that in this study, we avoided mentioning any names, titles or characteristics that cause the loss of privacy of the participants, and the participants were clearly informed of the possible uses of the results.

Results

Demographic and clinical characteristics

The enrolled COVID-19 patients(case) consisted of 53.4% (71) males and 46.6% (62) females with the medium age of 45.56 ± 18.55 years old and there were 50.04% (69) males and 49.6% (68) females with the medium age of 45.59 ± 17.0 years old for non COVID-19 patients (control).

There was no statistical evidence for different association in the ages and sex between two case and control groups (Table 1).

The percentage of lung abnormalities on CT imaging in COVID-19 patients was 91.0% (121), while, 4.4% (6) abnormalities was found in imaging of non COVID-19 patients ($p < 0.05$) (Table 1).

Also, Table 1 shows the frequency of RT-PCR test results in the case and control samples. The frequency of positive results for case and control groups were 88.0% (117/133) and 3.6% (5/137), respectively.

Table 1
Describe the clinical characteristics of patients with COVID-19 and non- COVID-19

Variables		Case N (%)	Control N (%)	P-Value
Gender	Male	71(53.4)	69(50.4)	0.628
	Female	62(46.6)	68(49.6)	
Age	40 > yrs	65(48.9)	58(42.3)	0.480
	40–60 yrs	40(30.1)	50(36.5)	
	60 < yrs	28(21.1)	29(21.2)	
Mean ± SD		45.56 ± 18.55	45.59 ± 17	
CT	positive	121(91.0)	6(4.4)	0.000
	negative	12(9.0)	131(95.6)	
RT-PCR	positive	117(88.0)	5(3.6)	0.000
	negative	16(12.0)	132(96.4)	

ELISA Detection And Blood Count Test

Significant differences were found between the case and control groups on ELISA detections. These studies also reported the levels of serum IL-6 and Ferritin, which are especially high in COVID-19 patients (283.53 ± 115.21 pg/ml and 399.87 ± 143.31 pg/ml) compared to non-COVID-19 patients (44.07 ± 29.80 pg/ml and 150.63 ± 93.44 pg/ml) ($p < 0.05$) (Table 2).

Also, data analysis showed significant differences in hematological parameters (WBC, Lymphocyte, Neutrophil, Platelet and Hb) between COVID-19 patients and non- COVID-19 group ($p < 0.05$) (Table 2).

Table 2
Comparison of the laboratory findings of patients with COVID-19 and non-COVID-19.

Variables		Case N (%)	Control N (%)	P-Value
Ferritin (pg/ml)	≤ 240	48(36.1)	117(85.4)	0.000
	> 240	85(63.9)	20(14.6)	
	Mean ± SD	399.87 ± 143.31	150.63 ± 93.44	
IL-6 (pg/ml)	≤ 20	3 (2.3)	56(40.9)	0.000
	> 20	130(97.7)	81(59.1)	
	Mean ± SD	283.53 ± 115.21	44.07 ± 29.80	
WBC (UL)	< 4	0(0.0)	5(3.6)	0.001
	4–10	(83.5) 111	131(95.6)	
	Mean ± SD	> 10 22(16.5)	1(0.7)	
		8.56 ± 1.50	6.10 ± 1.40	
Lymphocyte (%)	< 18	15(11.3)	17(12.5)	0.001
	18–35	50(37.6)	85(62.0)	
	Mean ± SD	> 35 68(51.1)	35(25.5)	
		34.48 ± 10.67	30.60 ± 9.90	
Neutrophil (%)	< 45	2(1.5)	22(16.1)	0.001
	45–65	25(18.8)	82(59.8)	
	Mean ± SD	> 65 106(79.7)	33(24.1)	
		72.67 ± 10.40	58.87 ± 12.06	
Platelet (× 1000 UL)	≤ 150	24(18.0)	0(0.0)	0.001
	150–450	109(82.0)	39(90.7)	
	Mean ± SD	> 450 0(0.0)	4(9.3)	
		184.54 ± 43.30	256.97 ± 81.11	
Hb (mg/dL)	< 12	8(6.0)	10(7.3)	0.000
	12–16	64(48.1)	113(82.5)	
	Mean ± SD	> 16 61(45.9)	14(10.2)	
		15.66 ± 1.88	14.32 ± 1.55	

The means comparison of IL-6, Ferritin and hematological parameters in different groups were showed in Table 3. There was a high statistically significant in mean concentrations of IL-6, Ferritin, WBC, Lymphocyte, Neutrophil, Platelet and Hb, in COVID-19 group compared to non- COVID-19 group. All findings between two groups were significant at level $p < 0.05$ (Table 3).

Table 3
The means comparison of IL-6, Ferritin and hematological parameters between COVID-19 and non- COVID-19 groups.

