Kufa Journal for Nursing Sciences
Open Access Full Text Article

Publisher
University of Kufa, Faculty of Nursing

The correlation between Interleukin-6 and D-dimer, Serum ferritin, CRP in COVID-19 patients in Al-Najaf province

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الخلاصة:

خلفية البحث: متلازمة الجهاز التنفسي الحادة الوخيمة فايروس كورونا-2 هو ثالث فايروس كورونا قاتل ظهر في العقدين الماضيين, بعد فايروس كورونا المتلازمة التنفسية الحادة الوخيمة -1 ومتلازمة الشرق الأوسط التنفسية.

الاهداف: للتحقيق في الارتباط بين عدوى كوفيد-19 والبيانات الوصفية للمريض مثل الجنس والعمر ومراقبة الارتباط بين serum ferritin, CRP, D-Dimer, IL-6, في مرضى كوفيد-19.

منهجية البحث: أجريت الدراسة خلال الفترة من 1 أكتوبر 2021 الى 1 مارس 2022, تم جمع 110 عينة من مرضى كوفيد-19, الذين حضروا إلى مستشفى الأمل التخصصي للأمراض الانتقالية في الأسبوع الأول من الإصابة. تتراوح أعمارهم بين 15 - 90 سنة ومقارنتهم ب 50 فردا صحيا كمجموعة سيطرة. تم تشخيص كل حالة باستخدام تفاعل البوليميراز المتسلسل العكسي. تم جمع 7 مل من عينات الدم الوريدي من مرضى كوفيد19 للاختبارات المصلية والمناعية لقياس مستويات serum ,CRP ferritin, D-Dimer, IL--6.

النتائج: في الدراسة الحالية, أنتشار شدة الإصابة بكوفيد-19 حسب العمر ازدادت لدى كبار السن واعلى إصابة في الفئة العمرية (71- 90) بنسبة 100% تليها الفئة العمرية (51- 70) بنسبة 87.2 % ثم سجل معدل أنتشار منخفض في الفئة العمرية (36-50) بنسبة 37.5 % وانخفاض معدل الانتشار 28.6 % في الفئة العمرية (15-35). من أجمالي المرضى, كان 79 (71.8 %) منهم من الذكور و 31 (28.1 %) من الأناس. يظهر متوسط مستوى المصل للفرتين فروقا معنوية في مصل مريض كوفيد-19 مقارنة بمجموعات السيطرة (392.4 مقابل 131.6 مايكرو غرام/لتر), كذلك أظهر متوسط مستوى المصل ل CRP فرقا معنويا عاليا في مصل مرضى كوفيد-19 بالمقارنة مع مجموعات السيطرة (59.26 مقابل 7.61 ملغرام/لتر), ايضا متوسط مستوى المصل ل D-dimer اظهر فرقا معنويا عاليا في مصل مرضى كوفيد-19 بالمقارنة مع مجموعات السيطرة (1104 مقابل 220 نانو غرام/مل). أظهر متوسط مستوى المصل لIL-6 في مرضى كوفيد-19 فرقا معنويا عاليا بالمقارنة مع مجموعات السيطرة (130 مقابل 9.2 بيكوغرام/مل), وكذلك أظهر فرقا معنويا عاليا في الحالات الشديدة والحالات الشديدة المصاحبة للوفاة عند مقارنتها بمجموعات السيطرة, بالإضافة الى ذلك أظهر مرضى كوفيد-19 ارتباطا ايجابيا مهما ل IL-6 مع CRP , D-dimer, ferritin بواسطة قيم r.

الاستنتاج: أنتشار الإصابات الشديدة بمرض كوفيد-19 حسب العمر تزداد عند كبار العمر من المرضى وأظهر الذكور نسب إصابة عالية اكثر من الإناث. ارتفاع مستوى المصل ل IL-6, CRP, D-dimer, and ferritin مرتبط مع النتائج السيئة في مرضى كوفيد-19.

التوصيات: يحتاج هذا النوع من الدراسة الى مزيد من التعاون بين المؤلفين, الأطباء المتخصصين والمختبرات الطبية لأن هذه الدراسة مفيدة جدا للمرضى والأطباء لتقييم الدور الدقيق لأدوية كوفيد-19 التي يمكن أن تتحكم في عاصفة السايتوكينات ومستوى CRP, serum ferritin, D-Dimer, IL-6, مما يؤدي الى إنقاذ حياة المرضى.

