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SARS-CoV-2 Induced Interleukin -18 Response among Presumptive Covid-19 Patients in Kano State, Nigeria

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Abstract

Coronaviruses have a history of causing severe outbreaks with life-threatening consequences, including Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS), and the recent coronavirus disease 2019 (COVID-19). COVID-19 first broke out in Wuhan (China) in December 2019). The disease was later declared a pandemic, and so far, more than 222 countries have been affected, with over 771 million confirmed cases and total deaths of over 7.05 million. Some immunological markers were reported elsewhere as directly related to COVID-19 pathophysiology and stand a chance to be considered biomarkers. Interleukin 18 (IL-18) is a proinflammatory cytokine and a member of the interleukin-1 family, produced by macrophages at the early stage of viral infections. However, aberrant IL-18 production can lead to severe pathological injury. Hence, there is a need to assess the feasibility of interleukin -18 as a biomarker for COVID-19. Forty-five individuals diagnosed with COVID-19 and 45 healthy controls screened using a COVID-19 antigen rapid test kit and confirmed by one-step real-time PCR were recruited for this study. Blood samples were collected from the patients and controls, and the samples were analyzed for IL-18 using the ELISA technique. This study revealed a higher level of IL-18 in COVID-19-positive patients (206.42 ± 13.2 pg/mL) compared to the control group (97.96 ± 14.4 pg/mL). Serum level IL-18 was statistically associated with COVID-19 infection (t value 6.16, p <0.00010). The study demonstrates the importance of IL-18 in the COVID-19 cohort, inferentially implying its potential in the prognosis and clinical management of COVID-19.

Keywords: Biomarker; COVID-19; Interleukin-18; Kano; SARS-CoV-2

INTRODUCTION

Coronaviruses have caused severe outbreaks with life-threatening consequences, including Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS), and the latest coronavirus disease (COVID-19) (Rothan and Byrareddy, 2020). SARS-CoV-2 is the latest respiratory infection caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (COVID-19) (Sohrabi *et al.*, 2020). The disease first broke out in Wuhan (from a wholesale market), China, in December 2019 and was reported by the China Centre for Disease Control and Prevention CDC. On January 30, 2020, the World Health Organization declared the outbreak an international public health emergency (Rothan and Byrareddy, 2020; WHO 2020).

The infection was later declared a pandemic, and so far, more than 222 countries have been affected by the disease, with over 771 million confirmed cases reported globally and a total of over 6.9 million deaths (WHO 2023). As of July 12, 2023, the number of confirmed cases in Africa amounted to 9,534,117, representing around 3.58 percent of the global cases (WHO 2023). Nigeria ranks 88th with 266,675 confirmed COVID-19 cases and 3,155 cumulative deaths as of July 12, 2023 (NCDC 2023). The

clinical manifestation and severity of COVID-19 can vary substantially. Most COVID-19 cases are asymptomatic or have mild to moderate symptoms that usually manifest as flu-like symptoms or mild pneumonia (Wu and McGoogan, 2020).

Interleukin-18 is a proinflammatory cytokine and a member of the interleukin-1 family. It is initially called IFN- (interferon-) inducing factor and plays an important role in innate and adaptive immune biology (Kaplanski, 2018). Macrophages produce interleukin-18 (IL-18) at the early stage of viral infections and induce the production of IL-6 and IFN-, which are critical for optimal viral host defense (Lagunas-Rand *et al.*, 2020). However, aberrant IL-18 production can also lead to severe pathological injury. Markedly elevated serum IL-18 levels have been linked to severe disease and mortality in some viral infections characterized by cytokine storms, such as dengue virus infection and possibly COVID-19 (Guo *et al.*, 2015). Identifying the role of IL-18 will shed light on the disease pathogenesis of COVID-19, which is also characterized by hyperferritinemia and cytokine storm. Moreover, serum concentrations of IL-18 might serve as a biomarker that may predict COVID-19 prognosis.

