DIAGNOSTIC VALUE OF INTERLEUKIN 6 (IL-6) IN COVID-19: A REPORT FROM KANO, NIGERIA

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ABSTRACT

Background: Severe Acute Respiratory Syndrome Coronavirus 2 (SASR-CoV-2), the aetiologic agent of Coronavirus Disease 2019 (COVID-19) causes dysregulation of the immune system, with excessive, ineffective immune stimulation, resulting in severe lung injury, immunosuppression, multi-organ failure and cytokine storm.

Aim: The study aimed to investigate the association between serum interleukin 6 (IL-6) level and clinical characteristics of a cohort of COVID-19 patients during the first wave of SARS-CoV-2 infection in Kano, northwestern Nigeria.

Methods: COVID-19 status was initially checked using SARS-CoV-2 point-of-care antigen test and confirmed by one-step real-time RT-PCR. Enzyme Link Immunosorbent Assay was used for the IL-6 quantification and the results obtained compared using independent student T and Mann Whitney U tests.

Results: The mean age of the participants was 39.5 ± 14.8 years, the minimum was 22 years and the maximum was 45 years, males constituted 64.4%. Fever and cough were the most common presenting symptoms and hypertension was the major comorbidity. Mean serum IL-6 level was significantly higher in COVID-19 individuals than the controls (mean diff = 11.62; 95%CI: -13.41 to 9.83; $P < 0.001$). Among the COVID-19 cases, IL-6 was significantly expressed in those with sore throat ($P = 0.013$) and bodyache ($P = 0.035$), and also among hypertensive individuals ($P = 0.029$).

Conclusion: This study provides several aspects of clinical data and highlights the relevance of IL-6 in COVID-19, thereby suggesting its role as a surrogate marker of SARS-CoV-2 infection, especially in places with limited clinical virology laboratory capacity.

Keywords: COVID-19, Cytokines, Inflammation, Interleukin 6, Kano, Nigeria, SARS-CoV-2.

84

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a systemic disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that primarily affects the respiratory tract, characterized by dysregulation of the immune response. Interleukin 6 (IL-6) is a multifunctional cytokine that transmits cell signaling and regulate the immune system. It plays multiple important biological functions; a sensitive biomarker of inflammation, infection, and tissue damage. Importantly, IL-6 is always in check and maintain at low level, but increases rapidly and significantly in an acute inflammatory response.

The novel SARS-CoV-2 causes dysregulation of the immune system, with excessive, ineffective immune stimulation. Studies have indicated that IL-6, is a critical mediator for respiratory failure and multi-organ dysfunction (Aziz *et al*., 2020, Mardani *et al*., 2022). Exaggerated level of IL-6 induces production of acute-phase reactants; C-reactive protein (CRP), fibrinogen, haptoglobin, and α1-antitrypsin. This result in severe lung injury, immunosuppression, multi-organ failure and cytokine storm (Qin *et al*., 2020, Mehta *et al*., 2020). Because of its strong proinflammatory effect, it was postulated that IL-6 can stand alone as an independent predictor of disease progression in COVID-19 (Yang *et al*., 2020a).

However, it is noteworthy that, case fatality rate and progression to severe disease in COVID-19 was significantly lower in Sub-Saharan Africa. Many speculated and attributed this to possible prior exposure to cross reactive viruses among other reasons. It is with this perspective that this study examined sera of a cohort of COVID-19 individuals from Kano, northwestern Nigeria to investigate IL-6 expression level. The study went further to compare serum level of the biomarker to that of apparently healthy controls.

MATERIALS AND METHODS

Ethical consideration

Prior to the commencement of the study, the protocol for the study was reviewed and approved by the ethics and research committee, Kano state ministry of health and provided the following ethical code: NHREC/17/03/2018. Informed consent was obtained from each participant before enrolling into the study.

