**Al-Kindy College Medical Journal (KCMJ)**

*Research Article*

# Interleukin 6 Levels and their Correlation with Various Hematological and Biochemical Parameters in Covid-19 Patients

Ruaa Salim Rasheed[1*], Subh Salim[2]

[1] Educational laboratories ,Medical City , Baghdad , Iraq
[2] College of Medicine, Nahrain University, Baghdad , Iraq
* Corresponding author's email: ruaa.salim1991@gmail.com

***Article history:***
Received 13 September 2022
Accepted 22 Novemer 2022
Available online 30 April 2023

https://doi.org/10.47723/kcmj.v19i1.893

***Keywords***: Covid 19, cytokine storm, interleukin 6.



**ABSTRACT**

***Background***: coronavirus 19 is a beta-coronavirus, enveloped and roughly spherical with approximately 60 to 140 nm in diameter with positive-sense single-stranded RNA genome.
***Objectives***: Measurement of interleukin 6 (IL6) level in a group of patients with confirmed Covid19 infection and its correlation with many hematological and biochemical parameters , mainly lymphocyte , neutrophil count and their ratio , platelet count , serum ferritin , C reactive protein as well as D-dimer level
***Subjects and Methods***: This study was conducted on 60 PCR positive patients variably affected by COVID-19 , cases collected sequentially from June till November 2021 from Medical City hospital (Private Nursing hospital/ Intensive Care Unit (ICU)) with outpatient mild cases collection.
***Results***: IL-6 levels showed a significant difference between the mild-moderate groups (p 0.001) and the mild- severe groups (p 0.009) ; additionally, there was significant statistical correlation between IL-6 and the other parameters included in the study.
***Conclusions***: IL6 level was highly elevated in most of moderately and severely affected COVID 19 patients ,It's level correlates well with the most important hematological and inflammatory parameters that enable it to be used as a marker to assess the severity of the infection and for following up patients.

## Introduction

In December 2019, the outbreak of coronavirus disease 2019 (COVID-19), was caused by a novel coronavirus that resulted in severe acute respiratory syndrome and had spread rapidly throughout the world ,considering this epidemic situation, the World Health Organisation (WHO) declared a global pandemic on March 11, 2020.(1)

SARS-CoV-2 is a beta-coronavirus ; it is an enveloped virus with roughly spherical or moderately pleomorphic virions of approximately 60 to 140 nm in diameter ,it has a positive-sense single-stranded RNA genome , It is believed to be transmitted from animals to humans' i.e. zoonotic disease, however, human to human transmission of the virus is the leading cause of this pandemic. (2)
Most patients with COVID-19 were asymptomatic or presented with mild to moderate symptoms, however, about 10–20% of cases developed severe symptoms, characterized by the rapid development of acute respiratory distress syndrome (ARDS), sepsis and/or multiple organ failure (3)

It has been speculated that the cytokine storm and immune dysfunction were closely related to the rapid disease progression, as well as to the level of infection-related biomarkers including IL6, D Dimer, s.ferritin and others which played vital role in severe cases of COVID-19 (4).

One of the biomarkers in this disease was IL 6 which is one of a group of related proteins made by leukocytes and other cells in the body mainly by macrophage, monocyte, T-lymphocyte and also by adipocyte ,endothelial cells, mast cell ,osteoclast as well as cardiac and skeletal muscle cells.

IL6 is a multifunctional cytokine that plays a central role in host defense due to its wide range of immune and hematopoietic activities and its potent ability to induce the acute phase response.

IL-6 has cytokine featuring pleiotropic activity ,it plays an important role in Covid 19 cytokine storm as it induces synthesis of acute phase proteins such as CRP, serum amyloid A, fibrinogen, and hepcidin in hepatocytes, whereas it inhibits production of albumin(5).

Aims of current study is to measure IL6 level in patients with confirmed Covid19 infection and to assess the correlation between IL6 and other hematological and biochemical parameters, mainly Lymphocyt , neutrophil count and their ratio, platelet count, serum ferritin , C reactive protein as well as D-dimer level.