MATERIALS AND METHODS

Study Area

The study was carried out at SARS-COV-2 testing sites, all located in Kano State, Northwestern Nigeria. Kano is an urban metropolis and the second-largest city in the country. It is located at latitude 12.000 N and longitude 8.300E (NPC, 2009). It has a population of more than twenty (20) million people (Kurawa, 2006), and it is inhabited mainly by the Hausa-Fulani tribe. Kano has forty-four (44) local governments with an area of 20,479, 6 square kilometres. The testing sites include Aminu Kano Teaching Hospital, Muhammad Abdullahi Wase Teaching Hospital, Murtala Muhammad Specialist Hospital, Infectious Disease Hospital, and Muhammadu Buhari Hospital.

Study Population

All symptomatic COVID-19-confirmed positive patients (Case) at the testing sites and asymptomatic COVID-19-confirmed negative patients (control group) that gave their consents were included as study participants.

Study Design

This study was a case-control study in design.

Sample Size Determination and Sampling Technique

Determination of sample size was performed using the formula proposed by Hennekens (1987), using the previously determined prevalence rate of symptomatic positive SARS-COV-2 patients in Lagos State, Nigeria, which is 89.6% (Otuonye *et al.*, 2021), and asymptomatic negative patients in Ibadan, Nigeria, which is 54.9% (Olayanju *et al.*, 2021).

$$,n = \frac{(Z_\alpha + Z_{1-\beta})^2\{(p_1\, q_1) + (p_2\, q_2)\}}{(p_1 - p_2)^2}$$

Where:

n = minimum sample size required in each group

Z_α = standard normal deviation corresponding to a 5% level of significance = 1.96

(Obtained from normal distribution table)

$Z_{1-\beta}$ = standard normal deviation corresponding to a power of 80% = 0.84

P_1 = the prevalence of symptomatic positive SARS-COV-2 patients (89.6%) = 0.896

P_2 = the prevalence of asymptomatic negative SARS-COV-2patients (54.9%) = 0.549

q_1 =1- P_1 = 0.104 q_2= 1- P_2 =0.451

Therefore

$$n = \frac{(1.96 + 0.84)^2\{(0.896 \times 0.104) + (0.549 \times 0.451)\}}{(0.896 - 0.549)^2}$$

n = 22.188 = 22 per group

Using the formula above, the minimum sample size calculated was twenty-two (22); however, 45 samples were collected from each group using systematic random sampling techniques to improve precision.

Ethical clearance

Ethical clearance was obtained from the Kano State Ministry of Health, Research, and Ethics Committee before the commencement of the study (NHREC/17/03/3029), and informed

consent was obtained from each participant before enrolling in the study.

Inclusion and Exclusion Criteria

Symptomatic individuals who showed positive test results for SARS-CoV-2 using rapid antigen test and asymptomatic individuals with SARS-CoV-2 negative test results who gave consent were included in the study, while those who met the inclusion criteria but declined consent were excluded from this study.

Data Collection

Data were collected from COVID-19-positive and negative patients using a pre-designed form based on standard clinical criteria. Socio-demographic variables and COVID-19-related clinical data were collected with the help of trained health professionals working in the testing sites. Data collected were kept confidential.

Sample Collection

Nasopharyngeal swab samples for the screening and confirmation of SARS-CoV-2 and 5mls of venous blood samples for IL-18 ELISA were collected aseptically from each patient and the control. The blood sample was dispensed into a labelled and sterile container containing a clot activator for a serological test. The blood samples were centrifuged at 3000 rpm for 10 minutes. The sera were separated from the whole blood and stored at 20^{o}C until used.

