Original Article

Role of TNF-α in Patients with Omicron Coronavirus: A Cross-Sectional Study

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Abstract

Background: Severe acute respiratory syndrome coronavirus (SARS-CoV-2) omicron variant has spread through the world and has caused a global pandemic. In order to make an accurate diagnosis of the disease's severity, several immunological and biomarkers (tumor necrosis factor alpha (TNF-α), D-dimer, serum ferritin, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase (GPT), and lactate dehydrogenase (LDH)) are evaluated immediately. **Objectives:** The current study aimed to determine the concentration of TNF-α level in the omicron coronavirus patients, and to determine the correlation of TNF-α with some biomarkers [TNF-α, D-dimer, serum ferritin, glutamic-oxaloacetic transaminase (GOT), GPT, and LDH]. **Materials and Methods:** Sixty patients (40 males and 20 females), with the age ranging from 23 to 71 years from the intensive care unit of a tertiary hospital in Babylon Iraq, were included in the current investigation. The period of study was between October and December 2021. Included criteria omicron coronaviruses patients with positive "Real-Time Polymerase Chain Reaction (RT-PCR)" results while the excluded criteria omicron coronaviruses patients with positive "RT-PCR" results were reviewed. Then the positive patient divided by using saturation oxygen (SPO2) into three groups (mild, moderate, and severe groups). Sex, age, and SPO2 were recorded, and biomarkers (GOT, GPT, and LDH) were measured in all patients using a bio-based device (ACCENT-200 ALAT KIT). The D-dimer and serum ferritin for all patients were done by VIDS instrument. **Results:** In this sample, the ages ranged from 23 to 71, with a mean of 60.32 ± 13.39. TNF-α concentrations were found to be significantly greater in the severe patient group compared to the mild patient group and the moderate patient group ($P = 0.06$). Severe patients had greater GPT and GOT concentrations than mild and moderate patients. There was a positive and statistically significant association between TNF-α and D-dimer in the mild group ($r = 0.734$**, $P = 0.000$). Neither TNF-α nor serum ferritin levels were correlated with liver function tests. **Conclusion:** Among omicron coronavirus patients, GOPT, GPT, and LDH were all elevated in the severe group, whereas there was no statistically significant difference between the mild and moderate groups (mild, moderate, and severe group). TNF-α levels were not associated with liver function tests in this research.

Keywords: D-dimer, ferritin, omicron, TNF-α

Introduction

Many countries throughout the world have been hit by the 2019 coronavirus sickness (COVID-19), which was caused by Severe acute respiratory syndrome coronavirus (SARS-CoV-2) and has devastated healthcare systems and claimed many lives.[1-3] There is a significant difference in the mutation rates of DNA and RNA viruses. SARS-CoV-2, like other RNA viruses, has a high adaptive mutation rate owing to its large 30 kb genome and low-fidelity RNA polymerase, both of which help the virus adapt to new human hosts (RdRp).[4] Between January 2021 and September 2021, multiple different SARS-CoV-2 strains appeared and spread fast over a wide range of nations. These strains include the Alpha, Beta, Gamma, and Delta kinds of volatile organic compounds identified by the World Health Organization (WHO).[5] In comparison to the early wild-type strain, the four variants of concerns (VOC) acquired multiple mutations in the spike protein, which resulted in the generation of immunological escape and made the VOCs more infectious.[6]

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The first samples of B.1.1.529 are discovered in South African soil on November 14, 2021, and in Botswana the following day, on November 11. The WHO was made aware of the situation on November 24. On November 26th, the WHO designated "Omicron" as VOC #5.[7] Among the SARS-CoV-2 variations (including VOCs and variants of interests) circulating during the COVID-19 pandemic, the omicron strain was the one that experienced the greatest changes. The amino acid variations that are characteristic of the Omicron type have an effect on proteins engaged in a broad array of functions. Nonstructural proteins are included with structural proteins such as spike (S) and envelope (E) proteins, membrane (M) proteins, and nucleocapsid (N) proteins (nonstructural proteins3, nonstructural proteins4, nonstructural proteins5, nonstructural proteins6, nonstructural proteins12, and nonstructural proteins1).[8] Two distinct segments, S1 and S2, and furin protease cleavage sites make up the SARS-CoV-2 spike protein. The S1 subunit has two distinct regions: the receptor-binding domain and the N-terminal domain. It is the receptor-binding motif that is principally responsible for making contact with the receptor and facilitating viral invasion into cells [human angiotensin-converting enzyme-2, angiotensin-converting enzyme 2 (ACE-2)].[9] Tumor necrosis factor alpha (TNF-α) has been linked to a number of negative outcomes, including increased neutrophilia in the respiratory epithelium, bronchial hyperreactivity, and airway constriction. When TNF-α directly damages the respiratory epithelium, the cells in that layer respond by producing mucin and releasing inflammatory cytokines such IL-8, GM-CSF, RANTES, and intercellular adhesion molecules.[10] In addition, TNF-α induces the production of matrix metalloproteinase-9 from neutrophils, which in turn drives pulmonary fibroblasts to manufacture glycosaminoglycans. Pulmonary fibrosis is a persistent condition caused by TNF-α, which directly stimulates collagen formation by myofibroblasts and fibroblasts.[10] Concurrently, the pandemic by omicron coronavirus is a live issue affecting people worldwide. Without fundamental therapeutic interventions, current management is to reduce the virus spread and provide supportive care for diseased patients. There is an urgent need to develop targeted therapies. Understanding the difference in pediatric and adult responses to this virus may help to direct immune based therapeutics.