Variables	Mean Difference	P-Value	CI (95%)	
			Lower	Upper
Ferritin	156.19	.000	129.69	182.70
IL-6	243.45	.000	222.27	264.6
WBC	2.45	.000	2.10	2.80
Lymphocyte	3.88	.002	1.40	6.35
Neutrophil	13.79	.000	11.09	16.50
Platelet	-71.80	.000	-89.25	-54.34
Hb	1.34	.000	0.93	1.75

Data analysis revealed that there was a direct positively correlation between the serum levels of IL-6 with Ferritin ($r = 0.509$, $p = 0.000$) and hematological markers includes WBC($r = 0.539$, $p = 0.000$), Lymphocyte ($r = 0.197$, $p = 0.001$), Neutrophil ($r = 0.431$, $p = 0.000$) and Hb($r = 0.344$, $p = 0.000$), while it was an inverse correlation with Platelet ($r = -0.361$, $p = 0.001$), (Table 4).

Based on our assessment, a significant correlation was identified between the serum levels of Ferritin and the following parameters: direct positively correlation with IL-6 ($r = 0.509$, $p = 0.000$), WBC($r = 0.433$, $p = 0.000$), Neutrophil ($r = 0.420$, $p = 0.000$) and Hb($r = 0.226$, $p = 0.000$), direct negatively correlation with Platelet ($r = -0.357$, $p = 0.000$) and non-correlation with Lymphocyte ($r = .076$, $p = .211$) (Table 4).

In our study, a negative direct relationship was identified between Platelets with IL-6($r=-0.361$, $p = 0.000$), Ferritin($r=-0.357$, $p = 0.000$) and all hematological indices WBC($r=-0.374$, $p = 0.000$), Lymphocyte ($r=-.159$, $p = 0.009$), Neutrophil ($r=-0.272$, $p = 0.000$) and Hb($r=-0.280$, $p = 0.000$). (Table 4).

In addition, data analysis revealed that there was non-significant correlation in Lymphocyte with WBC ($r = 0.118$, $p = 0.052$), Neutrophil ($r=-0.071$, $p = 0.243$) and Ferritin ($r=-.076$, $p = 0.211$) (Table 4).

Table 4. The Correlation between IL-6 and Ferritin and hematological parameters in COVID-19 patients.

Variables		Ferritin	IL-6	WBC	Lymphocyte	Neutrophil	Platelet	Hb
Ferritin	R	1	.509**	.433**	.076	.420**	-.357**	.226**
	P		.000	.000	.211	.000	.000	.000
IL-6	R		1	.539**	.197**	.431**	-.361**	.344**
	P			.000	.001	.000	.000	.000
WBC	R			1	.118	.491**	-.374**	.320**
	P				.052	.000	.000	.000
Lymphocyte	R				1	-.071	-.159**	.234**
	P					.243	.009	.000
Neutrophil	R					1	-.272**	.182**
	P						.000	.003
Platelet	R						1	-.280**
	P							.000
Hb	R							1
	P							

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.0f level (2-tailed).

R: Pearson correlation

P: P-Value

Discussion

COVID-19 is a cryptic disease that it could enable sickness ranging from a cold to cytokine storm and acute respiratory syndrome(6). To date, there is no specific treatment for COVID-19 and few data on the predictive variables of COVID-19 were obtained (16, 17). Therefore, diagnose of disease by measuring inflammatory mediators might help clinicians in identifying patients with poor prognosis at earlier stage (18). Present study demonstrated comprehensive data on the clinical, laboratory and image features of patients with nonsevere and severe COVID-19 in Kohgiluyeh and Boyer Ahmad (K&B) province.

In our data set of COVID-19, we revealed significant differences in positive chest CT scans and positive RT-PCR results, when comparing subjects with severe COVID-19 and hyper inflammation with those who did not show expression of hyper inflammation. The results from this review in similarly to other studies from Duan et al(19), Poortahmasebi et al (20), and Udugama et al.(21), indicate that the chest CT scan and RT-

PCR should be used for symptomatic and hospitalized covid19 patients. In the study by Tao et al, in the epidemic area, chest CT scan in comparison with RT-PCR, maybe a more reliable, practical, and rapid method to diagnose COVID-19(22). According to the latest guidelines released by the Chinese government, Chest CT scan is a common imaging tool for pneumonia diagnosis, and the key indicator for the confirmation of the diagnosis of COVID-19 is based on RT-PCR or sequencing of the gene for respiratory or blood specimens(20, 22). However, a rate of 10–40% RT-PCR false-negative results made this technique insufficient for proper detection(20, 23). We suggest a major obstacle for this low efficacy might be related to sampling errors, markedly inappropriate timing of sampling, which is reflected by variations in viral load in upper versus lower respiratory tract.