Screening of study participants

The study participants were screened using a COVID-19 antigen rapid test kit and polymerase chain reaction. The Panbio COVID-19 Ag rapid test device (Abbott, Germany) was used to screen participants based on the manufacturer's instructions. Three hundred (300µl) of the buffer fluid was used to extract the nasopharyngeal sample, and five (5) drops of the extracted specimens were dispensed vertically into the specimen well on the test device. The results were taken after 15 minutes while one step real time PCR was used to confirm the screened participants.

COVID-19 Genomic RNA Extraction

Pure viral RNA was extracted from one hundred and forty (140µl) of the sample mixed with Five point six (5.6 µl) of the carrier RNA using (Qiagen Nucleic Acid Extraction Kit, Germany) by following the manufacturer's directions. Pure extracted RNA was eluted using Sixty µl (60 µl) of the elusion buffer into the Eppendorf tube and stored at -20^{O}C until use (Lan *et al.*, 2020; Zhang *et al.*, 2020).

COVID-19 Nucleic Acid Amplification

The COVID-19 nucleic acid was amplified using (a GeneFinder kit) in Twenty (20µl) reaction volume containing 15µl master mix and 5µl extracted COVID-19 RNA. The entire component in the PCR tube was mixed well and transferred into the real-time PCR machine for amplification. The thermal cycling conditions were 20 minutes of reverse transcription at 50 ^{0}C, 5 minutes of initial denaturation at 95 ^{0}C, followed by 45 cycles of 15 seconds of denaturation at 95 ^{0}C, annealing for 60 seconds at 55 ^{0}C, and 5 minutes of extension at 68 ^{0}C (Lan *et al.*, 2020)

Serum level Interleukin-18(IL-18) ELISA Assay

Serum levels of Interleukin-18 were measured with Sandwich-ELISA (Sunlong Biotech Co. Ltd. China) according to the manufacturer's instructions. About 2 ml of blood samples were collected from both COVID-19-positive and negative patients. The blood samples were centrifuged at 4000 rpm for 10 minutes, and the serum was used for interleukin-18 ELISA assay. The standard was first diluted in a small tube, and 50 µl was added to the microplate well. Forty (40 µl) of dilution buffer and 10 µl of the sample were added into the microplate wells, mixed gently, and incubated for 30 minutes at 37 °C after sealing with a closure plate membrane. The closure membrane was carefully peeled off, and the wells were thoroughly washed with washing solution. Fifty (50 µl) of HRP-conjugate reagent was added to each well except the blank control well and incubated for another 30 minutes at 37 °C. The wells were then washed for the second time using a washing solution. Fifty (50 µl) of chromogen solutions A and B were added to each well, mixed gently, and incubated at 37 °C for 15 minutes in the dark. Fifty (50 µl) of stop solution was added to each well to terminate the reaction; the color in the well changed from blue to yellow. The optical density of the reaction was measured with a microtiter plate reader at 450 nm and compared with the optical density of the known standard samples to determine interleukin-18 concentrations. The concentrations of IL-18 in each sample were obtained through extrapolation on the standard curve (Fouda *et al.*, 2021).

Statistical Analysis

The data obtained were analyzed using Statistical Package for Social Sciences (SPSS) software (Version 25) USA. Categorical variables were described as each category's frequency rate and percentage, and continuous variables were expressed as the mean standard deviation. Normally distributed continuous variables were compared using the independent sample t-test; non-normally distributed continuous variables were compared with the Mann-Whitney U test. A P-value of less than 0.05 was considered significant.

RESULTS

Socio-demographic Characteristics of the Study Participants

The mean age of the participants was found to be 39.5±14.8. Most participants fell within the age range of 21-44 (71.1%), while the least fell within the age range of 65-80 (4.40%). Most of the study participants were males (64.4%) while females (35.6%) were the minority. In terms of marital status, most of them were married (71.1%), 24.4% were single, while 2.2% were found to be widowed, and 2.2% were divorced. Most of the participants had tertiary education (93.3%), followed by those with secondary education (4.4%) then those with only non-formal education (2.2%) Table 1.