الكلمات المفتاحية: كوفيد-19, IL-6, D-dimer, Ferritin, CRP.

Abstract

Background: Severe acute respiratory syndrome coronavirus-2 is the third fatal coronavirus that has emerged in the past two decades, following severe acute respiratory syndrome coronavirus-1 and the Middle East respiratory syndrome coronavirus.

Objectives: To investigate the correlation between COVID-19 infection and patient's descriptive data such as gender, age, and to observe the association of IL-6 and D-dimer, CRP, Serum ferritin in COVID-19 patients.

Methodology: A study is conducted during the period from 1st October 2021 to 1st March 2022, 110 samples are collected from COVID-19 patients, Who attended to AL-Amal Specialized Hospital for Communicable Diseases at first week of infection. Their age range between 15-90 years old, and compared them with 50 healthy individuals as control group. The diagnosis of each case was established using clinical diagnosis and confirmed by reverse transcriptase polymerase chain reaction (PCR). 7 ml of fresh venous blood samples were collected from COVID-19 infected patients for serological and immunological assay for measuring the levels of IL6, D-dimer, serum ferritin, and C-reactive protein.

Results: In the current study, the prevalence of severe COVID-19 infection according to age are increased in older aged patients, the highest one appeared at the (71-90) age group in 100% followed by (51-70) age group 87.2%, then the low prevalence frequency was recorded at (36-50) age group in (37.5%), and the low prevalence 28.6 % at the age group (15-35). From total patients, 79(71.8%) of them were males and 31(28.1%) were females. The mean serum level of ferritin show a significant differences in sera of patient with COVID-19 as compared with control groups (392.4 vs. 131.6 µg/l), as well as, the mean serum level of CRP show a highly significant difference in sera of patient with COVID-19 as compared with control groups (59.26 vs. 7.61 mg/L), also the mean serum level of D-dimer show a highly significant difference in sera of patient with COVID-19 as compared with control groups (1104 vs. 220 ng/ml). The mean serum level of IL-6 show a highly significant difference in sera of patient with COVID-19 as compared with control groups (130 vs. 9.2 pg/ml), also it show a highly significant differences in severe and severe with death cases when compared with control groups, in addition, COVID-19 patients show a significant positive correlation of IL-6 with CRP, Ferritin, and D-dimer by their r values.

Conclusion: The prevalence of severe COVID-19 infection according to age are increased in older aged patients, the male show high infected percentage than female. An elevated serum level of IL-6, CRP, D-dimer, and ferritin are associated with a poor outcome in COVID-19 patients.

Recommendations: This type of study need more cooperation between the authors, special physicians and medical laboratories because such study very useful to the patients and physicians to evaluate the exact role of COVID-19 drugs that can be control the cytokine storm and the level of D-dimer, serum ferritin, and CRP leading to save the lives of patients.

Keywords: COVID-19, IL-6, D-dimer, Ferritin, CRP.

INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the virus responsible for coronavirus disease 2019 (COVID-19) [(1)], a member of the species of severe acute respiratory syndrome related coronavirus, the family Coronaviridae [(2)]. COVID-19 is single strain RNA virus with typical crown-like appearance [(3)] due to the presence of the glycoprotein spikes on the envelope under an electron microscope [(4)], that infects many host, including human, mainly causes respiratory

infections [5]. COVID-19 already infected about 90 million people worldwide causes more than 1.9 million deaths and becoming a worldwide pandemic [6], although most cases had only mild symptoms, 20% of Coronavirus patients develop severe pathology with acute bilateral pneumonia that may evolve to acute respiratory distress syndrome, in addition to multi-organs failure. The risks of the severity of disease and death increase with age and presence of comorbidities [7].

