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The reduction of IL6 serum level after four months of COVID- 19 infection in the medical staff of Neurosurgery Teaching Hospital in Baghdad

Mawada N. Abdulla[1*], Ahmed R. Abdulla[2], Mustafa J. Al-Ghazi[3]

[1] Neurosurgery Teaching Hospital, Ministry of Health/Iraq
[2] The Medical Microbiology depart., College of Medicine, Al-Iraqia University /Iraq
[3]College of Medical Technology, Al- Farahidi University/Iraq
Email: masnaj2018@gmail.com

***Correspondence author:** Mawada N. Abdulla (masnaj2018@gmail.com)

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Abstract

Background: SARS-CoV-2, the causative agent of COVID-19, was first detected in Wuhan, China, in late 2019 in a cluster of patients with pneumonia. Accumulatively and until July 2022, Iraq registered 2,438,101 million COVID-19 cases and 25,304 deaths putting Iraq in third place among the Eastern Mediterranean Sea countries.The pathophysiological hallmark of COVID is closely associated with severe inflammatory responses; thereby, identifying the serum level of IL-6 may predict the progression of COVID-19 disease. Materials and Methods: This study included ninety volunteered medical staff who worked in the Neurosurgery Teaching Hospital and were diagnosed with COVID-19 by PCR and accepted to give 5 ml of their blood. The medical staff was categorized into two groups and every group contain forty-five, we collected blood from the first group after one month (30 days) from the day of COVID-19 infection diagnosis while the second group after 4 months (120 days). we used a Quantikine IL-6 ELISA kit (catalogs numbers D6050 56050 PD6050) which is a sandwich ELISA and according to the manufacturer to estimate the serum levels of Human IL-6. Results: The independent two-sample Mann−Whitney test was used which showed that there was a significant decrease ($P<0.05$) in the IL-6 serum levels of the volunteered Medical staff after 4 months when it is compared to the one month Conclusion:IL6 serum levels decreased significantly after 120 days (4months) of COVID-19 infection.

Keywords

Covid-19, Neurosurgery Teaching Hospital, IL-6 serum levels, One month, four months.

Coronaviruses (family Coronaviridae) are common pathogens of humans and animals. Four coronaviruses are endemic in humans (human coronavirus NL63 (HCoV-NL63), HCoV-229E, HCoV-OC43, and HCoV-HKU1) and typically infect the upper respiratory tract, causing common-cold symptoms. In the past two decades, three zoonotic coronaviruses (severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2) have infected humans, after spilling over from animal reservoirs. SARS-CoV originated in China and caused an epidemic in 2003, whereas MERS-CoV is currently causing intermit- tent outbreaks in the Middle East. SARS-CoV-2, the causative agent of COVID-19, was first detected in Wuhan, China, in late 2019 in a cluster of patients with pneumonia. These three viruses can replicate in the lower respiratory tract and may cause a potentially fatal acute respiratory distress syndrome (ARDS) (1). The first cells targeted by SARS-CoV-2 during natural infection in humans are likely to be multiciliated cells in the nasopharynx or trachea and sustentacular cells in the nasal olfactory mucosa. After entry, the positive-sense SARS-CoV-2 genome directly

initiates the production of viral proteins, including the replicase proteins that form replication factories from endoplasmic reticulum membranes These replication factories contain double-membrane vesicles in which transcription occurs, shielding the double-stranded RNA (dsRNA) transcription intermediates from detection by cytoplasmic pattern recognition receptors (PRRs). The main cytoplasmic PRR capable of detecting SARS-CoV-2 is thought to be MDA5 (ReFS27,28), which recognizes long dsRNAs and initiates signaling cascade to promote the transcription of type I and type III interferons. Interferons and chemokines are also produced by bystander epithelial cells and local immune cells (for example, neutrophils and macrophages) in response to the detection of SARS-CoV-2 using endosomal Toll-like receptors (TLRs) or paracrine effects of locally produced interferons. Interferons signal in an autocrine and paracrine fashion to induce an antiviral cellular state through the production of interferon-stimulated genes, which may have direct or indirect (attraction of immune cells) antiviral functions. At the same time, the production of cytokines like IL-1,6,8 and TNF-α promotes the development of adaptive B cell and T cell responses that help clear the virus (2).

Globally the WHO reported in July 2022 (572,239,451) confirmed cases of COVID-19 including (6, 390, 401) deaths, (3). In Iraq, COVID-19 infections began to appear in February 2020, specifically in Najaf province when an infected Iranian student who had entered Iraq before the prohibition was announced by COVID-19 there. Accumulatively and until July 2022, Iraq registered 2,438,101 million COVID-19 cases and 25,304 deaths putting Iraq in third place among the Eastern Mediterranean Sea countries (4). Healthcare workers have more risk of encountering infectious diseases such as COVID-19 than the normal population.

Based on recent studies, it was strikingly shown that the level of inflammatory cytokines is increased in COVID- 19. An overview of the literature indicates that IL-6, IL-2, IL-7, IL-10, granulocyte colony-stimulating factor (G-CSF), IFN- γ, inducible protein (IP)-10, TNF-α, MCP-1, macrophage inflammatory protein (MIP)-1α play a crucial role in the pathogenesis of COVID-19(5).

