How to Cite:
Noori, A. H., & Ali, M. H. (2023). IL6 as prognostic factor for severity of COVID-19. *International Journal of Health Sciences*, *6*(S9), 4753–4760. https://doi.org/10.53730/ijhs.v6nS9.14027

IL6 as prognostic factor for severity of COVID-19

Aseel Hamzah Noori
Babylon College of Medicine/University of Babylon, Babylon, Iraq
Corresponding author email: dr.ahn1977alkhawaja@gmail.com

Mohammed Hasan Ali
Babylon College of Medicine/University of Babylon, Babylon, Iraq

***Abstract*---**Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the viral illness known as coronavirus disease 2019. Several inflammatory and biochemical markers are elevated, and blood indices are altered, in COVID-19, a sign of a cytokine storm.(50) COVID-19 patients at Marjan medical city and Imam Sadiq Teaching hospitals between December -2021 and April -2022 were studied for the connection of IL-6 levels. Results of blood tests varied significantly depending on the severity of the condition. Interleukin-6 (IL-6) and SPO2 (percent) were significantly different between the two measurement periods at day 1 and day 3. Nearly three out of four patients had severe illness (N=34, 68.0 percent), and there was no one with moderate disease (N=16, 32.0 percent). According to the level of illness severity, there were large variations in the distribution of age groups. This study found a strong correlation between illness severity and immunization history and previous medical records.

***Keywords*---**IL-6 levels, COVID-19 patients, hematological and serological markers.

Introduction

Coronavirus disease 2019 (COVID-19) is an infectious illness caused by the coronavirus 2 that causes severe acute respiratory syndrome (SARS-CoV-2). First discovered in China's Hubei province capital of Wuhan, where it causes life-threatening respiratory illnesses such pneumonia and lung failure (Ahn et al., 2020).

The World Health Organization (WHO) proclaimed the outbreak of coronavirus disease 2019 (COVID-19) a worldwide pandemic in March 2020. The virus has

spread to almost every continent in the world as of this writing (Chen et al., 2020).

A member of the Coronavirinae subfamily of the Coronaviridae family and the Nidovirales order, this pathogen (Cong et al., 2017). This is the biggest known genome among all RNA viruses, and it is found in the coronaviruses, which are enclosed viruses. Under electron microscopy, the projecting spike proteins on the surface of coronavirus virions resemble a crown, thus the word "coronavirus" (Zhao et al., 2020).

When two people come into close physical contact, respiratory droplets are the primary mode of transmission for SARS-CoV-2. Asymptomatic, pre-symptomatic, and symptomatic carriers may all transmit the disease (Wiersinga et al., 2020). SARS-ability CoV2's to enter the body is dependent on the virion's ability to attach to the ACE2 receptor (Zhou et al., 2020). SARS-CoV-2 elicits an immunological response with inflammatory cytokine production and a modest interferon (IFN) response when it enters respiratory epithelial cells. A cytokine storm occurs as a consequence of the subsequent infiltration of macrophages and neutrophils into the lung tissue (Hussman, 2020).

It has been shown that SARS-CoV2 is particularly effective in activating pathogenic Th1 cells, which then release proinflammatory mediators such GM-CSF and interleukin 6 (IL-6). Tumor necrosis factor (TNF) and other cytokines are produced in considerable amounts by activated CD14+CD16+ inflammatory monocytes after exposure to GM-CSF (Zhou et al., 2020). By entering the bloodstream, these inflammatory mediators may cause harm to other organs as well as the epithelial cells that line the inside of the body (Rothan & Byrareddy, 2020). An major role in inflammation is played by interleukin-6 (IL-6), an important component of the cytokine network (Zhang et al., 2020).

A wide variety of stromal and immune system cells, as well as lymphocytes, monocytes, dendritic and mast cells, as well as non-lymphocytic ones including fibroblasts, endothelium and keratinocyte cells, as well as tumor cells, may all generate the interleukin-6 (IL-6) receptor agonist cytokine (Jones & Jenkins, 2018). More data suggests that critically sick individuals with severe respiratory failure and SARS-CoV-2 have either immunological dysregulation or macrophageactivation syndrome, both of which are marked by pro-inflammatory cytokines. Interleukin-6 (IL-6), not Interleukin-1beta (IL-1beta), is the driving force behind the immunological dysregulation (Giamarellos-Bourboulis et al., 2020).