Study population and setting

A comparative cross-sectional study involving 45 symptomatic COVID-19 patients and age- and sex-matched apparently healthy controls with SARS-CoV-2 negative test results was carried out during the first wave of SARS-CoV-2 infection in Kano, Nigeria.

Kano is located at latitude 12^0 3' North and longitude 8^0 31' East, situated within the semi-arid Sudan Savannah zone of West Africa, about 840 kilometers from the edge of the Sahara Desert, and has an estimated (2022) population of 14,363,776, projected at 2.6% per annum growth from the 2006 census. Kano is 486.5 meters (1,578 feet) above sea level, with a tropical savanna climate and a landmass of 20,131 km^2. It is the second largest industrial and commercial center and one of the most crowded cities in Nigeria. The city of Kano is cosmopolitan with a large migrant worker population that was estimated to be increasing at the rate of 30% to 40% per annum (Isa *et al*., 2016). Kano experiences a tropical wet and dry climate, categorized as AW by Koppen, exhibiting both annual and seasonal variations. Precipitation levels range from 750 mm to 850 mm annually, fluctuating between wet and dry years, with the highest rainfall being in the month of August (Umar *et al*., 2021). Temperature dynamics fluctuate from warm to hot seasons, with the advent of the harmattan prevailing from November to February, the weather is hot for most of the year and peaks in April-May.

Sample size determination and recruitment of participants

The sample size for the study was calculated based on the formula put forward by Charan and his colleague (Charan and Biswas, 2013). Proportion of symptomatic COVID-19 patients reported from Lagos state, Nigeria was used as P_1 (89.6% = 0.9) (Otuonye *et al*., 2021) and that of asymptomatic individuals that tested negative for SARS-CoV-2 in Ibadan, Nigeria, used as P_2 (54.9% = 0.55) (Olayanju *et al*., 2021). A minimum sample size of 22 was determined for each group and increased to 45 to improve precision. Participants (45 each for SARS-CoV-2 positive individuals and controls) were randomly selected and enrolled until the required sample size was achieved. The COVID-19 cases were recruited from the testing sites designated by the Federal Ministry of Health and Kano State taskforce for COVID-19 and the controls from the general population.

Screening for SARS-CoV-2 and confirmation of results

Initially, rapid antigen test (Ag RDT) was used to screen for SARS-CoV-2 from nasal samples using Panbio™ COVID-19 Ag RDT based on the World Health Organization interim guideline (WHO, 2020) and confirmed by one-step real-time RT-PCR (WHO, 2021).

Data collection and determination of serum IL-6

Data were collected from both COVID-19 individuals and the controls using a pre-designed form based on standard clinical criteria. The main aim and objectives of the study were explained to the care providers at the testing sites and the participants and consents obtained as appropriate. Socio-demographic, clinical and other relevant information (co-morbidities) were collected from the relevant groups and documented, and maximum confidentiality ensured. About 5mls of venous blood was collected from each participant based on standard laboratory protocol. Serum IL-6 was measured using Enzyme Linked Immunosorbent Assay

(Melsin Medical Company, Jilin, China and Calbiotech Inc. California, USA) according to the manufacturer's instructions and the results read using Rayto RT-2100C Micro plate Reader (Rayto Life and Analytical Sciences Co. Ltd, Shenzhen, China).

Statistics

Data obtained was checked for errors, outliers and completeness. The IBM Statistical Package for Social Sciences (SPSS) version 25.0 was used for the analysis. Categorical variables were described as frequencies and percentages, mean and standard deviation for continuous variables. Mean comparison was tested by independent T-test and Man-Whitney U test and a *P*-value < 0.05 considered significant.

