

Sero-prevalence of SARS CoV-2 IgM and IgG Antibodies Amongst Blood Donors in Nigeria

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Research

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Abstract

Background: Currently in Nigeria, SARS CoV-2 infection control measures rely solely on RT-PCR testing of cases with high index of suspicion for COVID-19. The sero-prevalence of SARS-CoV-2-specific antibodies in the populations remains unknown. We aimed to determine the sero-prevalence of SARS CoV-2 IgM and IgG in screened blood donors in two selected cities in Nigeria.

Methods: A total of 113 blood samples from screened and accepted blood donors were tested for both SARS CoV-2 IgM and IgG antibodies using NOVATECH ELISA kits from Eurofins Germany.

Results: The age range of donor was 21-48 years. Males were 96 %. Of the 113 blood donors 42% were positive for SARS CoV-2 IgG while 41% were positive for SARS CoV-2 IgM respectively. Only 11% and 19% of blood donors had equivocal test result for IgG and IgM respectively.

Conclusions: Nearly a half of accepted blood donors in Nigeria had been exposed to SARS CoV-2 infection. Although it is not a blood transmissible infection, the blood banks must take adequate preventive measures to prevent spread amongst the bank staff and potential donors. It also implies a wider spread of the infection in Nigeria.

Trial Registration: The study does not require registration.

Background

The emergence of Severe acute respiratory coronavirus 2 (SARS-CoV-2) and subsequent outbreak of COVID-19, is a global health challenge which has culminated in significant morbidity and mortality [1]. The virus can be transmitted in humans by direct or indirect contact with respiratory droplets of an infected person [2]. Although the viral RNA has been detected in stool, whole blood and urine of COVID-19 patients, transmission via these routes have not yet been documented [3, 4]. Infection with SARS-CoV-2 often presents with no symptom or a range of mild symptoms, including high temperature, dry cough, muscle pain, anosmia etc, and can progress into severe forms of the illness and possible death especially in the elderly and/or persons living with other comorbidities [5, 6]. Asymptomatic infections may be a substantial source of transmission and a challenge to infection control measures [7].

The first confirmed case of COVID-19 in sub-Saharan Africa was reported in Lagos, Nigeria on February 28, 2020, and as at 23rd November, 2020, a total of 743,298 RT-PCR tests had been performed in Nigeria, resulting in 66,383 onfirmed cases and 1,167 deaths (<https://www.worldometers.info/coronavirus/#countries>) [8]. Being a novel virus, sero-prevalence in a given population is presumed to be dynamic (negligible at onset and changing over the course of time). To date SARS-CoV-2 surveillance especially in Nigeria has focused primarily on individuals with high index of suspicion of COVID 19 even when it is apparent that there exist community transmissions. Although information on laboratory confirmed cases using RT PCR and deaths is important in monitoring the dynamics of the disease, they are less than ideal in estimating the proportion of infection in the entire

population [9]. Thus sero-prevalence studies are needed for an enhanced monitoring of the evolution of the outbreak and estimation of the actual infection fatality rate in the population

A quick search on SeroTracker [9]; a custom-built dashboard that systematically follows up on SARS-CoV-2 serological studies globally and displays findings on an interactive world map, shows paucity of data from sub-Saharan Africa. The SeroTracker enables easy comparison of estimates between regions and population groups clearly. Sero-surveillance of the populace allows inferences to be drawn on the extent of spread and cumulative incidence of infection in the population. Epidemiological studies focusing on the community will provide relevant data and expand our understanding of the spectrum of the disease.

Currently in Nigeria, given that SARS CoV-2 contact tracing relies solely on RT-PCR, the sero-prevalence of SARS-CoV-2-specific antibodies in different populations remains unclear. Consequently, the full spread of the disease, including the extent of mild or asymptomatic infections that do not require medical attention are not documented. Studies to determine the sero-prevalence SARS-CoV-2-specific antibodies in the community and specific population are paramount. These will provide means of measuring the extent of spread and appraise current control measures put in place by the authorities to curb the transmission of the virus. Additionally, knowledge provided by these studies will be found useful by policy makers in calibrating social interventions.

Although, there is frequently updated information on many aspects of the virus spread in Nigeria (e.g., numbers of patients tested positive for SARS-CoV-2, admitted and discharged from the hospital, mortalities) from the Nigeria Centre for Disease Control (NCDC), in contrast, there is very limited data on the percentage of the population with previous mild or asymptomatic infections. The proportion of the population with past and asymptomatic but active infection can fairly be estimated by testing for total antibody, IgG, IgA and IgM antibodies specific to SARS-CoV-2. These antibodies may confer immunity to repeat infection, although it remains unclear how long these immunities could last [10]

Healthy volunteers drawn from community blood donation units of major hospitals in Nigeria may provide vital sero-prevalence data on Covid-19 infection. Therefore, our study was aimed at sero-prevalence of SARS CoV-2 IgM and IgG in apparently healthy blood donors who had been recruited, screened and accepted to donate blood in the blood bank. This group of the population will provide a practical glimpse of the current situation of the virus spread at the community level.