Overproduction of pro-inflammatory cytokines by monocytes and lymphocyte dysregulation with CD4 lymphopenia are two major hallmarks of this immune dysregulation (Giamarellos-Bourboulis et al., 2020). When activated, tumor necrosis factor (TNF) is a potent pro-inflammatory agent that may be found in a wide range of tissues (Lai et al., 2016).

Lung damage is linked to TNF- in disorders like influenza and COVID-19. Patients with ARDS have been shown to have elevated levels of TNF- in plasma and alveolar fluid lavage. Endothelial permeability increases and alveolar fluid

clearance decreases when cytokines are elevated because sodium channels in the epithelium are downregulated (Feldmann et al., 2020).

Materials and Method

Human IL-6-specific antibodies were employed to cover the microliter wells in this test, which was performed using the Sandwich-ELISA method. The specified antibody was mixed with the standards or samples in the microliter wells. For each microliter well, a biotinylated detection antibody specific for Human IL-6 and an Avidin-Horse Radish Peroxidase (HRP) conjugate were added, then free components were washed away. After that, each well was filled with a substrate solution and incubated. The enzyme-substrate process was finally stopped by adding stop solution, resulting in a yellow color change. The IL-6 concentration was determined using a 450 nm standard curve. Each well received 100 l of standard or sample, which was then incubated on an orbital shaker for 1 hour at room temperature. Use 350 ml of wash buffer to wash five times. Detection of biotinylated compounds Biotinylated detection Ab was added and incubated on an orbital shaker for 1 hour at room temperature before being aspirated and washed five times with 350 l of wash buffer. On an orbital shaker at room temperature for 30 minutes, 100 l HRP Conjugate was added, and washed five times with 350 l of wash buffer.,100 l of the substrate solution was added and incubated for 30 minutes at room temperature under dark conditions before being discarded., At 450 nm, a stop solution of 100 l was added and the reading was immediately taken.

Results and Discussion

Results

Table 1 shows distribution of COVID-19 patients according to study variables including (age, gender and vaccination history).Mean age of patients were (59.20 ± 16.86) years, younger patient was 20 years and older patient was 90 years. Majority of patients were male (60.0%), vaccinated patients represented only (34.0%) of patients. Table 4 shows the association between severity of disease including (moderate and severe) and study variables including (gender, vaccination history and past medical history).There was significant association between severity of disease and vaccination history and past medical history. The mean differences of interleukin-6 at day 1 and interleukin-6 at day 3 according to severity of disease including (moderate and severe).There were significant differences between means of interleukin-6 at day 1 and interleukin-6 at day 3 according to severity of disease this result show that no significant difference at P. Value >0.05 as in the table.

Table 3.4 Association between severity of disease and study variables (N=50))

Study variables	Severity		Total	X^2	P-value
	Moderate (N=16)	Severe (N=34)			
Gender					
Male	11 (68.8)	19 (55.9)	30 (60.0)	0.751	0.386
Female	5 (31.2)	15 (44.1)	20 (40.0)		
Total	16 (100.0)	34 (100.0)	50 (100.0)		
Vaccination history					
Positive	11 (68.8)	6 (17.6)	17 (34.0)	**12.662**	**<0.001***
Negative	5 (31.2)	28 (82.4)	33 (66.0)		
Total	16 (100.0)	34 (100.0)	50 (100.0)		
PMH					
Positive	3 (18.8)	26 (76.5)	29 (58.0)	**14.880**	**<0.001***
Negative	13 (81.2)	8 (23.5)	21 (42.0)		
Total	16 (100.0)	34 (100.0)	50 (100.0)		

*P value ≤ 0.05 was significant.

