NEXUS OF MEDICINE AND LABORATORY SCIENCE JOURNAL

ISSN (ONLINE): 3027-2998

EVALUATION OF INFLAMMATORY CYTOKINES IN COVID-19 PATIENTS AT RIVERS STATE UNIVERSITY TEACHING HOSPITAL, NIGERIA

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Article type: Original

Cite as: Okpara KE, Elechi Q, Amadi CF, Onyekwere N. Evaluation of Inflammatory Cytokines in Covid-19 Patients at Rivers State University Teaching Hospital, NigeriA. Nexus Med. Lab. Sci. J. 2025;2(1):10-20

Received on 7th February, 2025; Accepted on 25th February, 2025; Published on 5th March, 2025

Publisher: ScholarlyFeed

https://doi.org/10.71462/sfpl2502002

ABSTRACT

The COVID-19 pandemic has highlighted the critical role of inflammatory cytokines in the disease's pathophysiology and outcomes. This study aimed to assess the serum levels of key inflammatory cytokines—IL-1β, IL-6, and TNF-α—in COVID-19 patients at Rivers State University Teaching Hospital, Nigeria. A cross-sectional study was conducted involving forty-one (41) COVID-19 positive patients and forty-one (41) control subjects aged between twenty (20) and seventy (70) years. Blood samples were collected from all consenting participants for cytokine analysis. The findings revealed a significant elevation in IL-6 levels among COVID-19 patients compared to controls ($p < 0.001$). Conversely, IL-1β and TNF-α levels did not show significant differences between the groups ($p = 0.07$ and $p = 0.77$, respectively). There was no significant difference ($p > 0.05$) in IL-6, IL-1 β and TNF- α based on gender. There was a significant difference in IL-1β levels across the age groups ($p = 0.04$) in the test group, while no significant differences were observed for IL-6 ($p = 0.95$) and TNF-α ($p = 0.92$). Post-hoc analysis for IL-1β revealed a significant difference between age groups 31-40 and 51-60 years ($p = 0.00$). In the control group, that there is a significant difference in the levels of IL-6 among the different age groups, with a p-value of 0.04. Post-hoc analysis shows a significant difference between the 31-40 years and 41-50 years groups ($p = 0.02$). This study has shown that COVID-19 patients experience heighten IL-6 level than those without the disease. Although gender may not affect cytokine levels in these patients but age may impact differently with or without the disease.

Keywords: *COVID-19, inflammatory cytokines, IL-1β, IL-6, TNF-α*

INTRODUCTION

Over the last two decades, our understanding of how inflammatory cytokines contribute to disease pathogenesis and their potential as therapeutic targets has grown substantially. Due to the pivotal role of cytokines in protective as well as toxic immune responses, they have also become key biomarkers when diagnosing inflammatory diseases and monitoring responses to treatment [1].

In order to orchestrate a response to infections, tissue injury, and inflammation, the immune system depends on small signaling proteins called inflammatory cytokines. Primarily produced by immune cells including macrophages, lymphocytes, and dendritic cells, they function to modulate the magnitude and the duration of the immune response [2]. The proper balance between pro- and anti-inflammatory cytokines is important for maintaining immune homeostasis, but a deviation from this equilibrium can contribute to pathological conditions such as chronic inflammation, autoimmune diseases, or, in the worst-case scenario, infections with SARS-CoV-2 (COVID-19) [3].

Cytokines, including interleukin (IL)-1, IL-6, tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ), are well-documented in promoting inflammation [4]. These pleiotropic activators trigger cytokine signaling cascades that lead to the recruitment and activation of immune cells at infected or injured sites, facilitating pathogen clearance and supporting tissue repair [5]. However, exaggerated production of pro-inflammatory cytokines, known as a "cytokine storm," can severely impact numerous tissues and organs, rapidly leading to life-threatening organ failure or mortality in conditions such as sepsis or viral infections, including SARS-CoV-2 [6].