Clinical Profile of Study Participants

The prevalence of COVID-19-related symptoms among the participants was determined. Among the 45 COVID-19-positive participants, Cough (71.1%), chest pain (71.1%), and headache (53.3%) were the most prevalent symptoms reported. Dizziness (46.7%), Sore throat (42.2%), fever (37.8%), runny nose (35.6%), and body pain (26.7%) were common among more than 30% of the participants. Shortness of breath (24.4%), nausea (20.0%), diarrhea (17.8%), and vomiting (13.3%) were also reported by some of the participants. The onset and duration of the symptoms are also described, respectively. The most common duration of symptoms onset is 7-10 days (55.6%), followed by 3-6 days (31.1%), then 11-14 days (11.1%) and 15-17 days (2.2%). Participants were also asked to report any chronic disease they had been suffering from, where 24.4% had diabetes, hypertension (15.6%), and kidney disease (4.4%), while 55.6% had none (Table 2).

Biomarker profile of COVID-19-positive patients and the control group

Analysis of the biomarker (IL-18) of COVID-19 showed that positive patients have higher serum IL-18 levels (206.42 ± 13.2) than the control group (97.96±14.4) at $P < 0.05$. Furthermore, a significant difference is observed in serum IL-18 levels of COVID-19 positive and control group (t value =6.16, p-value <0.0001) as shown in Table 3.

Table 1: Participant's Socio-demographic Characteristics

Variable	Frequency (n=45)	Percentage(%)
Gender		
Male	29	64.4
Female	16	35.6
Age group(in years)		
25-44	32	71.1
45-68	11	24.4
69-80	2	4.40
Marital Status		
Single	11	24.4
Married	32	71.1
Divorced	1	2.2
Widowed	1	2.2
Level of Education		
Primary	0	0
Secondary	2	4.4
Tertiary	42	93.3
Non-formal	1	2.2

Table 2a: Clinical Profile of COVID-19 Positive Participants

Variable	Frequency (n=45)	Percentage (%)
Fever		
Yes	17	37.8
No	28	62.2
Sore Throat		
Yes	19	42.2
No	26	57.8
Runny Nose		
Yes	16	35.6
No	29	64.4
Cough		
Yes	32	71.1
No	13	28.9
Shortness of Breath		
Yes	11	24.4
No	34	75.6
Vomiting		
Yes	6	13.3
No	39	86.7
Diarrhea		
Yes	8	17.8
No	37	82.2
Nausea		
Yes	9	20.0
No	36	80.0

Table 2b: Clinical Profile of COVID-19 Positive Participants

Variable	Frequency(n=45)	Percentage
Headache		
Yes	24	53.3
No	21	46.7
Dizziness		
Yes	21	46.7
No	24	53.3
Chest Pain		
Yes	32	71.1
No	13	28.9
Body Pain		
Yes	12	26.7
No	33	73.3
Disease Onset		
3-6 days	14	31.1
7-10 days	25	55.6
11-14 days	5	11.1
15-17 days	1	2.2
Comorbidity		
None	25	55.6
Hypertension	7	15.6
Diabetes	11	24.4
Kidney Infection	2	4.4

Table 3: Biomarker profile of Covid-19 positive patients and the control group

COVID-19				
Biomarkers	**Positive Mean ±SD**	**Negative Mean ±SD**	**t-value**	***P-value***
IL-18 (pg/ml)	206.42±13.2	97.96±14.4	6.16	**<0.0001**

DISCUSSION

Globally, COVID-19 disease has become a serious health concern since it emerged in Wuhan in 2019 (Khalid and Ali, 2020) after being declared a global pandemic by WHO. It affects millions of people, causing huge numbers of deaths and exhausting global health resources in the fight against COVID-19. Assessing clinical, and demographic indicators and biomarkers (Interleukin-18) that guide understanding the disease progression. Researchers and medical practitioners are looking for available predictive biomarkers to determine who would progress to critical conditions among COVID-19-positive patients. Based on this, a comparative cross-sectional study was conducted on 45 male and female patients with confirmed COVID-19-positive results recruited to assess serum levels of interleukin-18 to predict whether they could be biomarkers for COVID-19 disease progression. Another 45 healthy individuals matched in age and sex were considered the control group.