With this evidence, a great deal of attention has been paid to dampening cytokine signaling pathways of inflammatory ones aiming to reduce inflammatory responses and mortality in patients suffering from COVID-19. According to the literature, cytokines have a key role in regulating immunological and inflammatory profiles. Among the cytokines, IL-6 is known as a causative factor in the pathogenesis and severity of COVID-19 due to various pleiotropic functions. Therefore, continuous measurement of IL-6 levels is suggested in affected subjects with COVID-19. Multiple clinical trials are ongoing to evaluate the benefit of cytokine blockade by corresponding inhibitors (6).

IL-6 is a glycoprotein that can act as both pro-inflammatory and anti-inflammatory cytokines. It can be produced by stromal, almost all immune cells, and other cells such as endothelial cells, fibroblasts, keratinocytes, and tumor cells. It has been well established that IL-6 has a crucial role in the differentiation of B-cells and the production of antibodies. Other immunomodulatory roles of IL-6 are linked to the development of self-reactive pro-inflammatory CD4+ T-cell response, stimulation of cytotoxic T-lymphocyte activity, regulation of T-helper 17, and regulatory T-cell balance (7,8).

The pathophysiological hallmark of COVID is closely associated with severe inflammatory responses; thereby, identifying the serum level of IL-6 may predict the progression of COVID-19 disease (9).

The aim of our study is to evaluate the role of IL-6 in COVID-19 infection by achieving the objective of estimating IL-6 serum levels in the medical staff of Neurosurgery Teaching Hospital in Baghdad after 1 month and 4 months of infection.

Materials and Methods

The Ethical Review Committee of the College of Medicine in Al- Iraqia University reviewed and approved the study.This study included ninety volunteered medical staff who worked in the Neurosurgery Teaching Hospital and were diagnosed with COVID-19 by PCR and accepted to give 5 ml of their blood.Blood samples were collected from December 2021 to March 2022 in the virology laboratory inside the Neurosurgery Teaching Hospital and the demographic data have been collected by a questionnaire sheet. The medical staff was categorized into two groups and every group containforty-five, we collected bloodfrom the first group after one month (30 days) from the day of COVID-19 infection diagnosis while the second group after 4 months (120 days).The collected blood was put in a gel tube which was left at room temperature for about 10 minutes for clotting then centrifuged at 3600 Xg to gain serum. The collected serum was transferred into several Eppendorf tubes with 500 µL and kept at frozen temperature-20 Cє to be analyzed later for estimating the serum levels of Interleukin 6. we used quantikine IL-6ELISA kit (catalogs numbers D6050 56050 PD6050) which is a sandwich ELISA and according to the manufacturer, the sensitivity

of the quantification kit is less than 0.70 pg/ml.

The quantitative results have been studied according to the ratio of the extinction of the monitored or tested pattern to the extinction of the calibrator. The resulting data were counted by determination of the mean absorbance of each duplicated measurement. The mean calculation then was made by plotting the common logarithm of absorbance against concentration in pg/ml for each calibrator of the Human Interleukin-6 ELISA Kit.

Statistical analysis was made by STATISTICA version 12 in addition to SPSS statistical software v.26. The distribution standard was analyzed preliminarily by Kolmogorov–Smirnov, and Shapiro–Wilk tests. Categorical values havebeen expressed as absolute and relative frequencies. The distinction between the groups for persistent as well as categorical variables was made by the non-parametric Kruskal–Wallis test which deals with more than two groups and by the Mann–Whitney U-test which deals with Bonferroni's correction when necessary, to find out the differences between 1 and 4 months of Human Interleukin-6 plasma levels ($P<0.05$).

Results

Ninety medical staff volunteered from the surgery teaching hospital to conduct our study and their demographic data showed thattheir mean age was 35.29 ± 34.02 years old while their gender distribution in 1 month (30 days) and 4 months (120 days) can be seen in table 1 and 2.

Table 1. The Frequency and Percentage of Gender after one month

Gender	Frequency	Percent	Valid Percent	Cumulative Percent
Female	11	24.4	24.4	24.4
Male	34	75.6	75.6	100.0
Total	45	100.0	100.0	

Table 2. The Frequency and Percentage of Gender after four months

Gender	Frequency	Percent	Valid Percent	Cumulative Percent
Female	16	35.6	35.6	35.6
Male	29	64.4	64.4	100.0
Total	45	100.0	100.0	

After obtaining the ELISA results of IL-6 serum levels (1months) and (4 months), the data were analyzed according to the Kolmogorov– Smirnov, and Shapiro–Wilk tests as they were non-parametric. Accordingly, the independent two-sample Mann–Whitney test was used which showed that there was a significant decrease ($P<0.05$) in the IL-6 serum levels of the volunteered Medical staff after 4 months when it is compared to the one month as shown in tables 3 and 4.