IL-6 is a cytokine first produced by monocyte, dendritic cells (DCs) [8], mast cell, macrophage, B lymphocytes, T-cell, and many non-lymphocyte, like fibroblast and endothelial cells [9], a key biomarker of inflammation [10]. IL-6 contributes to host protection by inducing the acute phase response, hematopoiesis and immune reaction. It is developed rapidly and transiently in response to infection and tissue injuries. IL-6 is the main cytokine whose development has been linked to many inflammatory diseases [11]. High level of IL-6 were seen in patient with COVID-19, and this level are linked to pulmonary inflammations and severe lung injuries [12]. Previous review have shown that in the severe COVID-19 disease, high level of serum IL-6 are significantly related to severity of COVID-19 and adverse clinical outcome , which include admission to the intensive care unit, ARDS and death [13]. IL-6 play pivotal role in COVID-19 induced the cytokine storm and to participate in the interstitial pneumonia observed in severe COVID-19 [14]. IL-6 and IL-10 are synthesized from regulatory T cell and even TH 1 cell, that have been reportedly implicated in immune regulation and inflammation [15], IL-10 exerts anti-inflammatory function by directly limiting innate immune-related function of macrophage [16], Indeed, number of the clinical studies have unveiled that the circulating IL-6 level was elevated in COVID-19 patient, particularly in those with severe stage and was positively associated with the severity and mortality of COVID-19 [17].

Ferritin is key mediator of the immune dysregulation, especially under the extreme hyperferritinemia, via direct immune suppressive and the pro-inflammatory effects, contributing to cytokine storm [18]. It was reported that the fatal outcomes by COVID-19 are accompanied by cytokine storm syndrome, thereby it has been suggested that the disease severity is dependent of cytokine storm syndrome [19], on this basis, we briefly review evidence supporting hypothesis that the ferritin level might be crucial factor influencing severity of COVID-19 [20].

D-dimer is fibrin degradation product (FDP), releases into the circulation when blood clot dissolves via the fibrinolysis. It has two D fragments of fibrin protein attached by cross-link [21], widely used as biomarker for the thrombotic disorders. D-dimer value less than 0.5 µg/mL usually considers normal, and values increases with increasing age [22]. Researchers suggest that high D-dimer level could predict disease severity, lung complication, and thromboembolic events before they even occur. In this way, they aim to reduce disease's morbidity and mortality with the early diagnosis and treatment [23].

In response to infections, liver synthesizes significant quantities of acute-phase proteins (APPs), like CRP [24]. This acute inflammatory protein is highly sensitive biomarker for the inflammation, infection, and tissue damage [25]. It has been shown that CRP level are correlated with the level of inflammation [26]. C-reactive protein has association with severity of disease [27]. It may has pivotal role in the severe respiratory failure and death caused by COVID-19 [28], research have reported that the CRP level can be used in early diagnosis of pneumonia and higher CRP level are associated with the severe pneumonia [29].

Aims of the Study

To investigate the correlation between COVID-19 infection and patient's descriptive data such as gender, age, and to observe the association of IL-6

and D-dimer, CRP, Serum ferritin in COVID-19 patients.