Table 3.5: The mean differences of interleukin-6 at day 1 and interleukin-6 at day 3 according to severity of disease (N=50)

Study variables	Severity	N	Mean ± SD	t-test	P-value
IL-6 at day 1 (pg/ml)	Moderate	16	14.04 ± 11.00	**-5.483**	**<0.001***
	Severe	34	41.18 ± 23.99		
IL-6 at day 3 (pg/ml)	Moderate	16	4.74 ± 3.33	**-5.204**	**<0.001***
	Severe	34	14.59 ± 9.92		

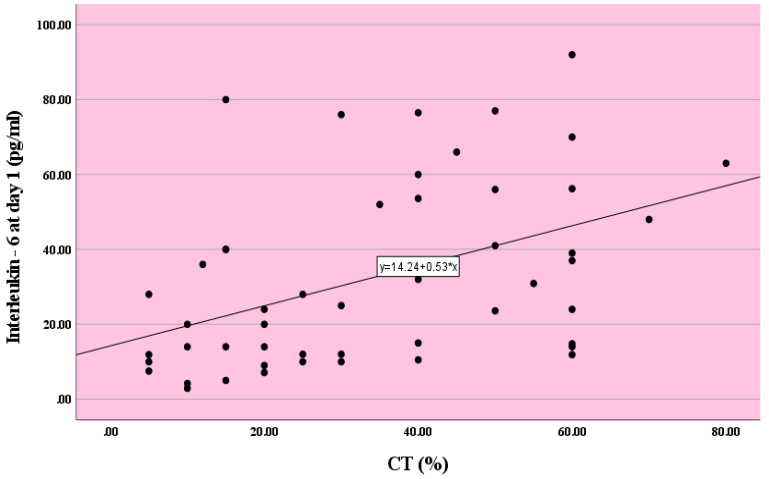

Figure 3.6: The correlation between interleukin-6 at day 1 and CT involvement (N=50, r = 0.451, P= 0.001*)

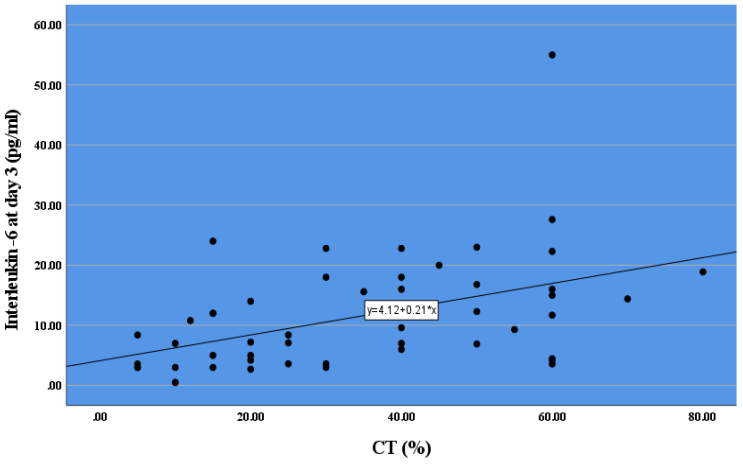

Figure 3.7: The correlation between interleukin-6 at day 3 and CT involvement (N=50, r = 0.46, P<0.001*)

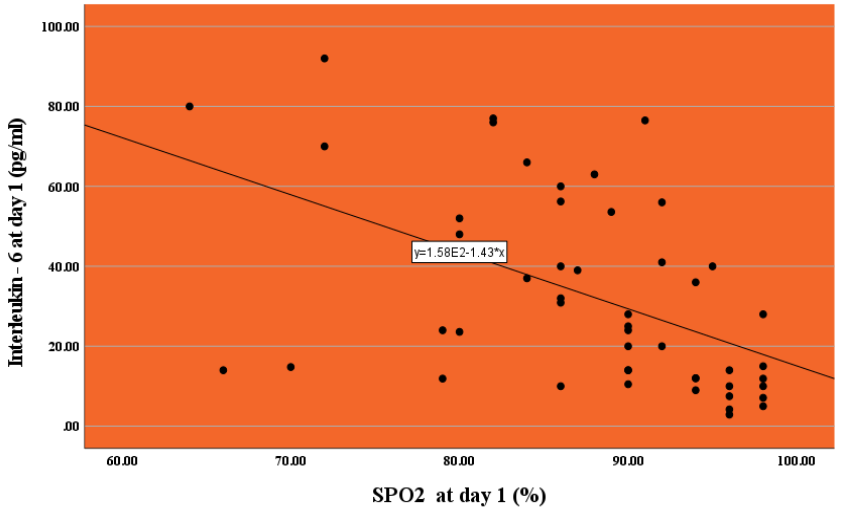

Figure 3.8: The correlation between interleukin-6 and SPO2 (%) at day 1 (N=50, r = -0.503, P<0.001*)