## Subjects and Methods

This descriptive cross sectional study was conducted on 60 PCR positive patients who were variably affected by covid-19 infection . Depending on the WHO criteria for classification of covid-19 patients(6) cases were grouped into :

Mild cases …in which there was respiratory symptoms without evidence of pneumonia or hypoxia.

Moderate cases…in which there was clinical or radiological evidence of pneumonia with SpO2 ≥ 90 on room air

Severe cases…which was like the moderate but SpO2 ≤ 90 on room air or respiratory rate > 30 breaths /min.

Selection of Covid-19 patients were based on the following criteria :

- Age more than 20 years of both gender.
- Patients proved to have Covid -19 by positive PCR .
- Hospitalized Covid19 patients who were severely or critically ill.
- Outpatient Covid19 cases who were mildly affected.

Cases were collected sequentially over a period of time from June till November 2021 from Medical City hospital (Private Nursing hospital/ Intensive Care Unit (ICU)) with outpatient collected as mild cases.

**Table 1:** Description of hematological and inflammatory parameters of 60 patients according to severity:

| | | AGE (Year) | Platelets *109/L | Neutrophils *109/L | Lymphocytes *109/L | Neutrophils to lymphocytes ratio | S.ferritin Ng/ml | CRP Mg/dl | D.dimer Ng/ml | IL 6 Pg/ml |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mild | 32.5 | 191.5 | 5.2 | 2 | 2.2 | 156 | 4.6 | 280.5 | 5.2 |
| **Mdn** | Moderate | 55 | 205.5 | 6.5 | 0.785 | 7 | 463.5 | 31.5 | 928.5 | 14.4 |
| | Severe | 54 | 157 | 7.7 | 1.15 | 6.35 | 1400 | 65.5 | 4250 | 9.55 |
| | Mild | 6.5 | 50.5 | 1.15 | 0.85 | 0.85 | 52 | 1.05 | 125 | 1.8 |
| **MAD** | Moderate | 14 | 68.5 | 2.4 | 0.37 | 4.55 | 236.5 | 23.05 | 685.5 | 9.9 |
| | Severe | 15 | 34.5 | 1.4 | 0.615 | 4.15 | 600 | 48 | 2950 | 4.8 |
| | Mild | 20 | 110 | 2.1 | 0.7 | 0.58 | 38.9 | 0.5 | 46 | 2.1 |
| **Min.** | Moderate | 28 | 96 | 1.5 | 0.2 | 0.98 | 67 | 2.66 | 52 | 3.8 |
| | Severe | 27 | 100 | 1.2 | 0.2 | 0.6 | 400 | 2 | 1049 | 2.7 |
| | mild | 71 | 371 | 10.4 | 4.3 | 9.4 | 261 | 16 | 992 | 11 |
| **Max.** | Moderate | 82 | 340 | 11.4 | 5.8 | 52 | 3400 | 214 | 10000 | 69.7 |
| | severe | 77 | 251 | 13.1 | 3.1 | 56 | 2700 | 270 | 10030 | 80 |

,MAD: Median Absolute Deviation, Min: minimum, Max: maximum Median: Mdn

**Materials , reagents , equipment and instruments used in this study are** :

gel tube: Used for separation and examination of blood serum.

A verbal consent was obtained from the patient him/herself or their caregiver before enrollment in the study. Also, the study was approved by the ethical committee of Scientific Council of Pathology at the Arabic Board for Medical Specializations.

The leftover of the samples taken from patients were collected in serum separation tube (SST) and stored at -20 C till time of analysis, on day of analysis, the samples were thawed; the system needs only 30 ul from each serum sample to perform the test.

The assay principle combines a two steps electro-chemiluminescence immunoassay, sandwich method, all steps were done automatically in the Nipigon health Corp system , each test required 18 min. to be completely done.