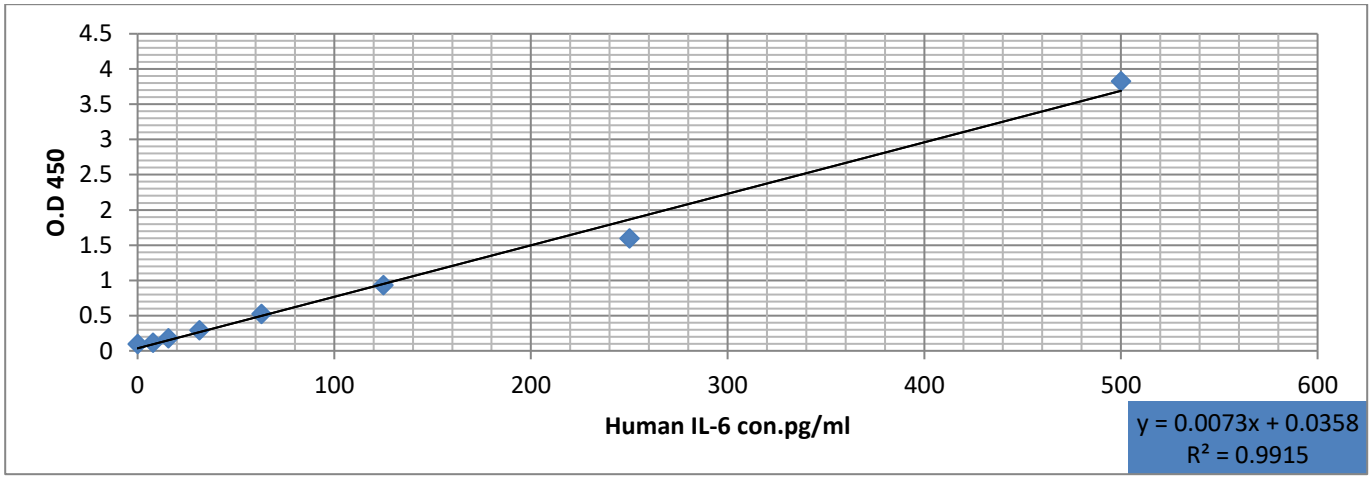
METHODOLOGY

Sample collection and preparation

Samples Collection During the period from 1st October 2021 to 1st March 2022, 110 sample are collected from COVID-19 patients, Who attended to AL-Amal Specialized Hospital for Communicable Diseases at first week of infection with symptoms (fever, Headache, cough, shortness of breath, diarrhea, loss of taste and smell) (30). Their ages range between 15-90 years old, 79 were males and 31 were females and compared with 50 apparently healthy individuals as control group. The diagnosis of each case was established using clinical diagnosis and confirmed by reverse transcriptase polymerase chain reaction (rt-PCR). 7 ml of fresh venous blood samples were collected from COVID-19 infected patients by sterile syringes which divided into (1.8 ml) saved in sodium citrate tube for D-dimer test, (5.2 ml) saved in serum tube for serological and immunological assay (IL6, ferritin, CRP). Blood samples were obtained from each patient and placed in serum separator tube (SST) with specific gel to easy sorting the serum, then left for 20 minutes at room temperature. After coagulation, sera were separated by centrifuge 4000xg for 15 minutes and directly stored at -20 ºC to be analyzed later for IL-6 and other blood parameters.

Standard Curve: For calculation, average the OD 450 nm readings for each standard, and each sample, and the average control (zero) OD reading.(Relative OD) = (OD of each well) - (OD of zero well). Known concentrations of IL-6 standard and its corresponding reading OD were plotted on the (x-axis) and (y-axis) respectively. The concentration of IL-6 in sample is determined by plotting the sample OD on the Y-axis. The original concentration is calculated by multiplying the dilution factor (Figure 1).

Figure (1): Standard Curve to Determine the Level of Interleukin-



Hematological assays Methods:

The Mindray uses the multi-angle polarized scatter separation (MAPSS) technique only A measurement is made by laser light flow depolarization cytometer.

D-dimer: D-dimer are detected by nephlometry technique using scatter light according to manufacturer company (Genis/China).

Ferritin: This assay doing automated according by manufacturer company (Cobas E411/Germany).

C-Reactive Proteins: This test done by nephlometry technique (Genius).

Statistical Analysis:

The Statistical Analysis System- SAS (2012) program was used to detect the effect of different factors in study parameters. Chi-square test was used

to significantly compare between percentages (0.05 and 0.01 probability) in this study. The results are presented as mean ± standard deviation (SD) or median and interquartile range. Differences between groups were analyzed using the T-test. Statistical analyses were performed using Statistical significance was determined to be $P < 0.05$.

RESULTS

During the period from 1st October 2021 to 1 st March 2022 , 110 patients were diagnosed as COVID-19. Their ages range between 15-90 years old, 79 were males and 31 were females and compared with 50 apparently healthy individuals as control group. The diagnosis of each case was established using clinical diagnosis and confirmed by reverse transcriptase polymerase chain reaction (rt-PCR). In the current study, There are no significant differences between the age groups in total patients and control (table 1), the prevalence of severe COVID-19 infection according to age are increased in older aged patients, the highest one appeared at the 71-90 age group in 100% followed by (51- 70) age group 87.2%, then the low prevalence frequency was recorded at (36-50) age group in (37.5%), and the low prevalence 28.6 % at the age group (15-35) (table 2).

Table (1): Distribution of COVID-19 patients and controls according to age groups.