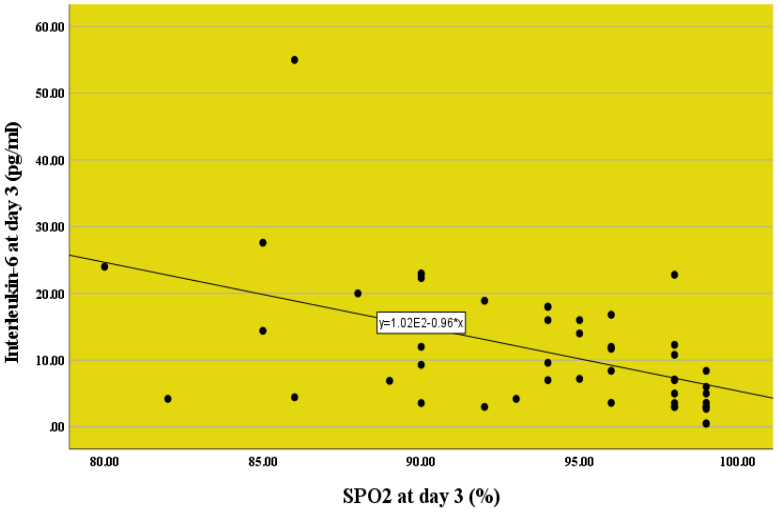

Figure 3.9: The correlation between interleukin-6 and SPO2 (%) at day 3 (N=50, r = -0.503, P<0.001*)

Discussion

Although blood levels of IL-6 were higher in mild instances in this investigation, the rise in cytokine levels was significantly greater in moderate and severe patients. These findings are in line with those of (Liu et al., 2020; Udomsinprasert et al., 2020), who observed that IL-6 levels rose with illness severity.It's worth noting that their increased levels in moderate instances make them more trustworthy indicators than D. dimer, S. ferritin, and LDH.

Serum cytokine levels have increased in patients with COVID-19, especially in the most severe instances, showing that cytokine storm is associated to disease severity (Liu et al., 2020). Baseline IL-6, CRP, LDH and ferritin was closely related to severity of COVID-19. Elevated IL-6 was significantly related to clinical manifestations of severe type patients (Liu et al., 2020). Also, Qin et al. 2020) reported that a positive correlation between CRP, IL-6 and LDH and with severity of infection. Levels of LDH, CRP,D-dimer, IL-6 increased over normal ranges in COVID-19- induced pneumonia patients (Chen et al., 2020). A Spanish group, on the other hand, provided a mortality risk model based on IL-6 at admission (the variable with the best specificity), SpO2/FiO2 ratio, Lactate dehydrogenase (LDH) level, neutrophil-to-lymphocyte ratio, and age collected from 443 patients (Rocio et al., 2020).

Following this notion of change over time, an Italian retrospective research demonstrated the use of IL-6 in combination with CRP and SpO2/FiO2 in identifying patients who will have clinical deterioration in the near term (within the first three days of arrival) (Vultaggio et al., 2020). There was significant positive linear correlation between interleukin-6 at day 1 and day 3 and CT involvement. Further study demonstrated that the alterations in IL-6 were linked with severity and illness course of severe COVID-19. Higher baseline IL-6 was

related with more advanced chest CT evaluation. Consistently, severe COVID-19 patients who required more extensive care and therapy, perhaps because to more severe lung injury, had greater baseline IL-6 level. In this research, CRP, ferritin, IL-6, and LDH dropped considerably after recovery. In connection with illness development demonstrated by aggravating pulmonary lesions on chest CT scan, IL-6 elevated to a further degree. Collectively, our data show that IL-6 would be a useful choice for monitoring severe type COVID-19 (Liu et al., 2020). When treating SARS patients, dynamic variations in IL-6 levels have been shown to occur simultaneously with changes in radiographic scores (Chien et al., 2006).

Conclusion

First, mild COVID-19 patients are younger than intermediate and severe COVID-19 patients. In the most severe instances (moderate to severe), lymphopenia and neutrophilia, as well as a rise in total blood cell counts, are present. It has been shown that only C.reative protein is raised in mild instances, as well as in other severity categories. However, other biomarkers, including as D. dimer and S. ferritin, were raised in more severe types of COVID-19 illness. All severity categories had increased levels of the cytokines IL-6, although moderate and severe versions had the highest amounts.