COVID-19 can occur in any age group. This study showed that most participants fell within the age range of 25–44, and the majority were males. This shows that younger individuals were more susceptible to COVID-19, but the earlier study on COVID-19 had observed a high infection rate among the elderly (Zhang *et al.*, 2020). But the emerging trend showed increased prevalence among the middle-aged population (Wang *et al.*, 2020). In this study, the mean age of the 45 patients was 39.5±14.8, lower than the 45–68 years range reported in most studies (Chen *et al.*, 2020; Guan *et al.*, 2020).

This result was similar to a report by Jibrin *et al.* (2020) that indicated a mean age of 41.5–10.5 and that most of the patients fell within the age range of 21–40, most of whom were males. Moreover, this report is similar to the previous study conducted in Lagos State by Bowale *et al.* (2020), which suggested that this condition affects children at a younger age. Another result reported in a meta-analysis that was conducted in China to compare the epidemiological variations in COVID-19 patients shows that the male population has a higher proportion in all included studies, suggesting a higher prevalence of the disease in the male population than in the female population (Sun *et al.*, 2020; Xu *et al.*, 2020). However, this result contradicted the report of El-Sagheer *et al.* (2022), where they reported a high prevalence among the female population.

Some studies have also indicated that males are more susceptible to COVID-19 than females and have accounted for this in terms of the lower expression in females of angiotensin-converting enzyme-2 receptors for coronavirus (Guan *et al.*, 2020; Mardani *et al.*, 2020). Innate and adaptive immunity were suggested as factors responsible for the low susceptibility of females to the infection (Bwire *et al.*, 2020). Another explanation of the male gender prevalent in this study could be that more females stayed at home during the curfew law than males, which made males more exposed to the infection.

Among the excess cytokines produced by activated macrophages, IL-18 is one of the key cytokines. However, markedly elevated serum IL-18 levels have been linked to severe disease and mortality in some viral infections characterized by cytokine storms, such as avian influenza and dengue (Hotchkiss *et al.*, 2016). In this study, we found out that serum IL-18 in COVID-19 patients was remarkably higher (206.42±13.2 pg/ml), which suggested that IL-18 levels could be used in the clinical monitoring of COVID-19 patients when compared to apparently healthy individuals, which is comparable to the finding in the earlier studies. This study's result agrees with Kerget *et al.* (2021), who reported elevated IL-18 levels among non-survivors compared to surviving COVID-19 patients.

A similar result was reported by Afrah and Abdulhameed (2022), who reported high levels of IL-18 among the COVID-19 patients compared to the control group. Another previous study conducted by Satis *et al.* (2021) found that the IL-18 level on admission continuously increased across the severe groups, and it was higher in those with a worse outcome. However, in contrast to the findings of this study, Rodrigues *et al.* (2022) found no significant difference in IL-18 levels between the patients with severe, mild, and moderate COVID-19, highlighting the importance of IL-18 as a possible biomarker for

the late stages of COVID-19 rather than the early cases.

CONCLUSION

We systematically analysed important biomarkers (IL-18) associated with COVID-19 patients and healthy controls. Based on the research, the results showed higher serum levels of IL-18 in COVID-19-positive participants (206.42±13.2(pg/ml) compared to negative participants. The increased interleukin 18 (IL-18) level in COVID-19-positive participants was statistically associated with COVID-19 infection.

RECOMMENDATIONS

This study suggested that IL-18 levels could be a possible candidate for monitoring COVID-19 patients. Future studies with a larger sample size are highly recommended to get more robust data on cytokines' effects on patients with COVID-19.

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