Age groups Years	Patients No	Patients (%)	Control No	Control (%)	P-Value
15-35	14	12.7	12	24	0.976
36-50	32	29.1	14	28	
51-70	39	35.5	18	36	
71-90	25	22.7	6	12	
Total	**110**	**100**	**50**	**100**	

* Significantly at 0.05, ** Highly Significantly at 0.01 and 0.001.

Table (2): Distribution of Severe cases of COVID-19 patients according to age groups.

Age groups Years	Patients No	Severe cases No	Severe cases (%)	P-Value
15-35	14	4	28.6	0.976
36-50	32	12	37.5	0.604
51-70	39	34	87.2*	0.01
71-90	25	25	100**	0.001
Total	**110**	**75**	**68.2**	**0.05**

* Significantly at 0.05, ** Highly Significantly at 0.01 and 0.001.

A total of 110 patients with COVID-19 were included in this study.79(71.8%) of them were males and 31(28.1%) were females, in comparing with 50 apparently healthy control individuals, males were 22(44%) and 28(56%) were females. The result of statistical analysis demonstrated that statistically significant differences were found in male patients as compared with female patients, as well as when compared with male of control groups as shown in (table 3).

Table (3): Distribution of study population according to gender.

Gender	Patients No	Patients (%)	Control No	Control (%)	P- Value
Males	79	71.8*	22	44	0.05*
Females	31	28.1	28	56	0.06
Total	**110**	**100**	**50**	**100**	

* Significantly at 0.05, ** Highly Significantly at 0.01.

The mean serum level of Ferritin show a significant difference in sera of patient with COVID-19 as compared with control groups (392.4 vs. 131.6 µg/l), also it show a highly significant differences in severe and severe with death cases when compared with control as well as it show non-significant differences in moderate and mild cases as compared with controls as shown in (table 4).

Table (4): Serum Ferritin level among Study Population.

Group		Mean ± SD (µg/l)	P-value
Control		131.6 ±47.96	<0.05
Patient	Milled	207.9 ±78.09	
	moderate	262.1 ± 82.94	
	Severe	906.2** ±261.5	
	Severe with	1123.0** ±394.1	
	Total	**392.4* ±156.6**	

*Significantly at 0.05, ** Highly Significantly at 0.01 and 0.001.

The mean serum level of CRP show a highly significant difference in sera of patient with COVID-19 as compared with control groups (59.26 vs. 7.61 mg/L), also it show a highly significant differences in severe and severe with death cases when compared with control, as well as it show non-significant differences in moderate and mild cases as compared with controls (table 5).

Table (5): C-reactive protein level among Study Population

Group		Mean ± SD (mg/L)	P-value
Control		7.61 ±1.366	<0.001
Patient	**Milled**	15.51 ±1.706	
	moderate	22.75 ±2.027	
	Severe	67.55** ±5.241	
	Severe with	99.92** ± 6.425	
	Total	**59.26 ** ± 21.41**	

*Significant at 0.05, ** Highly Significant at 0.01 and 0.001.

The mean serum level of D-dimer show a highly significant difference in sera of patient with COVID-19 as compared with control groups (1104 vs. 220 ng/ml), also it show a highly significant differences in severe and severe with death cases when compared with control, as well as it show non-significant differences in moderate and mild cases as compared with controls (table 6).

Table (6): D-dimer level among Study Population

Group		Mean ± SD (ng/ml)	P-value
Control		220 ±73.26	<0.001
Patient	Milled	305 ±117.4	
	Moderate	442 ±124.4	
	Severe	1614** ±1193	
	Severe with death	8183** ±1063	
	Total	1104** ±1998	

*Significant at 0.05, ** Highly Significant at 0.01 and 0.001

The mean serum level of IL-6 show a highly significant difference in sera of patient with COVID-19 as compared with control groups (130 vs. 9.2 pg/ml), also it show a highly significant differences in severe and severe with death cases when compared with control, as well as it show non-significant differences in moderate and mild cases as compared with controls (table 7).

Table (7): Serum level of IL-6 among Study Population

Group		Mean ± SD (pg/ml)	P-value
Control		9.2 ±2.37	<0.001
Patient	Milled	19.7 ±3.98	
	Moderate	38.7 ±4.96	
	Severe	105.6** ±14.63	
	Severe with death	299.4** ± 43.6	
	Total	130** ± 17.30	

*Significant at 0.05, ** Highly Significant at 0.01 and 0.001.