The cut off value according to the used IL6 kit was < 6.6 pg / ml , system limits of detection was 1.5 – 5000 pg /ml .

Other parameters including CBC, D-dimer, s.ferritin and C reactive protein were obtained from patients' files .

Data were entered into computer and processed by Microsoft excel program version 2018 and Statistical Package for the Social Sciences version 25 (spss 25).

Tests of normality of data were done by The Kolmogorov–Smirnov test and Shapiro–Wilk test which showed that data have non- normal distribution; therefore, we used nonparametric methods and tests to describe the data ( Median, Median Absolute Deviation , in addition to minimum, maximum for range) ,Spearman's correlation test used to find correlation coefficient among different study variables , Kruskal–Wallis test used for analysis of study variables median according to the severity of the cases (mild, moderate, severe) , post -hoc test was done then to specify which pair of severity groups was responsible for the significance. In all statistical tests we chose significance level of P value ≤ 0.05.

## Results:

Table 1 showed that the median of IL6 and CRP in mild cases were within normal reference limit unlike moderate and severe median values , moreover the median absolute lymphocyte count in mild group was within normal limit unlike those in moderate and severe groups

Statistical correlations between the 3 groups of patients according to the severity of the disease regarding age and hematological parameters are shown in table 2. Kruskal–Wallis test was done between the 3 groups regarding median of each parameter and only when showed significance, post -hoc test was done then in between the groups

**Table 2:** Statistical correlations between the 3 groups of patients according to the severity of the disease regarding age and hematological parameters

| Variable | Statistical test | Mild | Moderate | Severe | P value |
|---|---|---|---|---|---|
| **AGE (Year)** | Kruskal–Wallis test | 32.5 | 55 | 54 | 0.009 (significant) |
| | Post hoc test | | | | |
| | Mild-moderate | | | | 0.014 (significant) |
| | Mild- severe | | | | 0.041 (significant) |
| | Moderate-severe | | | | 1.000 (non-significant) |
| **Platelets *109/L** | Kruskal–Wallis test | 191.5 | 205.5 | 157 | 0.190 (non-significant) |
| | Post hoc test | No Post hoc test performed because there was no significant difference. | | | |
| **Neutrophils *109/L** | Kruskal–Wallis test | 5.2 | 6.5 | 7.7 | 0.007 (significant) |
| | Post hoc test | | | | |
| | Mild-moderate | | | | 0.458 (non-significant) |
| | Mild-severe | | | | 0.005 (significant) |
| | Moderate-severe | | | | 0.251 (non-significant) |
| **Lymphocytes *109/L** | Kruskal–Wallis test | 2 | 0.785 | 1.15 | 0.014 (significant) |
| | Post hoc test | | | | |
| | Mild-moderate | | | | 0.021 (significant) |
| | Mild- severe | | | | 0.064 (non-significant) |
| | Moderate-severe | | | | 1.000 (non-significant) |
| **Neutrophils to lymphocytes ratio** | Kruskal–Wallis test | 0.85 | 4.55 | 4.15 | 0.009 (significant) |
| | Post hoc test | | | | |
| | Mild-moderate | | | | 0.061 (non-significant) |
| | Mild- severe | | | | 0.010 (significant) |
| | Moderate-severe | | | | 1.000 (non-significant) |

.

**Table 3:** Statistical correlations between the 3 groups of patients according to the severity of the disease regarding inflammatory parameters

