West African Journal of Allied Health Sciences (2025), 1,23–27

ORIGINAL ARTICLE

An Elevated Interleukin-6 and TNF-α Levels as Predictors of Severity and Poor Outcomes in Critically Ill COVID-19 Patients

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(Received: June 1, 2025 Revised: June 12, 2025 Accepted: June 15, 2025)

Abstract

Introduction: This current research measured Interleukin-6 (IL-6) and Tumor necrosis factor alpha (TNF-α) in an attempt to explore their involvement in progression and clinical outcomes of SARS-CoV-2 among critically infected individuals.
Methods: The current study comprises 49 critically ill and 27 mildly infected COVID-19 patients in an isolation centre in Jigawa State, north-western Nigeria. COVID-19 was confirmed using RT-PCR, while IL-6 and TNF-α were measured using enzyme-linked immunosorbent assay techniques (ELISA).
Results: The findings of this study revealed a statistically significant difference in cytokine levels between the critically ill patients and those with mild infections. In addition, cytokine levels were found to be lower in survivors compared to those who succumbed to the disease. **Conclusion:** Increased cytokines were observed in critically ill patients 'compared to mildly infected ones. Furthermore, the study suggests hypercytokinemia in those patients who lost their lives than those who survived and subsequently discharged.

Keywords: COVID-19, critically ill, IL-6, TNF-α

Introduction

The devastating COVID-19 outbreak, which began in China and rapidly spread to every continent, prompted the World Health Organization (WHO) to declare it a global pandemic in the first quarter of 2020. The outbreak cost many lives and left the world in total chaos World Health Organization 2020. As at the time of this report, WHO has recorded a total of 645,192,987 confirmed COVID-19 cases globally, resulting in 6,640,834 deaths World Health Organization 2022.

Since the report of Nigeria's index COVID-19 case in March 2020, the virus continued to spread across the country. By July 13, all 36 states and the Federal Capital Territory had been impacted by the outbreak Nigeria Centre for Disease Control 2020. The disease is associated with high fatality rates, particularly affecting individuals with comorbidities and the elderly due to their weakened immune systems Roy et al. 2020; Chen et al. 2020; Ali and Rosmilah 2019. Abdelhafiz et al. Abdelhafiz et al. 2020 noted that individuals infected with the virus commonly present with high body temperature and cough, which may progress to shortness of breath.

Despite the fact that the majority of COVID-19 patients are asymptomatic or have mild symptoms, a significant proportion require hospitalization and admission to intensive care units (ICUs) Frank, Veerdonk, and Netea 2020.

Interleukin-6 (IL-6) plays a crucial role in the cytokine network and is primarily produced by stromal and immune cells Zhu et al. 2020. In critically ill patients infected with SARS-CoV-2, immune dysfunction or overactivity may lead to multi-organ failure, both influenced by IL-6 levels Mudhafar and Alsailawi 2019; Jones and Jenkins 2018. Two primary features of this immune dysfunction include excessive cytokine production and lymphocyte dysregulation.

Tumour necrosis factor-alpha (TNF-α), a multifunctional

pro-inflammatory cytokine of the TNF superfamily, is often upregulated during acute lung injury and may trigger a cytokine storm via SARS-CoV-2's interaction with ACE2 Abbas, Lichtman, and Pillai 2018; Baran et al. 2018. This study aimed to examine the roles of IL-6 and TNF-α in the progression and adverse clinical outcomes of patients severely affected by SARS-CoV-2.

Materials and Methods

Study Design and Population

This retrospective cohort study was conducted at an accredited Nigeria Centre for Disease Control and Prevention (NCDC) COVID-19 centre in Dutse, Jigawa State. The study included 76 patients, of which 49 were critically ill and 27 had mild infections.

Definition of Terms

Critically ill COVID-19 patients were defined as those requiring admission to an ICU, including cases of respiratory failure necessitating intubation or mechanical ventilation, shock, or multiple organ failures Centers for Disease Control and Prevention 2024.

Specimen Collection

Blood Samples: Three millilitres (3 mL) of blood were collected into lithium heparin containers and centrifuged at 15,000 revolutions per minute (rpm) for 5 minutes to obtain serum.

Respiratory Extracts: Nasopharyngeal and bronchoalveolar lavage fluids were collected and placed into virus transport medium for immediate processing.

Confirmatory Testing

COVID-19 confirmation was performed using one-step reverse transcription polymerase chain reaction (RT-PCR) as per the Dangene protocol. This method targeted the ORFab and N gene regions using specific primers and fluorescent probes. Nucleic acid extraction followed Liver River (China) protocols.

Cytokine Assay

The concentrations of TNF-α and IL-6 in serum were measured using ELISA kits (Elabscience, USA) via the Double Antibody Sandwich ELISA method. A pre-coated antibody captured the cytokines from samples, after which a biotinylated detection antibody and avidin-enzyme conjugate were added. Following the enzymatic reaction, a color change from blue to yellow was observed upon the addition of stop solution. The color intensity, measured spectrophotometrically, corresponded to cytokine concentration, with quantification done using a standard curve.