Viral entry into the host activates innate immune mechanisms and the synthesis of inflammatory mediators. Specific viral components interact with pattern recognition receptors of the host immune system, activating cellular signaling pathways that culminate in the production of various cytokines [7]. Major cytokines produced include IL-1α/β, IL-18, IL-6, TNF-α, IFN-α/β, and transforming growth factor (TGF)-β, all of which play crucial roles in host antiviral responses. For instance, IFN-α/β inhibits viral replication, while TNF-α promotes immune cell localization at the infection site and apoptosis of infected cells [8]. IL-1 enhances inflammatory cell infiltration by increasing membrane permeability through the activation of mast cells and histamine release. IL-6 mediates monocyte differentiation into macrophages and enhances mononuclear cell infiltration to sites of inflammation, and it, along with TGF-β, induces the maturation of naive T cells into T helper 17 (TH17) cells, which are vital for immune defense against pathogens [9]. IL-18 enhances type 1 immunity and activates natural killer T cells [10].

Excessive cytokine release can lead to severe complications, including acute respiratory distress syndrome (ARDS), multi-organ failure, and death, a phenomenon observed in infections such as SARS, MERS, and SARS-CoV-2 (COVID-19) [11]. Specifically, hypercytokinemia in COVID-19 contributes to disease severity and a hyper-inflammatory state, which is a key driver of pathology [12]. Endothelial cell injury induced by viral infection amplifies the release of pro-inflammatory cytokines like IL-1, IL-6, and TNF-α, further contributing to systemic inflammation [13]. This cytokine storm leads to a prothrombotic state, endothelial damage, and multi-organ dysfunction in some patients [14,15].

The purpose of this article is to compare the levels of pro-inflammatory cytokines in patients with COVID-19 and those without the infection. This will enable one understand the role of the viral infection in cytokine homeostasis among those infected with the virus.

METHODOLOGY

Study Design

A cross-sectional study was employed for the evaluation of inflammatory cytokines in COVID-19 patient in Port-Harcourt City

Isolation Centres as a way to understand the effect of COVID-19 on inflammatory cytokines parameters.

Eligibility Criteria

Inclusion Criteria

Subjects (males or females) who tested positive for COVID-19, confirmed for the disease, and are currently at the isolation centre were included in the study were included in the study. Also, those who tested negative for the disease were recruited for the study to serve as the control subjects.

Exclusion Criteria

On the other hand, unconscious subjects or those experiencing severe difficulty in breathing as a result of COVID-19 were excluded from the study as obtaining consent from them was complex.

Study Population

The population of interest were those infected with coronavirus without gender restrictions. A total of fifty-five (55) COVID-19 positive subjects and fifty-five (55) control subjects were recruited for this study between the ages of twenty (20) and sixty-five (65).

Ethical Consideration

The design of this study was approved by the Ethics Committee Rivers State Ministry of Health for the commencement of the study. Also, written consent was obtained from the participants before their enrollment into the study.

Sampling Method

The consenting participants in the population of interest were randomly selected via simple random sampling technique where all interested participants were given equal opportunities to be selected. They were provided with a list of numbers, having equal number of even and odd numbers. All those who picked even numbers were recruited while those who picked odd numbers were excluded.

Sample Collection

To analyze inflammatory cytokines, a total of 10 mL of whole blood was collected from each subject using sterile hypodermic syringes and needles, adhering to standard venipuncture techniques. This collection aimed to ensure a sufficient volume of blood for various analyses.

For the assessment of inflammatory cytokines, 3 mL of blood was collected and dispensed into a plain bottle. This particular portion was then centrifuged to separate the serum from the cellular components of the blood. Resultantly, the serum was utilized for the analysis of inflammatory cytokines, specifically interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha (TNF-α), using an enzyme-linked immunosorbent assay (ELISA) test kit.

Sample Analysis

Enzyme-Linked Immunosorbent Assay (ELISA) [16] [17].

Statistical Analysis

Data analysis involved descriptive statistics to summarize the data generally and by sex and age subgroups, presenting means and standard deviations. A T-test was used to compare inflammatory cytokine levels between COVID-19 positive and negative subjects. Additionally, Analysis of Variance (ANOVA) examined interaction effects between COVID-19 test outcomes and demographic characteristics. If significant differences were found, Tukey's Honestly Significant Difference (HSD) post hoc tests were applied. All statistical tests were two-tailed, with a significance threshold of $p<0.05$.