Methods

Ethical Approval: The study received ethical approval from two Health Research Ethics Committees: from the Federal Capital Territory Health Research Ethics Committee Abuja, Approval Number **FHREC/2020/01/77/11-08-20**; and The Molecular Pathology Institute Health Research Ethics Committee Enugu, Approval Number **MPIHREC/2020/08/02/0002**. The study was carried out in compliance with the WMA declaration of Helsinki as amended in 2013 and the Nigerian National Code of Health Research Ethics 2007.

Aim of study: The aim of this study was to determine the seroprevalence of SARS CoV-2 IgM and IgG antibodies amongst blood donors in Nigeria.

Study Design: This was a cross sectional study with non-probability sampling.

Sample Size: Sample size was calculated using the Raosoft Sample size Calculator (<https://raosoft.com>). We used a prevalence of 15 % since the pandemic was still evolving. At 5 % alpha level of significance and 95% confidence level, a sample size of **113** had 80 % power to determine seroprevalence up to estimated prevalence of 15%.

Sampling: A hundred and thirteen informed consented blood donors were recruited during the month of August 2020 to participate in the study. Specifically, these blood donors were drawn from blood donation centers in the city of Abuja and Enugu located in the north central and south eastern part of Nigeria respectively. Briefly about 5 ml of blood was collected from each participant after having been screened and certified eligible to donate according to National Blood Transfusion Service (NBTS) guidelines. The blood was centrifuged at 6000 rpm for 10 minutes and plasma was harvested. Plasma samples were frozen in minus 20°C freezer and later transferred on cold chain to the testing laboratory, Safety Molecular Pathology Laboratory, where samples were frozen at – 86°C until required for testing. Storage period was no more than 1 month. All samples were screened for SARS-CoV-2 IgM and IgG antibodies using a semi quantitative Enzyme Linked Immunosorbent Assays (ELISA) test kits (NOVA TECH, Germany).

Enzyme Linked Immunosorbent Assays (ELISA): ELISA kits for SARS CoV-2 IgM and IgG antibodies were obtained from Eurofins, Germany, they were manufactured by NOVA TECH Germany. Both assays were semi-quantitative and had a cut of 11 NTU (Nova Tech Units) for positivity. SARS CoV-2 IgM was tested separately from SARS CoV-2 IgG. Tests were carried out according to the Manufacturer's instructions. Briefly, microwell titre plates pre-coated with specific antigens were selected according to the number of samples to be analyzed and 100 ul of diluted sample or control was added into appropriate well. Each sample was diluted in 1 in 100. Positive, cut-off and negative controls were prediluted ready to use. Wells were covered with a foil and incubated for 1 hour at 37°C. After incubation, the foil was removed, and the contents of each well was aspirated and 300 ul of 1x Wash Buffer was added. Overflows were avoided. The microwell plates were washed thrice. Plates were tapped on tissue paper to ensure that all moisture was removed and 100 ul Conjugate solution was added into all wells. The wells were incubated for 30 minutes at room temperature. After the incubation, the washing steps were repeated using 300 ul of the 1x Wash buffer. Then 100 ul of TMB Substrate Solution was added into each well and the wells incubated for 15 minutes at room temperature in the dark. At this point blue colours appeared in the wells due to enzymatic reaction. Then 100 ul of Stop Solution was added into all the wells, with the colours changing to yellow. The absorbance of the wells was read at 450/620 nm using Mindray M96 Microwell Plate Reader. In each run a substrate blank was included, in addition to the positive, negative and cut-off controls. The results were calculated according to the manufacturer's instruction and expressed in Nova

Tech Units (NTU) as follows: > 11 NTU: Positive for the specific antibody, < 9 NTU: Negative for the specific antibody, 9-11 NTU: Equivocal for the specific antibody.

Assay Characteristics: Assay specific characteristics as stated by the manufacturer, for SARS CoV-2 IgG: the assay diagnostic specificity was 100% (95% CI: 95.82-99.98) and diagnostic sensitivity at > 12 days post symptom onset was 100 %. SARS CoV-2 IgM: the assay diagnostic specificity was 100% (95% CI: 97.26- 100.00) and diagnostic sensitivity at > 12 days post symptom onset was 57.14 % (95% CI: 18.84 – 73.37)).