Materials and Methods

Study design

This study is quantitative in nature, and it employs a cross-sectional technique and a descriptive correlational research design. Research was conducted between October and December 2021. Patients were divided into three categories depending on their saturation oxygen (SPO2) levels: "mild" (SPO2 levels 95%), "moderate" (SPO2 levels 91%–94%), and "severe" (SPO2 levels less than 90%). According to demographic information such as age and sex from standard data collection forms. However bio-based instruments were used to measure bio markers [glutamic-oxaloacetic transaminase (GOT), glutamate pyruvate transaminase (GPT), lactate dehydrogenase (LDH)] for identify the damage in organs functions in all cases (ACCENT-200 ALAT KIT). The VIDS (French) equipment was used to measure D-dimer and serum ferritin levels in all patients. Patient real-time polymerase chain reaction (PCR) omicron coronavirus positives were considered for inclusion. Patients whose real-time PCR results were negative for omicron coronavirus were not included.

Blood sample processing

In order to collect blood samples from 60 patients, a syringe was used to take 5 µL of venous blood under sterilization and placed into a gel tube, which was then allowed to coagulate for 5 min before centrifugation at 1500 rpm. The concentrations of TNF-α in the blood were measured using commercially available ELISA test kits (Elabscience Biotechnology, United States). All testing was performed in accordance with the manufacturer's guidelines.

Statistical analyses

The Statistical Package for the Social Sciences (SPSS) was used in order to carry out the statistical analyses (version 25.0 for Windows; SPSS, Chicago, Illinois). For the purpose of illustrating qualitative information, counts and percentages are utilized. An investigation of TNF alpha's relationships to a variety of other factors was carried out with the use of correlation analysis. An analysis of variance test was used to do the comparisons and discover the differences between the groups. A *P*-value of ≤0.05 was considered statistically significant.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 4930 in September 23, 2021 to get this approval.

Results

The ages ranged from 23 to 71, with a mean of 60.32 ± 13.39. The inpatient cases were included in this study, which had 40 male patients (66.7%) and 20 female patients (33.3%). TNF-α concentrations were found to be significantly greater in the severe patient group compared

to the mild patient group and the moderate patient group ($P = 0.06$).

Table 1 displays the patient's demographic characteristics in this status of the inpatient cases which included 40 male patients and 20 female patients. The mean of age was 60.32 ± 13.39 with minimum 23 years and maximum 71 years. Sixteen inpatient cases were included in this study and distributed according to a severity score: 25 severe, 15 moderate and 20 mild SARS-CoV-2 Omicron variant according to oxygen saturation. Demographic characteristics of the subjects are presented in Table 1.

Table 2 shows in patients with SARS-CoV-2 omicron variant coronavirus, TNF-α was compared to a variety of other parameters, and this was the mean of those comparisons. In comparison to other patient groups, those with severe illness had significantly higher levels of TNF-α (mild, moderate). Serum levels of GPT and GOT are elevated in the severe group (60.17 ± 56.62 and 45.98 ± 25.29, respectively) compared to the moderate group (42.73 ± 24.45 and 34.27 ± 14.96, respectively) when testing liver function. In contrast, somewhat elevated LDH levels (531.76 ± 182) were seen in the moderate group compared to the mild and severe groups. Serum ferritin levels were within healthy ranges throughout the board, including for the D-dimer.

Table 1: Baseline characters of the studied group

Total number		60	
Gender		Male.40 (66.7%) Female.20 (33.3%)	
Age/Mean ± SD		60.32 ± 13.39	
Oxygen saturation	Patient groups	Frequency	Percent
	Severe group	25	41.7%
	Moderate group	15	25%
	Mild group	20	33.3%

Analyses of statistical correlations were carried out between TNF-α and tests of liver function. There was no a significant association between TNF-α levels and liver function tests, as a results in Table 3.

Statistical correlations were made between TNF-α and others parameters. In the mild patients group, TNF-α was significantly correlated with D-dimer ($r = 0.734^{**}$, $P = 0.000$). In contrast, there was a negative significant correlation notably among TNF-α with age ($r = –0.542^{*}$, $P = 0.045$), and results are shown in Table 4.