| | | | | | |
|---|---|---|---|---|---|
| | Kruskal–Wallis test | 156 | 463.5 | 1400 | 0.000 (significant) |
| S.ferritin Ng/ml | Post hoc test | | | | |
| | Mild-moderate | | | | 0.001 (significant) |
| | Mild- severe | | | | 0.000 (significant) |
| | Moderate-severe | | | | 0.034 (significant) |
| **CRP Mg/dl** | Kruskal–Wallis test | 4.6 | 31.5 | 65.5 | 0.000 (significant) |
| | Post hoc test | | | | |
| | Mild-moderate | | | | 0.000 (significant) |
| | Mild- severe | | | | 0.000 (significant) |
| | Moderate-severe | | | | 1.000 (non-significant) |
| **D.dimer Ng/ml** | **Kruskal–Wallis test** | **280.5** | **928.5** | **4250** | **0.000 (significant)** |
| | Post hoc test | | | | |
| | Mild-moderate | | | | 0.003 (significant) |
| | Mild- severe | | | | 0.000 (significant) |
| | Moderate-severe | | | | 0.033 (significant) |
| **IL 6 Pg/ml** | Kruskal–Wallis test | 5.2 | 14.4 | 9.55 | 0.001 (significant) |
| | Post hoc test | | | | |
| | Mild-moderate | | | | 0.001 (significant) |
| | Mild- severe | | | | 0.009 (significant) |
| | Moderate-severe | | | | 1.000 (non-significant) |

Table 3 shows statistical correlations between the 3 groups of patients according to the severity of the disease regarding inflammatory parameters**.** Kruskal–Wallis test was done between the 3 groups regarding median of each parameter and only when showed significance, post -hoc test was done then in between the groups.

As shown in table 4, there was significant positive statistical correlation between IL-6 and the other parameters included in the study ( all P values are <0.05 ) ,and there was an inverse correlation between IL-6 and both platelets and lymphocytes values.

**Table 4**: Spearman's correlation test for IL 6 with hematological and inflammatory parameters in the 60 cases included in the study

| | | |
|---|---|---|
| interleukin 6 with platelets | Sparman's Correlation Coefficient (rho) | - 0.265 (negative correlation) |
| | P value | 0.041 (Significant) |
| interleukin 6 with neutrophils | Spearman's Correlation Coefficient (rho) | 0.457 (positive correlation) |
| | P value | 0.000 (Significant) |
| interleukin 6 with lymphocytes | Spearman's Correlation Coefficient (rho) | - 0.665 (negative correlation) |
| | P value | 0.000 (Significant) |
| interleukin 6 with neutrophils lymphocyte ratio | Spearman's Correlation Coefficient (rho) | 0.637 (positive correlation) |
| | P value | 0.000 (Significant) |
| interleukin 6 with serum ferritin | Spearman's Correlation Coefficient (rho) | 0.471 (positive correlation) |
| | P value | 0.000 (Significant) |
| interleukin 6 with c reactive protein | Spearman's Correlation Coefficient (rho) | 0.500 (positive correlation) |
| | P value | 0.000 (Significant) |
| interleukin 6 with d dimer | Spearman's Correlation Coefficient (rho) | 0.361 (positive correlation) |
| | P value | 0.005 (Significant) |

## Discussion

This study revealed that male to female patients ratio was 1.4:1 . However male gender consisted 55% of mild cases while it form 65% of sever cases . This observation may had happen by chance since cases were collected randomly or it may due to the way of male living that increase the risk of infection or it may reflect the effect of gender that maybe consider as one of the risk factors for severe Covid infection ; in line with Li et al and Zhou et al studies. (7)

The minimum age included in the study was 20 years old and the maximum age was 82 years old , the median age of mild group was much lower than that of moderate and severe groups which was expected because advanced age is one of the most common risk factors for severe infection and mortality and old age individual usually had many commodities. (7)

Neutrophilia was observed in severe group which can be due to that as inflammatory process becomes more severe thus more neutrophils will be recruited, such finding was in agreement with a study done by Chen et al who stated that neutrophilia can be considered as a marker for severity of Covid 19 infection as well as can be consider as a prognostic marker. (8)

Reduction of lymphocytes count in moderate and severe groups was noticed . A previous study published in International Journal for Vitamin and Nutrition Research clarified that absolute counts of major subsets of lymphocytes were significantly lower as the severity of COVID-19 increases , however, the level of this decrement differs according to the definition of disease severity that varies across those studies , the variations in blood specimen collection times as well as the laboratory practices, and clinical care. (9)