RESULTS

Description of the study participants

A total of 45 each of COVID-19 cases and apparently healthy controls with SARS-CoV-2 negative results were enrolled. Their mean age was 39.5±14.8 years, the minimum was 22 years and the maximum was 45 years. Most of them (71.1%) were within the age range 21-44 years and males constituted the majority. Cases of COVID-19 were confirmed by SARS-CoV-2 one-step real time RT-PCR using nasopharyngeal swab. The clinical profile of the COVID-19 cases is summarized in Table 1 and the symptoms recorded in decreasing order of frequency; fever (71.1%), cough (71.1%), sore throat (53.3%), dizziness (46.7%), headache (42.2%), chest pain (37.8%), runny nose (35.6%), bodyache (26.7%), shortness of breath (24.2%), and fatigue (17.8%). Others include nausea, diarrhoea and vomiting. Major co-morbidities were hypertension and diabetes mellitus.

Serum level of IL-6 in COVID-19 cases and the controls

Table 2 depicts and compare the results of IL-6 and CRP for the COVID-19 cases and the controls. Mean serum IL-6 level was significantly higher in the COVID-19 cases with a mean difference of -11.62 (95%CI: -13.41 to -9.83; *P* < 0.001).

Multiple regression analysis was used to compare level of expression of IL-6 based on age and sex and no statistically significant difference was observed (Table 3).

Comparing IL-6 expression level by COVID-19 symptoms

Mann-Whitney U test was used to compare serum levels of IL-6 in relation COVID-19 symptoms (Table 4). According to the results, mean serum IL-6 level was significantly higher in patients who presented with sore throat ($P = 0.013$) and bodyache ($P = 0.035$). Similarly, those with hypertension as a co-morbidity had lower mean IL-6 when compared with their non-hypertensive counterparts and the difference was statistically significant ($P = 0.029$).

Table 1. Summary of Clinical profile of the COVID-19 cases

Variable	Frequency (%), n = 45
Age (years)	
21-44	32 (71.1)
45-64	11 (24.4)
65-84	2 (4.4)
Mean ± SD = 39.5 ± 14.8.	
Min. = 21, Max.= 82.	
Sex	
Male	29 (64.4)
Female	16 (35.6)
Fever	
Yes	32 (71.1)
No	13 (28.9)
Sore throat	
Yes	24 (53.3)
No	21 (45.7)
Runny Nose	
Yes	16 (35.6)
No	29 (64.4)
Cough	
Yes	32 (71.1)
No	13 (28.9)
Shortness of Breath	
Yes	11 (24.2)
No	34 (75.6)
Vomiting	
Yes	6 (13.3)
No	39 (86.7)
Diarrhea	
Yes	6 (13.3)
No	39 (86.7)
Nausea	
Yes	9 (20.0)
No	36 (80.0)
Headache	
Yes	19 (42.2)
No	26 (57.8)

Table 1 Continue

Dizziness	
Yes	21 (46.7)
No	24 (53.3)
Fatigue	
Yes	8 (17.8)
No	37 (82.2)
Chest Pain	
Yes	17 (37.8)
No	28 (62.2)
Bodyache	
Yes	12 (26.7)
No	33 (73.3)
Period of 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	
Diabetes mellitus	11 (24.4)
Hypertension	7 (15.6)
Pyelonephritis	2 (4.4)
None	25 (55.6)

Table 2. Comparing serum IL-6 level in COVID-19 cases and controls

Study group	Serum Interleukin 6 (Pg/mL)			
	Mean±SD	Mean Difference	95%CI	*P*-value
COVID-19 (n=45)	15.86±5.80	-11.62	-13.41 to 19.83	< 0.001
Controls (n=45)	4.24±1.70			

Table 3. Expression level of IL-6 by age and sex

Variable	Coefficient	Standard Error	t-value	*P*-value
Age (years)	20.8718	32.3226	0.6457	0.523
21-44				
45-64				
65-84				
Interleukin 6	-0.3184	0.389	-0.8185	0.419
Gender				
Male	0.4582	1.0446	0.4386	0.664
Female				
Interleukin 6	-0.012	0.013	-0.928	0.360