References

Abady, N. R., Al-Khafaji, Z. A., & Baay, A. S. (2022). Clinical features associated with Severe Acute Respiratory Syndrome (SARS-COV2). International Journal of Health Sciences, 6(S1), 5679–5688. https://doi.org/10.53730/ijhs.v6nS1.6239

Akira, S., Taga, T., & Kishimoto, T. (1993). Interleukin-6 in biology and medicine. Advances in immunology, 54, 1-78

Al-Khafaji, Z., N. Abady, and H. Al-Kafaji, Epidemiological and Clinical Comparative Study for COVID-19 Patients in Babylon Province, Iraq. Archives of Razi Institute, 2022. 77(1): p. 101-105.

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., ... & Zhang, L. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The lancet, 395(10223), 507-513

Delerive, P., De Bosscher, K., Besnard, S., Berghe, W. V., Peters, J. M., Gonzalez, F. J., ... & Staels, B. (1999). Peroxisome proliferator-activated receptor α negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-κB and AP-1. Journal of Biological Chemistry, 274(45), 32048-32054

Diao, B., Wen, K., Chen, J., Liu, Y., Yuan, Z., Han, C., ... & Wu, Y. (2020). Diagnosis of acute respiratory syndrome coronavirus 2 infection by detection of nucleocapsid protein. MedRxiv.

Fielding, R. A., Vellas, B., Evans, W. J., Bhasin, S., Morley, J. E., Newman, A. B., ... & Zamboni, M. (2011). Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia. Journal of the American Medical Directors Association, 12(4), 249-256

Fishman, D., Faulds, G., Jeffery, R., Mohamed-Ali, V., Yudkin, J. S., Humphries, S., & Woo, P. (1998). The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. The Journal of clinical investigation, 102(7), 1369-1376

Hashizume, M., Hayakawa, N., & Mihara, M. (2008). IL-6 trans-signalling directly induces RANKL on fibroblast-like synovial cells and is involved in RANKL induction by TNF-α and IL-17. Rheumatology, 47(11), 1635-1640

Heinrich, P. C., Castell, J. V., & Andus, T. (1990). Interleukin-6 and the acute phase response. Biochemical journal, 265(3), 621

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., ... & Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The lancet, 395(10223), 497-506.

Jilka, R. L., Hangoc, G., Girasole, G., Passeri, G., Williams, D. C., Abrams, J. S., ... & Manolagas, S. C. (1992). Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science, 257(5066), 88-91

Kimura, A., & Kishimoto, T. (2010). IL-6: regulator of Treg/Th17 balance. European journal of immunology, 40(7), 1830-1835

Kotake, S., Sato, K., Kim, K. J., Takahashi, N., Udagawa, N., Nakamura, I., ... & Kashiwazaki, S. (1996). Interleukin-6 and soluble interleukin-6 receptors in the synovial fluids from rheumatoid arthritis patients are responsible for osteoclast-like cell formation. Journal of Bone and Mineral Research, 11(1), 88-95

Libermann, T. A., & Baltimore, D. (1990). Activation of interleukin-6 gene expression through the NF-kappa B transcription factor. Molecular and cellular biology, 10(5), 2327-2334

Magro, G. (2020). SARS-CoV-2 and COVID-19: is interleukin-6 (IL-6) the'culprit lesion'of ARDS onset? What is there besides Tocilizumab

Noor Abady, Zaytoon A Alkhafaji, Ali Saleh Baay [2] Genetic Detection and Phylogenetic Tree Study for SARS COV-2 (N Gene) In Iraq. Journal of Complementary Medicine Research, 12 (4), 131-135. doi:10.5455/jcmr.2021.12.04.20

Noor. R. A., zaytoon. A & Baay, . A. s. (2022) Detection of missense and frame shift mutations in envelope proteins using molecular phylogenetic SARS-COV-2. Journal of Complementary Medicine Research, 13 [2], 92-97. doi:10.5455/jcmr.2022.13.01.17

Poli, V., Balena, R., Fattori, E., Markatos, A., Yamamoto, M., Tanaka, H., ... & Costantini, F. (1994). Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion. The EMBO journal, 13(5), 1189-1196.