Neutrophils to lymphocytes ratio showed significant difference between mild and severe groups, which reflects the association of both components in predicting patients outcome , as reported by Zeng et al , who stated that NL ratio strongly associated with patient prognosis. (10,11)

Regarding platelets , count is lower in severe group but there was no obvious thrombocytopenia and no significant difference shown between patients groups; similar finding reported in a Brazilian study where it was found that there was no correlation between platelet level and disease severity or mortality ,however, they reported that non-survivors had lower platelet counts than survivors but this difference was not statistically significant(12) ; in addition other studies reported that platelet counts were found to be normal in many Covid patients at the time of hospital admission. (13)

Regarding CRP, their levels were significantly higher in moderate and severe groups ,similar to Zhong Z et al study , which was attributed to a more prominent inflammation in severe patients . Additionally ,CRP was significantly higher in those who die during follow-up rather than those who survive. (14,15)

Serum ferritin levels were significantly higher in moderate and severe groups compared to mild group , this finding was similar to Zeng F. and Huang Y. et al study which also revealed that higher serum ferritin level commonly associated with mortality in COVID-19 patients . This hyperferritinemia was most likely caused by the cytokine storm and to the secondary hemophagocytic lymphohistiocytosis phenomena. (16)

D- dimer level also significantly increased with increased severity of case, It is considered to have a vital role in diagnosis, prognosis and predicting outcomes in those patients , in line with Zhang et al study. (17)

The current study revealed that IL-6, median levels were significantly higher in sever and moderate cases than in mild cases , this result was in agreement with Chen et al. study (18) , who also found that raised IL-6 levels closely correlated with COVID-19-related mortality.

Chen X et al had propose that estimation of IL-6 levels could be an important tool to assess disease severity and further to decide treatment protocol in COVID-19 patients . This due to that in severe cases the virus replicates rapidly triggering cytokine storm that characterized by increased levels of cytokines mainly IL-6 . This inflammatory response will increase vascular permeability and flaring inflammation in respiratory system and other body systems, with subsequent occurrence of ARDS or respiratory failure and death. (19)

In relation to other parameters , IL-6 increment was associated with significant positive increment in neutrophils, neutrophils to lymphocytes ratio, serum ferritin, CRP and D-dimer with inverse decrement in lymphocytes and platelets which is similar to study done by Zhu et al and Huang et al .Those findings supported the important IL6 for assessing the severity and predicting the outcome of risky covid 19 patients as well as the use of suitable management protocols accordingly. (20).

## Conclusion:

IL6 level was highly elevated in most of moderately and severely affected COVID 19 patients and its level was closely related to the underlying inflammatory burden and cytokine storm ,thus it could be considered as a marker to assess the severity of the infection and to follow up the patients ; elevated IL6 correlates well with elevated CRP, D dimer, high neutrophil count, low lymphocyte count and high neutrophil/ lymphocyte ratio (NLR) that enable it to be used as a marker for following up those patients.

**Funding**

This research did not receive any specific fund.

**Conflict of Interest**

No conflict of interest

## References

[1] Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD. A pneumonia outbreak associated with a new coronavirus of probable bat origin. nature. 2020 Mar;579(7798):270-3.

[2] Seah I, Agrawal R. Can the coronavirus disease 2019 (COVID-19) affect the eyes? A review of coronaviruses and ocular implications in humans and animals. Ocular immunology and inflammation. 2020 Apr 2;28(3):391-5.

[3] Chang MC, Park YK, Kim BO, Park D. Risk factors for disease progression in COVID-19 patients. BMC infectious diseases. 2020 Dec;20(1):1-6.

[4] Tang L, Yin Z, Hu Y, Mei H. Controlling cytokine storm is vital in COVID-19. Frontiers in Immunology. 2020 Nov 30;11:570993.