Correlations

patient show a significant positive correlation of IL-6 with CRP, Ferritin, and D-dimer by their r values (table 8).

Table (8): Correlation coefficient (r value) between IL-6 and parameters of immunological and biochemical evaluation in COVID-19 patients

Parameter Investigated	IL-6	
	r - value	Probability ≤
CRP	0.944	0.001
Ferritin	0.963	0.001
D-dimer	0.972	0.001

*Significant at 0.05, ** Highly Significant at 0.01 and 0.001

DISCUSSION

The severity of the clinical picture seems to be correlated with age older than 70 years [31]. Due to the age-dependent alterations of the immune system that may be implemented by nutritional deficits, a relevant percentage of elderly patients progresses to insidious systemic inflammation, mainly affecting the lung, heart, renal function and coagulation system, the second stage of hyperinflammation is characterized by massive production of pleiotropic cytokines (e.g., IL-6) by lung resident (e.g., macrophages) and circulating immune cells [32].

Article demonstrate effect of sex hormones, testosterone and estrogen, on immune system response and engagement, resulting in less robust immunological response in males and subsequent increase the morbidity and mortality from the viral respiratory illnesses [33]. In addition, X chromosome carries largest number of immune-related genes in human genome, perhaps also contributing to female's superior immune response as well as female preponderance in the autoimmune diseases [34]. ACE2 and its role in viral transmission and associated morbidity has also been topic of recent COVID-19 associated discussion. ACE2 receptors on pulmonary endothelium serve as main entry point for coronavirus. Several previous animal models have demonstrate increasing ACE2 activity in male [35]. The gene for ACE2 receptor is also, interestingly, on X chromosome [36].

The result is an agreement with recent literature advocates hyperferritinemic syndromes as one of main modifications in COVID-19 infection [37], suggesting evaluation of ferritin level as parameter of infections [38]. Elevated inflammatory marker, including ferritin which has been associated with the critical and life threatening illness [39].

The current study revealed significantly higher CRP level in severe cases than in non-severe patient suggesting that CRP level may be biomarker of disease severity and progression in patient with COVID-19, this result is agreement with the result of Liu et al., who reported that more severe cases of covid-19 expressed higher CRP level than non-severe patient [40], as well as Qin et al., observed higher CRP level in severe COVID-19 patient, suggesting that this biomarker can be monitored to evaluate the disease progression [41].

The results are in agreement with previous studies about abnormal coagulation function including the elevated D-dimer has been demonstrated to be involved in disease progression of COVID-19 [42]. elevated D-dimer is associated with increased composite poor outcome especially mortality and severe COVID-19, this finding supports hypothesis that the severe acute respiratory syndrome coronavirus 2 infection could induce dysfunction of hemostatic system, leading to hypercoagulable state which we commonly encounter in sepsis [43]. As well as the result are in agreement with the result of Kim et al., who reported that IL-6 contributes to the host defense against infection and tissue injury however, the exaggerated, excessive synthesis of IL-6 while fighting COVID-19 leads to acute severe systemic inflammatory response cytokine storms [44]. The results are in agreement with the results of Ruetsch et al., who reported that IL-6 and D-dimer testing provide early sensitive and specific predictor of severe course of COVID-19 [45]. This stage consists of most severe manifestation of cytokine storm, in which the excessive hyperinflammation may lead to the cardiopulmonary collapse and multi-organ failures [46], we provided evidences that the inflammation reflected by the cytokine storms and CRP in COVID-19 patient could have contributed to the disease worsening.

CONCLUSION

The prevalence of severe COVID-19 infection according to age are increased in older aged patients, the male show high infected percentage than female. An elevated serum level of IL-6, CRP, D-dimer, and ferritin are associated with a poor outcome in COVID-19 patients.

RECOMMENDATIONS

This type of study need more cooperation between the authors, special physicians and medical laboratories because such study very useful to the patients and physicians to evaluate the exact role of COVID-19 drugs that can be control the cytokine

storm and the level of D-dimer, serum ferritin, and CRP leading to save the lives of patients.

Ethical Clearance: All experimental protocols were approved under the Faculty of science.

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