RESULTS

TABLE 1: Comparison of Mean and Standard Deviation of Serum Levels of some Inflammatory Cytokines in Patients with COVID-19 Infection and Control Subjects

Group	N	Mean	SD	df	T-value	p-value	Remarks
IL-1β							
Test	41	5.36	7.21	80	1.82	0.07	NSS
Control	41	3.67	5.39				
IL-6							
Test	41	4.90	2.12	80	6.12	0.00	SS
Control	41	2.84	0.44				
TNF-α							
Test	41	2.25	2.28	80	0.29	0.77	NSS
Control	41	0.62	0.36				

NSS: Non-statistically significant; SS: Statistically significant

The results in table 1 indicate that IL-6 levels were significantly higher in the test group compared to the control group, with a p-value of 0.00, signifying statistical significance. However, IL-1β and TNF-α levels did not show statistically significant differences between the test and control groups, with p-values of 0.07 and 0.77, respectively.

Table 2: Comparison of Mean and Standard Deviation of Serum Levels of some Inflammatory Cytokines among the Study Subjects According to Sex

Group	N	Mean	Sd	Df	T	P	Remarks
IL-1β							
Male	40	6.73	5.25	80	0.78	0.44	NSS
Female	42	5.93	3.98				
IL-6							
Male	40	3.40	1.08	80	1.59	0.12	NSS
Female	42	3.83	1.36				
TNF-α							
Male	40	2.33	0.62	80	1.9	0.06	NSS
Female	42	2.12	0.33				

NSS: Non-statistically significant; SS: Statistically significant

The results in table 2 show that there were no statistically significant differences in the levels of IL-1β, IL-6, and TNF-α between males and females. IL-1β had a p-value of 0.44, IL-6 had a p-value of 0.12, and TNF-α had a p-value of 0.06, all indicating no significant gender-based differences in these inflammatory cytokines.

Table 3: Comparison of Mean and Standard Deviation of Serum Levels of some Inflammatory Cytokines among the Total Study Subjects according to Age

Age groups (yrs)	N	IL-1β	IL-6	TNF-α
		Mean±Sd	**Mean**±Sd	**Mean**±Sd
21-30 (T1)	38	6.78±5.23	4.09±1.31	2.23±0.35
31-40 (T2)	23	5.12±2.54	3.09±0.74	2.24±0.47
41-50 (T3)	11	8.15±6.17	2.98±0.56	2.37±0.95
51-60 (T4)	5	5.79±1.64	4.27±1.37	2.59±0.53
61-70 (T5)	5	4.25±1.30	3.32±1.23	2.03±0.25
F-value		1.16	4.57	0.94
P-value		0.34	0.00	0.44
Remarks		NSS	SS	NSS
Post Hoc				
T_1 vs T_2		NSS (0.94)	NSS (0.32)	NSS (0.10)
T_1 vs T_3		NSS (0.97)	NSS (0.21)	NSS (0.98)
T_1 vs T_4		NSS (0.99)	NSS (0.99)	NSS (0.57)
T_1 vs T_5		NSS (0.78)	NSS (0.58)	NSS (0.92)
T_2 vs T_3		NSS (0.65)	NSS (0.99)	NSS (0.98)
T_2 vs T_4		NSS (0.99)	NSS (0.16)	NSS (0.60)
T_2 vs T_5		NSS (0.99)	NSS 90.99)	NSS (0.91)
T_3 vs T_4		NSS (0.82)	SS (0.01)	NSS (0.89)
T_3 vs T_5		NSS (0.40)	NSS (0.97)	NSS (0.63)
T_4 vs T_5		NSS (0.96)	NSS (0.36)	NSS (0.15)

NSS: Non-statistically significant; SS: Statistically significant

The results in table 3 show no statistically significant differences in IL-1β (p = 0.34) and TNF-α (p = 0.44) across and between the age groups. However, IL-6 showed a significant difference among the age groups (p = 0.00). The significant difference was found between age group 41-50 and 51-60 years (p = 0.01).