Discussion

This study investigated how helpful it is to keep an eye on inflammatory marker levels in order to identify especially hazardous cases of omicron coronaviruses among hospitalized patients. Specifically, looked at how effective it is. It was shown that severe patients had

Table 3: Pearson's correlation coefficient of TNF-α among liver functions in studied groups

Parameters	PC and P-value	TNF alpha		
		Mild N. 20	Moderate N. 15	Severe N. 25
ALT (GPT)	R	-.272	.187	-.196
	P	.275	.540	.395
AST(GOT)	R	-.167	.107	.022
	P	.508	.727	.925
ALP	R	.436	-.370	-.131
	P	.070	.213	.572
LDH	R	.033	-.192	.334
	P	.889	.511	.110

GOT = glutamic-oxaloacetic transaminase, GTP = glutamate pyruvate transaminase, LDH = lactate dehydrogenase

Values expressed as mean ± SD significant differences at the (P≤0.05) level

Table 2: Comparison mean of parameter of the studied groups

Variable	Normal values	Mild patients group *N* = 20 Mean ± SD	Moderate patients group *N* = 15 Mean ± SD	Severe patients group *N* = 25 Mean ± SD	*P* value
TNF-α pg/mL	0.0–8.1	60.73 ± 65.15	85.05 ± 91.15	116.60 ± 132.89	.06
ALT (GPT) U/L	5–40	42.73 ± 24.45	57.60 ± 45.65	60.17 ± 56.62	.205
AST (GOT) U/L	5–40	34.27 ± 14.96	40.92 ± 24.61	45.98 ± 25.29	.071
ALP U/L	40–150	97.51 ± 40.76	66.60 ± 23.93	88.68 ± 65.07	.043
LDH U/L	100–250	427 ± 220.47	531.76 ± 182	466.64 ± 228.91	.160
D-dimer ng/mL	0–500	145.40 ± 170.72	379.38 ± 647.48	248.08 ± 210.97	.036
S. ferritin ng/mL	0–250	124.91 ± 106.84	196.07 ± 241.61	136.47 ± 147.09	.037

GOT = glutamic-oxaloacetic transaminase, GTP = glutamate pyruvate transaminase, LDH = lactate dehydrogenase

Values expressed as mean ± SD significant differences at the ($P \leq 0.05$) level

Table 4: Pearson's correlation coefficient of TNF-α with other parameters

Parameters	PC and P-value	TNF alpha		
		Mild N. 20	Moderate N. 15	Severe N. 25
Age	R	.198	-.542*	-.362
	P	.403	.045	.075
Sex	R	.398	-.240	.069
	P	.082	.409	.743
S. ferritin	R	-.217-	.026	-.241
	P	.371	.932	.269
D-dimer	R	.734**	-.178	-.309
	P	.000	.543	.132

Values expressed as mean ± SD significant differences at the (P≤0.05) level

considerably greater serum TNF-α levels compared to mild ones. This study gives additional evidence that elevated levels of inflammatory cytokines are a prominent feature of the patients studied as well as a predictor of liver damage in those studies. The general mean age was 60.32 ± 13.39, as shown in Table 1. These results agreed on with D'ascanio *et al.*,[11] that found the older age (>80) was more susceptible to infection. The current study showed elevated levels of GOT, GPT, LDH, and D-dimer in severe cases more than mild cases, and this result agreed on with Chen *et al.*, who observed higher concentrations of ALT, AST, and LDH in non-survivors compared to survival.[12] Additionally, it has been shown that the patients' liver function is a strong predictor of mortality from COVID-19. Cholangiocyte dysfunction may be to blame for liver issues in COVID-19 patients, since recent study has shown that SARS-CoV-2 may directly attach to ACE2-positive cholangiocytes. This finding suggests that cholangiocyte dysfunction may be related to COVID-19.[13] When compared to mild instances, moderate, and severe cases had significantly higher serum ferritin concentrations than mild patients did. These findings are in line with the conclusions reached by Henry *et al*.[14] research reports that the serum ferritin levels of patients with severe illness are considerably higher than those of patients with less severe disease. When comparing the moderate and severe patient groups, both D-dimer and S. ferritin exhibited normal fold in the present study, which contradicts the findings of Taj *et al*.[15] All the patients had increased levels of TNF-α cytokine, yet those with moderate to severe disease showed much higher increases. These result are consistent with Liu *et al*.,[16] that found the TNF-α increased with severity of disease TNF ($P < 0.0001$). Cytokine storms, which may swiftly lead to organ failure and put the patients' lives in danger, are thought to be a significant contributing factor in the fatal outcomes of COVID-19 patients. Recent research has shown a rise in serum cytokine levels in COVID-19 patients, particularly in severe individuals, and the findings imply that cytokine storm is connected with the severity of the illness.[16,17]

Conclusion

TNF-α concentration was found to be significantly higher in the severe omicron coronavirus group compared to the mild group ($P = 0.06$). Serum ferritin and D-dimer concentrations remained within the normal range across all patient subsets. The research found TNF-α levels were not correlated with biomarkers (liver function tests) in individuals with "omicron coronavirus". This work demonstrates unequivocally that the emergence of omicron coronaviruses is not accompanied by alterations in biochemical profiles, which may not aid in their early detection. We recommend further study of TNF-α to determine its function and impact in omicron coronaviruses.

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Conflicts of interest

There are no conflicts of interest.

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