[5] Tanaka T, Kishimoto T. The biology and medical implications of interleukin-6. Cancer immunology research. 2014 Apr;2(4):288-94.

[6] Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, Hu Y, Tao ZW, Tian JH, Pei YY, Yuan ML. A new coronavirus associated with human respiratory disease in China. Nature. 2020 Mar;579(7798):265-9.

[7] Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y, Shi J, Zhou M, Wu B, Yang Z, Zhang C. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. Journal of Allergy and Clinical Immunology. 2020 Jul 1;146(1):110-8.

[8] Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Yu T. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The lancet. 2020 Feb 15;395(10223):507-13.

[9] Ekinci I, Uzun H, Utku IK, Ozkan H, Buyukkaba M, Cinar A, Akarsu M, Kumbasar A, Tabak O. Prognostic nutritional index as indicator of immune nutritional status of patients with COVID-19. International Journal for Vitamin and Nutrition Research. 2021 Sep 20.

[10] Yang AP, Liu JP, Tao WQ, Li HM. The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. International immunopharmacology. 2020 Jul 1;84:106504.
[11] Zeng F, Li L, Zeng J, Deng Y, Huang H, Chen B, Deng G. Can we predict the severity of coronavirus disease 2019 with a routine blood test. Pol Arch Intern Med. 2020 May 29;130(5):400-6.
[12] Güçlü E, Kocayiğit H, Okan HD, Erkorkmaz U, Yürümez Y, Yaylacı S, Koroglu M, Uzun C, Karabay O. Effect of COVID-19 on platelet count and its indices. Revista da Associação Médica Brasileira. 2020 Sep 11;66:1122-7.
[13] Fan BE. Hematologic parameters in patients with COVID- 19 infection: a reply. American Journal of Hematology. 2020 Aug;95(8):E215.
[14] Khalid I, Alraddadi BM, Dairi Y, Khalid TJ, Kadri M, Alshukairi AN, Qushmaq IA. Acute management and long-term survival among subjects with severe Middle East respiratory syndrome coronavirus pneumonia and ARDS. Respiratory care. 2016 Mar 1;61(3):340-8.
[15] Ji P, Zhu J, Zhong Z, Li H, Pang J, Li B, Zhang J. Association of elevated inflammatory markers and severe COVID-19: a meta-analysis. Medicine. 2020 Nov 11;99(47).
[16] Zeng F, Huang Y, Guo Y, Yin M, Chen X, Xiao L, Deng G. Association of inflammatory markers with the severity of COVID-19: a meta-analysis. International Journal of Infectious Diseases. 2020 Jul 1;96:467-74.
[17] Zhang JJ, Dong X, Cao YY, Yuan YD, Yang YB, Yan YQ, Akdis CA, Gao YD. Clinical characteristics of 140 patients infected with SARS- CoV- 2 in Wuhan, China. Allergy. 2020 Jul;75(7):1730-41.
[18] Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Yu T. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The lancet. 2020 Feb 15;395(10223):507-13.
[19] Chen X, Zhao B, Qu Y, Chen Y, Xiong J, Feng Y, Men D, Huang Q, Liu Y, Yang B, Ding J. Detectable serum severe acute respiratory syndrome coronavirus 2 viral load (RNAemia) is closely correlated with drastically elevated interleukin 6 level in critically ill patients with coronavirus disease 2019. Clinical Infectious Diseases. 2020 Oct 15;71(8):1937-42.
[20] Kaftan AN, Hussain MK, Algenabi AA, Naser FH, Enaya MA. Predictive Value of C–reactive Protein, Lactate Dehydrogenase, Ferritin and D-dimer Levels in Diagnosing COVID-19 Patients: a Retrospective Study. Acta Informatica Medica. 2021 Mar;29(1):45.

**To cite this article:** Rasheed RS, Salim S. Interleukin 6 Levels and their Correlation with Various Hematological and Biochemical Parameters in Covid-19 Patients . Al-Kindy College Medical Journal. 2023;19(1):75–80.