Table 4: **Comparison of Mean and Standard Deviation of Serum Levels of some Inflammatory Cytokines among the Test Group of Study Subjects according to Age**

Age groups (yrs)	**N**	**IL-1β**	**IL-6**	**TNF-α**
		Mean±Sd	**Mean**±Sd	**Mean**±Sd
21-30 (T1)	9	4.32±0.98	2.78±0.32	2.18±0.40
31-40 (T2)	12	4.62±1.57	2.84±0.55	2.27±0.57
41-50 (T3)	10	8.40±6.44	2.92±0.56	2.30±0.97
51-60 (T4)	5	4.58±1.15	2.88±0.29	2.39±0.59
61-70 (T5)	5	3.68±0.33	2.75±0.33	2.05±0.27
F-value		2.73	0.17	0.23
P-value		0.04	0.95	0.92
Remarks		SS	NSS	NSS
Post Hoc				
T_1 vs T_2		NSS (0.99)	NSS (1.00)	NSS (1.00)
T_1 vs T_3		NSS (0.18)	NSS (0.98)	NSS (0.10)
T_1 vs T_4		NSS (1.00)	NSS (0.99)	NSS (0.97)
T_1 vs T_5		NSS (1.00)	NSS (1.00)	NSS (0.99)
T_2 vs T_3		NSS (0.23)	NSS (1.00)	NSS (1.00)
T_2 vs T_4		SS (0.00)	NSS (1.00)	NSS (1.00)
T_2 vs T_5		NSS (0.98)	NSS (1.00)	NSS (0.96)
T_3 vs T_4		NSS (0.23)	NSS (1.00)	NSS (1.00)
T_3 vs T_5		NSS (0.08)	NSS (0.95)	NSS (0.94)
T_4 vs T_5		NSS (0.99)	NSS (0.98)	NSS (0.85)

NSS: Non-statistically significant; SS: Statistically significant

The results in table 4 showed a statistically significant difference in IL-1β levels across the age groups (p = 0.04), while no significant differences were observed for IL-6 (p = 0.95) and TNF-α (p = 0.92).

Post-hoc analysis for IL-1β revealed a significant difference between age groups 31-40 and 51-60 years (p = 0.00). However, there were no statistically significant differences between any of the age groups for IL-6 and TNF-α.

Table 5: Comparison of Mean and Standard Deviation of Serum Levels of some Inflammatory Cytokines among the Control Group of Study Subjects according to Age

Age groups (yrs)	N	IL-1β	IL-6	TNF-α
		Mean±Sd	Mean±Sd	Mean±Sd
21-30 (T1)	29	7.59±6.13	4.59±1.89	2.28±0.39
31-40 (T2)	7	6.29±3.99	3.65±0.94	2.17±0.25
41-50 (T3)	5	6.34±0.40	5.29±1.15	2.43±0.34
F-value		0.23	3.15	0.77
P-value		0.8	0.04	0.47
Remarks		NSS	SS	NSS
Post Hoc				
T_1 vs T_2		NSS (0.89)	NSS (0.24)	NSS (0.81)
T_1 vs T_3		NSS (0.89)	NSS (0.45)	NSS (0.69)
T_2 vs T_3		NSS (1.00)	SS (0.02)	NSS (0.33)

NSS: Non-statistically significant; SS: Statistically significant

The results presented in table 5 show that there is a significant difference in the levels of IL-6 among the different age groups, with a p-value of 0.04. Post-hoc analysis shows a significant difference between the 31-40 years and 41-50 years groups (p = 0.02).

For IL-1β and TNF-α, there were no significant differences among the groups, with p-values of 0.8 and 0.47, respectively.

DISCUSSION

The analysis of serum levels of inflammatory cytokines in COVID-19 patients reveals notable distinctions between those infected and healthy controls, particularly concerning interleukin-6 (IL-6). The significant elevation of IL-6 in COVID-19 patients underscores its critical role in the pathophysiology of the disease [18, 14]. IL-6 is a pro-inflammatory cytokine that serves as a key mediator in the immune response, often produced by activated macrophages and T cells [20]. Its elevation is indicative of an ongoing inflammatory process, which is characteristic of severe COVID-19 infections [21].