Statistical Analysis

Data analysis was conducted using SPSS version 28. Outliers were identified and managed using box plots. Categorical variables were expressed as frequencies and percentages, while continuous variables were reported as means ± standard deviation (SD). A significance threshold of $p < 0.05$ was used. Multiple linear regression analysis was conducted with mortality as the dependent variable and TNF-α and IL-6 levels as independent variables.

Methods

This retrospective cohort study was conducted at an NCDC-accredited COVID-19 center in Dutse, Jigawa State, Nigeria. The study included 76 patients: 49 critically ill and 27 with mild infection.

Definition of Terms

Critically ill COVID-19 patients are defined as those requiring ICU admission, including those with respiratory failure requiring intubation or mechanical ventilation, shock, or multi-organ failure Centers for Disease Control and Prevention 2024.

Specimen Collection

Blood samples: Three millilitres (3 mL) of blood were collected in lithium heparin tubes and centrifuged at 15,000 rpm for 5 minutes to obtain serum.

Respiratory samples: Nasopharyngeal and bronchoalveolar lavage samples were collected and placed in viral transport medium.

Confirmatory Testing

COVID-19 diagnosis was confirmed using one-step reverse transcription polymerase chain reaction (RT-PCR), targeting ORFab and N genes using specific primers and fluorescent probes, following the protocol by Dangene.

Cytokine Quantification

Serum TNF-α and IL-6 concentrations were determined using ELISA kits from Elabscience, USA, employing a Double Antibody Sandwich method. Optical density was measured, and concentrations were extrapolated from a standard curve.

Statistical Analysis

Data were analyzed using SPSS version 28. Outliers were handled via boxplot analysis. Categorical variables were presented as frequencies and percentages, while continuous variables were expressed as mean ± standard deviation (SD). A significance threshold was set at $p < 0.05$. Multiple linear regression was used with mortality as the dependent variable, and TNF-α and IL-6 as predictors.

Ethical Approval

The Health Research Ethics Committee (HREC) of the Jigawa State Department of Infectious Diseases vide *REF* : *RSSH/GEN/226/V*.1/11 waived ethical approval in line with isolation center policy. Informed consent was obtained from all participants prior to inclusion.

Table 1. Socio-demographic Characteristics of the Study Population

Parameter	Critically Ill (n=49)	Mild (n=27)
Age Group		
18–24 yrs	0 (0%)	2 (7.4%)
25–33 yrs	10 (20.4%)	11 (40.8%)
34–41 yrs	7 (14.3%)	12 (44.4%)
42–50 yrs	23 (46.9%)	1 (3.7%)
Above 50 yrs	9 (18.4%)	1 (3.7%)
Gender		
Male	31 (63.3%)	16 (59.3%)
Female	18 (36.7%)	11 (40.7%)
Mortality		
Alive	32 (65.3%)	27 (100%)
Death	17 (34.7%)	0 (0%)
Prognosis		
Good	28 (57.1%)	27 (100%)
Poor	21 (42.9%)	0 (0%)
Comorbidity		
DM	14 (28.6%)	7 (25.9%)
HTN	11 (22.4%)	4 (14.8%)
MP	9 (18.4%)	8 (29.6%)
Others	4 (8.2%)	2 (7.4%)
None	11 (22.4%)	6 (22.2%)

DM: Diabetes Mellitus; HTN: Hypertension; MP: Malaria Parasite.

Table 2. Comparison of IL-6 and TNF- Levels among COVID-19 Patients

Parameter	Critical (n = 49)	Mild (n = 27)	P-value
TNF- (pg/mL)	69.0 ± 15.4	33.3 ± 2.6	.000
IL-6 (pg/mL)	690.1 ± 135.6	491.9 ± 19.4	.000

TNF-: Tumour Necrosis Factor Alpha; IL-6: Interleukin-6; n: number of subjects.

Table 3. Cytokine Levels by Mortality Status among Critically Ill COVID-19 Patients

Mortality Status	IL-6 (pg/mL)	TNF- (pg/mL)	P-value
Alive (n = 32)	598.0 ± 46.8	58.9 ± 6.7	.000
Dead (n = 17)	863.5 ± 45.0	87.9 ± 6.4	.000

IL-6: Interleukin-6; TNF-: Tumour Necrosis Factor Alpha.

Results

A total of 49 severely critical patients were included in the study. The majority of the critically ill patients were males, comprising 31 (63.3%), while females constituted 18 (36.7%). About 32 (65.3%) critically ill COVID-19 patients survived, while 17 (34.7%) succumbed during the study period. Patients with good prognosis were 28 (57.1%) and those with poor prognosis were 21 (42.9%). Among the patients, 14 (28.6%) had diabetes mellitus (DM), 11 (22.4%) had hypertension (HTN), 9 (18.4%) had malaria (MP), 4 (8.2%) had other comorbidities, and 11 (22.4%) had no comorbidities. Among the 27 mildly ill COVID-19 patients, 16 (59.3%) were males and 11 (40.7%) were females. Within this group, 7 had DM, 4 had HTN, 8 had malaria, 2 had other comorbidities, and 6 had none (Table 1).

Interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) levels were significantly elevated in critically ill patients compared to those mildly infected (Table 2). Our findings indicated that patients who died displayed markedly higher levels of cytokines in comparison to those who survived (see Table 3). The adjusted R^2 value indicates that 91% of the variation in critical illness severity is explained by elevated cytokine levels (IL-6 and TNF-α). Statistically significant differences were observed in cytokine concentrations between groups, as depicted in Figure 1 and Figure 2 .

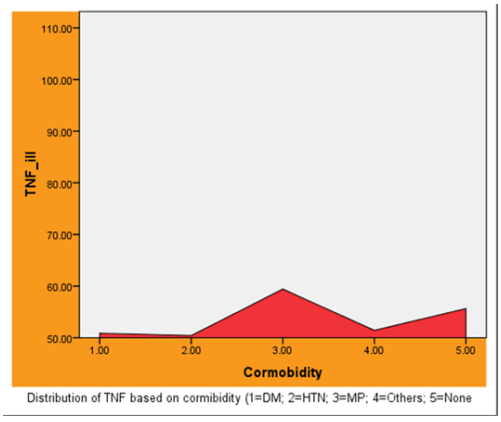

Figure 1. Distribution of Serum levels of (TNF-) based on comorbidities among study subjects

Discussion

COVID-19, caused by *SARS-CoV-2*, manifests with diverse clinical severity. In this study, the large number of critically ill patients underscores the importance of individualized clinical management. Elevated pro-inflammatory cytokines, including TNF-α and IL-6, play a pivotal role in disease severity.

TNF-α is known to promote ACE2 ectodomain shedding, enhancing viral entry. Our results show higher TNF-α in critically ill patients, consistent with the findings of Atal and Fatima Atal and Fatima 2020, Zeng et al. 2020, Cheng et al. 2020, and Xiong et al. 2020. (2020). However, Merza et al. 2021 reported conflicting results.

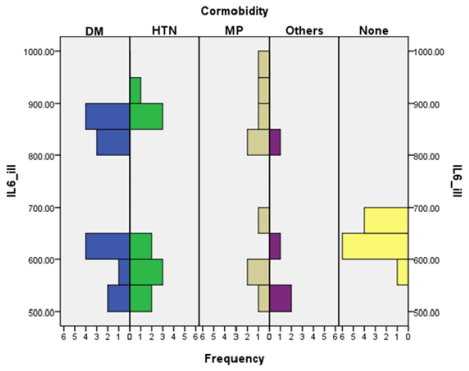

Figure 2. Distribution of Serum levels of IL-6 based on comorbidities among study subjects

IL-6 contributes to cytokine storms, lung injury, and systemic inflammation. Our findings of elevated IL-6 levels in critically ill patients align with previous reports Wu et al. 2020; Gao et al. 2020 Zhu et al. 2020; Fu et al. 2020; He et al. 2020. Notably, IL-6 $\geq$ 24 pg/mL was associated with poor outcomes.

Furthermore, non-survivors exhibited significantly higher IL-6 and TNF-α levels compared to survivors. These findings support Han et al. 2020 and Del Valle et al. 2020, who identified IL-6 and TNF-α as predictors of poor outcomes. Meta-analyses by Ji et al. 2020 and Coomes and Haghbayan 2020 and Haga et al. 2008 confirmed the prognostic significance of IL-6.

Study limitations include a small sample size, limited patient admission during data collection, and inability to measure viral load. Mortality could also be influenced by age and comorbidities.

Conclusion

This study found significantly elevated levels of TNF-α and IL-6 in critically ill COVID-19 patients compared to those with mild infections. These findings highlight hypercytokinemia as a critical inflammatory signature in severely affected individuals. The exaggerated inflammatory responses may play a central role in inducing apoptosis of alveolar epithelial cells, contributing to acute respiratory distress syndrome (ARDS) and leading to rapid disease progression and higher mortality rates among severely ill COVID-19 patients. Based on these results, it is recommended that clinicians monitor cytokine levels, particularly IL-6 and TNF-α, to assess disease severity and inform treatment decisions. Targeting these pro-inflammatory cytokines could be considered in therapeutic strategies aimed at improving patient outcomes. Future research should explore the mechanisms underlying these cytokine elevations and assess potential interventions that effectively mitigate hypercytokinemia's impacts on patient prognosis.

Recommendations: Clinicians should monitor IL-6 and TNF-α levels for early identification of severe disease and guide therapeutic interventions. Targeting cytokine pathways may improve outcomes. Future research should investigate the underlying mechanisms of hypercytokinemia and therapeutic options to mitigate its impact.

What is known about the topic

1. Hypercytokinemia results from elevated cytokine levels due to exaggerated immune responses.
2. Comorbidities increase the severity of COVID-19 infection.
3. The pandemic has strained global healthcare systems and economies.

Author Contributions

YM, YAK, and SI conceptualized the research, collected samples, and provided tools for statistical analysis. BM and BMK performed data curation and analysis. MAM handled participant recruitment. JM, RM, and MMH reviewed and finalized the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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