Table 4. Comparing IL-6 level and COVID-19 symptoms

Symptom	Interleukin 6 (Pg/mL)	
	Mean Rank	*P*-value
Fever		
Yes	25.74	0.276
No	21.34	
Sore throat		
Yes	28.68	0.013*
No	18.85	
Runny nose		
Yes	18.41	0.81
No	25.53	
Cough		
Yes	22.30	0.573
No	24.73	
Shortness of breath		
Yes	26.50	0.314
No	21.87	
Vomiting		
Yes	26.56	0.350
No	22.23	
Diarrhea		
Yes	31.89	0.405
No	20.78	
Nausea		
Yes	22.56	0.022
No	23.50	
Headache		
Yes	20.02	0.811
No	25.60	
Dizziness		
Yes	29.75	0.155
No	21.54	
Fatigue		
Yes	22.30	0.113
No	24.72	
Chest pain		
Yes	22.00	0.573
No	27.83	
Bodyache		
Yes	29.79	0.035*
No	20.53	
Comorbidity		
Diabetes mellitus		
Yes	31.23	0.415
No	26.57	
Hypertension		
Yes	29.83	0.029*
No	23.00	
Pyelonephritis		
Yes	19.86	0.391
No	20.78	

DISCUSSION

The hallmark of COVID-19 depends mainly on the expression levels of inflammatory markers and coagulation factors (Huang *et al*., 2020) and IL-6 has been suggested as a very sensitive marker for diagnosis of infection, monitoring and prognostication (Yin *et al*., 2023). However, there is dearth of information on the role of IL-6 in COVID-19 in Nigeria. This cross-sectional study described a cohort of COVID-19 patients recruited from testing sites and their serum IL-6 examined in comparison to age- and sex-matched control group in Kano, Nigeria. It is important to note that all the COVID-19 cases were clinically stable and recruited as outpatients, indicating mild disease presentation.

Analysis of symptoms revealed that fever and cough were the most common symptoms which is consistent with other reports from Nigeria (Bowale *et al*., 2020, Akerele *et al*., 2021) and also from China (Guan *et al*., 2020). Similarly, the fact that hypertension and diabetes mellitus were the most prevalent co-morbidities is in line with reports from Akwa Ibom South-south, Nigeria *(*Onukak *et al*., 2021), China (Yang *et al*., 2020b), and the USA (Jackson *et al*., 2022).

In apparently healthy individuals, serum IL-6 was estimated at 5.186 pg/mL (Said *et al*., 2021). This study demonstrates that, COVID-19 is associated with increasing level of IL-6 as its level was observed to be significantly elevated in COVID-19 compared to the controls. This corroborates well with the results of previous studies (Al-Hatemy *et al*., 2022, Ganesh *et al*., 2022, Mohamed *et al*., 2023, Wolszczak-Biedrzycka *et al*., 2023, Yin *et al*., 2023). On comparing the expression level of IL-6 in relation to COVID-19 symptoms, it was observed that patients who presented with sore throat and

bodyache had significantly higher IL-6 level. This finding is similar to that of Lai and his colleagues (Lai *et al*., 2023). Furthermore, this study revealed that the expression level of IL-6 is independent of age and sex which is in agreement with a report from the United Arab Emirates (Hafez *et al*., 2023). Littlefield and colleagues also reported no association between IL-6 level and sex, however, they noted higher IL-6 in elderly COVID-19 cases in Denver, Colorado metropolitan area, United States of America (Littlefield *et al*., 2022). This difference can be attributed to variation in age distribution of the study participants with elderly constituting majority of the COVID-19 cases studied in Colorado. Further analysis revealed that there was a significant difference in IL-6 level based on pre-existing condition as those with hypertension had higher serum IL-6 (P =0.029).

CONCLUSION AND RECOMMENDATION

This study revealed that COVID-19 is associated with significant increase in serum IL-6 regardless of disease severity, sex and geographical location. Together, these findings suggest the role of IL-6 as a surrogate marker of SARS-CoV-2 infection, most especially in places with limited clinical virology laboratory capacity.

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