The pronounced increase in IL-6 is consistent with the notion that COVID-19 can induce a hyper-inflammatory state, contributing to the cytokine storm frequently observed in severe cases [22, 23]. This inflammatory cascade can lead to systemic effects, including acute

respiratory distress syndrome (ARDS) and multi-organ dysfunction, often seen in critically ill patients [24]. As such, monitoring IL-6 levels may provide valuable prognostic information, guiding clinical decision-making and treatment strategies, including the potential use of IL-6 inhibitors in managing severe COVID-19 cases [25].

In contrast, the lack of statistically significant differences in IL-1β and tumor necrosis factor-alpha (TNF-α) levels between COVID-19 patients and controls suggests a nuanced inflammatory response [26]. IL-1β is another pro-inflammatory cytokine known to play a crucial role in the immune response; however, its levels did not rise significantly in infected individuals [6]. This finding may imply that the inflammatory response in COVID-19 patients is selective, with IL-6 emerging as a more critical player in the context of severe infection [28]. The similar observation for TNF-α further emphasizes the complexity of the cytokine milieu in COVID-19, where some cytokines may be upregulated while others remain stable [29, 8].

The analysis of serum levels of inflammatory cytokines based on sex reveals no statistically significant differences in IL-1β, IL-6, and TNF-α levels between male and female participants. The p-values for IL-1β (0.44), IL-6 (0.12), and TNF-α (0.06) indicate that the variations in cytokine levels among genders are not substantial enough to suggest meaningful differences in the inflammatory response [31].

The absence of significant differences in IL-1β levels suggests that the inflammatory response may be similarly regulated in both sexes, indicating that both males and females may experience comparable immune reactions to COVID-19 regarding this particular cytokine [32]. This finding challenges the notion that sex-based differences would substantially impact IL-1β production and may imply that other factors, such as genetic predispositions or external environmental influences, could equally modulate inflammatory responses across genders [33, 34].

Similarly, the lack of significant differences in IL-6 levels indicates that the pro-inflammatory responses associated with COVID-19 infection may not be inherently influenced by sex [35]. Although some studies suggest that males may exhibit more severe forms of COVID-19, the current results suggest that this phenomenon might not directly correlate with elevated IL-6 levels [36]. Instead, factors such as comorbidities, age, and individual immune profiles could play a more pivotal role in determining disease severity rather than sex alone [37].

The significant elevation of IL-6 levels in younger age groups compared to older counterparts could reflect an adaptive immune response or an increased production of this pro-inflammatory cytokine in response to viral infection [38]. This finding aligns with literature indicating that younger individuals may experience a hyper-inflammatory state during infections, potentially contributing to disease severity [39]. The role of IL-6 as a mediator of inflammation also underscores its potential as a therapeutic target, with implications for treatments that modulate IL-6 signaling pathways [40].

Conversely, IL-1β and TNF-α levels did not demonstrate statistically significant differences among the age groups. This lack of variation may suggest a more consistent production of these cytokines across ages in response to COVID-19 [41]. It may also indicate that IL-1β and TNF-α are less influenced by age in the context of this viral infection, implying a need for further investigation into the mechanisms governing their production [42, 43].

CONCLUSION

In conclusion, the inflammatory cytokine profile in COVID-19 patients reveals significant elevation of IL-6, which is linked to severe disease outcomes and the phenomenon of cytokine storms. Elevated IL-6 suggests a hyperactive immune response aimed at controlling the virus, highlighting the potential of IL-6 inhibitors as a therapeutic strategy. IL-1β and TNF-α levels did not show significant differences compared to controls. In addition, there was no gender bias in the cytokine levels in COVID-19 patients. However, certain cytokines may be affected based on the age in those with and without the disease.

LIMITATION

The limitations of this study include a small sample size, which may affect the generalizability of the results, and the cross-sectional design, which limits causal inferences. Additionally, potential confounding variables, such as underlying health conditions and medication use, may influence the observed hematological changes. Finally, the focus on specific parameters might overlook other important factors impacting patient outcomes.

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