Table 3. The normality Test for IL-6 serum level after 30 and 120 days

Variable		IL-6 (1 month)	IL-6(4 month)
N		45	45
Normal Parameters[a,b]	Mean	.5139	.3321
	Std. Deviation	.17300	.12213
Most Extreme Differences	Absolute	.139	.102
	Positive	.066	.102
	Negative	-.139	-.054
Test Statistic		.139	.102
P (2-tailed)		.029[c]	.200[cd]

Table 4. Independent two-sample Mann-Whitney test for IL-6 serum levels after 1 and 4 months

Variable	The Medical Staff infected with COVID-19	Mean Rank	Z	P-value. (2-tailed)
IL-6	1 Month	59.04	-4.919	.000
	4 Months	31.96		

Discussion

Cytokines are vital in regulating immunological and inflammatory responses. Among them, IL-6 is of major importance because of its multifunctional (pleiotropic) effects. It is synthesized by immune and stromal cells in response to activation of toll-like receptors mediated by "molecular patterns"

associated with pathogens and damage (pathogen-associated molecular patterns and damage-associated molecular patterns-PAMP & DAMP).

The biological activity of IL-6 is determined by its potential to activate target genes that regulate cell differentiation, survival, proliferation, and apoptosis. IL6 functions as an autocrine, paracrine, and "hormone-like" regulator of various normal and pathological biological processes associated with local and systemic inflammation, metabolism, and tumorigenesis.

MIl-6Rα is expressed only in particular types of cells (macrophages, neutrophils, CD4 T cells, hepatocytes, podocytes, megakaryocytes, and specialized intestinal epithelial cells); while gp130 (IL6-RЯ) is present in almost all cells of the human body. Initiation of the IL6-induced signaling cascade begins after binding of the IL-6 andIL-6-R complex to gp130, which, when dimerized, leads to activation of Janus kinases 1 and 2, via phosphorylation of tyrosine residues of the gp130 cytoplasmic site. Most human cells do not express mIl6-Rα, and therefore are resistant to the biological effects of IL6.

However, the bloodstream and tissues also contain a soluble (s) form of IL6-Rα, which is formed by proteolytic cleavage mediated by Zn2+ metalloprotease ADAM (a Disintegrin and Metalloproteinase domain) 10 and 17, and, to a lesser extent, by "alternative splicing" of messenger RNA. SIl6-Rα protects IL-6 from enzymatic cleavage, and therefore, prolongs its circulation in the blood and, most importantly, in tandem with IL6, sIL-6Rα can bind to gp130, therefore activating many cell types that do not express mIL-6Rα. This process is called "trans-signaling", while cell activation mediated by the interaction of IL6 with mIl6-R is defined as cis-signaling. Hypothetically pathogenic effects of IL-6 are mostly determined by trans-signaling rather than cis-signaling (10).

At the same time, "classical" (cis) signaling is also involved in the induction of acute-phase response, the production of pathogenic Th17 and Th22 cells, and the suppression of T regulatory cells. Therefore, trans and cis-signaling provide a multidirectional contribution to the development of the immunopathological process in the course of disease progression. "Trans-presentation" as a new mechanism of IL-6 signaling has been described recently when IL6 binds to IL-6Rα on the membrane of specialized dendritic cells and is "presented" to the gp130 homodimer, expressed on the surface of cognate T cells. This mechanism is believed to play a major role in actualizing IL-6 potential to induce differentiation of the pathogenic subpopulation of Th17 cells (11).

An increase in IL-6 levels has previously been observed in patients with respiratory dysfunction, implying a possible shared mechanism of cytokine-mediated lung damage caused by COVID-9 infection. Furthermore, it seems that the highly pathogenic SARS-CoV-2 is associated with rapid virus replication and a tendency to infect the lower respiratory tract, resulting in an elevated response of IL-6-induced severe respiratory distress (12) (13).

A study done by Del Valle et al. in 2020 found that IL-6 was one of the most robust prognostic markers of survival, eclipsing or outperforming CRP, D-dimer, and ferritin after adjusting for the demographic features and comorbidities in COVID-19. It remained independently associated with severity and predictive of the outcome when including information about ventilation and end-organ damage (14).

IL-6 plays a crucial role in the immunopathogenesis of COVID-19 and is supported by data from numerous studies reporting increased serum concentrations of this cytokine, foremost in severe cases (15) (16).

A meta-analysis of COVID-19 cases ($n = 1302$) indicates that the level of IL-6 was 3-fold higher in patients with severe vs mild/moderate COVID-19 ($p < 0.001$), and that high baseline IL-6 concentration correlates with the development of bilateral lung damage ($p = 0.001$) and pyrexia ($p = 0.001$). Also,Meta-regression indicates that increased IL-6 concentrations were significantly associated with an increase in mortality ($p = 0.03$) (17).

According to our study results, IL-6 showed a significant decrease after 4 months of COVID-19 Infection which can be explained because of an absence of systemic inflammation which other studies confirmed by indicating IL-6 as a central player in SARS-CoV-2 induced disease compared with thenetwork of other cytokines, and is a better representative of systemic inflammation (18) (19).

Conclusion

IL6 serum levels decreased significantly after 120 days (4 months) of COVID-19 infection.

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