Data Analysis: Data was analyzed in GraphPad Prism version 9 (www.graphpad.com). Gaussian distribution was tested for using D'Agostino Omnibus Normality test. Based on the outcome of the normality test, non-parametric statistics was used to analyze the data. P value was set at 0.05. For descriptive statistics, median, 95% confidence interval of the median and inter-quartile range were reported. For inferential statistics comparing positive, negative and equivocal cases, Kruskal Wallis test and Dunn's multiple comparison were performed.

Results

Of the 113 blood donors, 96% were males while 4 % were female. Table 1 shows the demographics of the blood donors. The age range for the blood donors were 21–48 years, median age was 33 years. Descriptive statistics of SARS CoV-2 IgM and IgG are shown in Table 2. Of the 113 blood donors studied, 48 (42 %; 95% CI: 34–52%) were positive for IgG with a median antibody level of 19 NTU; 46 (41 %; 95% CI: 32–50 %) were positive for IgM level with a median antibody level of 5 NTU. Only 11 (11%; 95 % CI: 05 – 17%) donors were equivocal (indeterminate) for SARS CoV-2 IgG, with a median antibody level of 10 NTU. Only 21 (19%; 95% CI: 12–27%) donors were equivocal (indeterminate) for SARS CoV-2 IgM, with a median antibody level of 10 NTU. Inferential comparison of antibody levels in positive, negative and equivocal cases are shown in Table 3. Analysed independently, Abuja (n = 60) had SARS CoV-2 IgG prevalence of 53 % (95% CI: 41–65%) while Enugu (n = 53) had SARS CoV-2 IgG prevalence of 31 % (95% CI: 19–44 %). Whilst 45 % of Abuja blood donors were SAR CoV-2 IgM positive (95% CI: 33–58%), but 37% of Enugu blood donors were SARS CoV-2 IgM positive (95% CI: 26–51%).

Discussion

The SARS-CoV-2 infection in most sub-Saharan African nations appeared to have a less fatal clinical outcome in comparison with other nations in many parts of the world ¹¹. Several reasons have been speculated to be responsible for the 'supposed' milder infections in individuals with COVID 19 in the region [11–13]. As at the time of our cross-sectional study in August 2020, Nigeria's COVID 19 situations had plateaued with the society actively re-engaged. Our serological survey showed IgG and IgM seropositivities of 42% and 41% respectively indicating that a greater proportion of individuals in our study population had been either in an active but asymptomatic infection or a previous exposure to SARS-CoV-2. But RT-PCR based results, from the NCDC reports, had suggested a low rate of COVID 19

infection in Nigeria. The NCDC reported low rate was expected as the country only tested individuals with only high suspicion index for COVID 19 while leaving out those who were probably infected but asymptomatic. The high rate of seropositivity to SARS-CoV-2 antibodies in our study suggests a wider circulation of the virus at the community level in the two cities that were sampled. By implication, more Nigerians had been infected than officially reported and more were still infected as indicated by the high SARS CoV-IgM sero-prevalence.

Our finding is comparable to rates ranging from 24.4% – 33.7% obtained at different points in time among blood donors in Pakistan and France [14, 15]. Our result however contrasted with the rates of 0.02 % – 10.9% from blood donors in other parts of the world including those with worst experiences during the initial phase of the pandemic ^{16–20}. Differences in health policies and adherence to social interventions by the general population in the various countries may have contributed to the variations in the seroprevalence recorded. Furthermore, the testing strategy in Nigeria which did not include antibody testing of asymptomatic persons might have led to many missed cases that continued to interact with other people in the community thus fueling the transmission of the virus. In addition, adherence to social control measures and other COVID 19 protocols was generally poor in most cities in Nigeria [21]. These might have contributed to the high sero-prevalence reported in this study. This poor adherence could be due to many reasons including but not limited to the living conditions of many urban dwellers in Nigeria that might make it impractical to observe physical distancing guidelines and sheer apathy or ignorance toward measures to limit virus spread.

The high rate of actively infected yet asymptomatic persons as shown by the IgM seropositivity in our study confirms the speculation that most infections in sub-Saharan Africa may be asymptomatic due to a large number of younger people in the population [12]. Although blood banking policies in Nigeria allowed for blood donation by individuals within the age range 15–65 years, our study shows the blood donors to be in the range of 21–48 years (young and middle aged). Studies have shown that all ages are susceptible to infection by SARS CoV-2, but older age is a major indicator for symptomatic active cases [22–25]. Significantly, this may have a considerable public health implication as many of these young actively infected individuals who are asymptomatic will most likely infect more people than symptomatic individuals [26]. Therefore, it becomes imperative that peculiarities of Nigerian population and situations be put into consideration while building a country specific predictive model that will provide policymakers with several prediction scenarios, based on different actions they can take to address the pandemic.