In a recent study on patients with COVID-19 in K&B province, our data reported a higher rate of IL-6 in patients with infection than in the general population. Probably, this findings indicate that IL-6 cytokine was positively associated with the severity of COVID-19. In accordance with present study, Coomes et al. suggested that in patients with COVID-19, IL-6 levels are significantly elevated and associated with noxious clinical subsequences including: ICU admission, asthma, and death (24). In the analysis by Zhang et al. IL-6 level in patients with COVID-19 have dramatically enhanced (25). Similarly, in research by Ruan et al. reported higher levels of IL-6 in COVID-19 patients compared to non-patients(26). Overall, elevations in IL-6 levels between patients with COVID-19 were identified in all previous studies (13, 27–29). This suggests that dynamic changes in level of IL-6 can be used as a diagnosis factor in patients with severe COVID-19 and also, the progression of COVID-19 to complexed disease may be the result of an over-response of the immune system to inflammatory mediators.

In our study cohort, data analysis revealed that there was a high significant levels of ferritin in COVID-19 patients compared to that of non-COVID-19 patients. Similar observations have been reported by scientists from China, Italian and the United States. In Yehuda et al. study, high levels of Ferritin (hyperferritinemia) has been associated with increased illness severity and adverse outcomes, including COVID-19(30). In the study with Guan et al, Ferritin was significantly elevated in more severe cases of COVID-19 Compared with control group (19). Accordance with retrospective cohort study by Zhou, et al, serum ferritin levels have been increased in non-survivors patients with COVID-19 from Wuhan as compared with survivors.(31). About ferritin, Wu et al. reported that higher serum ferritin was related with ARDS development and was able to predict an increased risk of COVID-19 illness(28). Recently, some scientific secrets by Ruscitti et al reported. they discovered the role of the H-chain of ferritin in activating macrophages (macrophage activating syndrome; MAS) to increase the secretion of inflammatory cytokines and cytokine storms in COVID-19 patients. (32). Overall, in accordance with previous studies, it turns out that elevated ferritin concentrations play a critical role in innate immunity and associated with an increase in production of special signaling molecules of the body(13). These studies complete our understanding of the pathogenesis of the high levels of ferritin including the infection with Covid-19, and may contribut clinicians to apply more aggressive treatment for those patients.

In the our study, COVID-19 severe cases had elevated levels of hematological indices (except for Platelet), compared with non-severe cases. The results showed, the COVID-19 can cause some hematological

indices changes between cases. Several studies have shown the common presenting hematological manifestations of COVID-19. Wu et al. retrospectively demonstrated risk factors for the clinical outcomes of COVID-19 pneumonia and death in China patients. The study showed that several factors related to the development of disease which included, neutrophils, lymphocyte and etc (28). Guan et al. found that lymphocyte had significantly increased in most patients of COVID-19, while platelets had decreased (19). In Huang et al. research, COVID-19 infection is associated with alterations in the WBC and lymphocyte count(33). In Dawei et al study, Compared COVID-19 patients received ICU care with non-ICU patients who had significantly elevated neutrophil and WBC count, as well as lymphocyte were significantly decreased(34). Interestingly, Hu Yun et al. showed that among Covid-19 Positive Patients, the platelet count during the disease course were decreased(35). Similarity, Lippi et al. was showed that a low platelet count correlated with higher disease severity(36). So, the Results of this study reported the changes in hematological markers in covid-19 patients might help to developed the pathophysiology Knowledge of this disease and provide early guides to diagnosis of coronavirus based on routine laboratory tests.

We have some limitations in this study. There were not complete clinical information for all patients with COVID-19 diseases admitted to Jalil hospital during the study term. The study was conducted with limited sample size, therefore, we were unable to do a multivariate analysis and generalize the results. There may also be a selection bias when identify factors that influence the clinical outcomes.

We have an opinion that the findings of this epidemiological study, in a region in southeast of Iran with a high number of COVID-19 cases, was one of the strengths of the present study.

Conclusion

In conclusion, inflammatory markers especially IL-6 and Ferritin and hematological parameters (WBC, Lymphocyte, Neutrophil, Platelet and Hb) were positively correlated with the severity of COVID-19. Measurement of IL-6, Ferritin and hematological indices might be workable tests to diagnosis, monitoring and prognosis of patients with COVID-19.

Declarations

Ethics approval and consent to participate

The Research project (No. 990000) was approved by the Ethic Committee of Yasuj University of medical sciences; IR.YUMS. REC.1399.003. Informed consent form was obtained from all subjects.

Consent for publication

Informed consent was obtained from all Subjects. This Informed Consent Form had two parts:

Information Sheet (to share information about the study).Certificate of Consent (for signatures if participants choose to participate).

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

none declared

Conflicts of Interest

The authors have no actual or potential conflict of interest to declare in relation to this study.

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Contribution Author

Parviz Yazdanpanah and Jalal Pournafard conceived the study hypothesis. Farzad Vafaei and Sajad Afrouz, designed the study and undertook the literature search, study selection and data abstraction. Saeed javidan sirat and Sajad Afrouz, analyzed the data. All authors interpreted the data, wrote the manuscript, and edited the manuscript critically for important intellectual content.

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