In this cross-sectional study, the seroprevalence in Abuja being higher than Enugu is expected as Abuja was one of the epicenters of Nigeria COVID 19 experience during the initial phase of the pandemic. Thus, individuals in Abuja may have been at a greater risk of exposure to the virus than Enugu. Although the point of surveillance was about a month after the ban on inter-city movement of people was lifted in Nigeria, possibility exists that in many months to come such may not be the picture of the epidemics. Therefore, point to point sero-surveillance of the population is needed to monitor trends in the virus transmission to inform appropriate health policies. Although, no association between sex and

susceptibility to SARS-CoV-2 infection has been reported [24], we could not make any inference on this as over 95% of our study population were males.

There is still no consensus among the scientific community on the implication of IgG antibodies to SARS-CoV-2 in terms of protection and correlates of such protection (if it exists) [27]. It is therefore difficult to extrapolate if these individuals with previous exposure to the virus are protected from future infection and if so, how long such protection will last. With documented cases [28–31] of reinfection after initial encounter with the virus and in some of the cases with worse symptoms than the initial encounter, one will suggest that the concept of immunity passport or herd immunity be treated with caution until more data on immune correlates of protection are available. There is possibility that reinfections are currently being underestimated as it is biased towards detection of symptomatic cases. Consequently, the extent to which reinfection occur in the general population among asymptomatic cases (who may have recovered from their previous infection albeit unknowingly) is still not clear. Hence, calculated utilization of antibody testing will help shed more light to immune correlates of SARS-CoV-2 infection.

One of the limitations of the study was the predominant number of young to middle-aged men, with fewer women involved. Secondly, our study was limited to events at the point of surveillance. Therefore, more serological studies are still required to uncover the rate of asymptomatic infections in the Nigerian population.

Conclusions

the high prevalence of IgG and IgM antibodies to SARS-CoV-2 reported in this study showed a wider spread of SARS CoV 2 infection in Nigeria. This highlights the need for more elaborate testing protocol that would include antibody testing of the population for epidemiological purposes, especially to uncover the actual rate of infections in Nigeria.

Abbreviations

ELISA: Enzyme linked immunosorbent assay

SARS CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

COVID 19: Coronavirus Disease (2019)

IgM: Immunoglobulin M

IgG: Immunoglobulin G

Declarations

Ethics approval and Consent to participate:

Favourable ethical opinions were obtained from two Health Research Ethics Committees. Written informed consent was obtained from each participant.

The Federal Capital Territory Health Research Ethics Committee Abuja, Approval Number **FHREC/2020/01/77/11-08-20**.

The Molecular Pathology Institute Health Research Ethics Committee Enugu, Approval Number **MPIHREC/2020/08/02/0002**.

Consent for Publication:

All authors consented to publication

Availability of data and materials:

All data sets and materials will be made public unhindered.

Competing Interest:

None

Funding:

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Authors' Contributions:

EN and II conceived and designed the study, UO, EI and OA reviewed the study and sampling method, OA, TY, OO and NU obtained ethical approval and sampled donors, HO, AN, NI, CO AND IO did lab testing, data collection. Early manuscript was drafted by II, EN expanded the manuscript fully, all authors contributed to revising the manuscript, EN and II did statistical analysis of data; all authors reviewed the manuscript.

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Tables

Table 1
Demographics of blood donors

Variable	Number	Percentage
Sex		
Male	106	94
Female	7	6
Location		
Abuja	67	59
Enugu	46	41
Sample size	113	100

Table 2
Descriptive statistics of SARS CoV-2 IgG and IgM antibodies in blood donors

Variable	SARS CoV-2 IgG	SARS CoV-2 IgM
Median antibody level (NTU) (POSITIVE cases)	19 (n = 48)	15 (n = 44)
95% CI of Median	17–21	14–17
IQR antibody level (NTU)	9	6
Median antibody level (NTU) (NEGATIVE cases)	5 (n = 46)	6 (n = 48)
95% CI of Median	4–6	5–7
IQR antibody level (NTU)	2	2
Median antibody level (NTU) (EQUIVOCAL cases)	10 (n = 12)	10 (n = 21)
95% CI of Median	9–10	9–10
IQR antibody level (NTU)	1	1
<i>NTU = NovaTech Unit. IQR is inter-quartile range. Cut off point is 11 NTU; > 11 NTU is positive, < 9 NTU is negative while 9–11 NTU is equivocal.</i>		

Table 3
Inferential Statistics on SARS CoV-2 IgG and IgM antibodies in blood donors

Variable	P value for IgG	P value for IgM
Positive vs. Negative cases	< 0.0001	< 0.0001
Positive vs. Equivocal cases	0.0071	0.0005
Negative vs. Equivocal cases	0.0113	0.0002
<i>Kruskal wallis and Dunn's multiple comparison tests were performed. Kruskal wallis test showed significant differences in IgG and IgM antibodies for positive, negative and equivocal cases (p value = 0.0001 for IgG and p value = 0.0001 for IgM).</i>		