



Distribution of Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2) Immunoglobulins G and M among Frontline Health Workers in Eleme Local Government of Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: The fight against Corona Virus Disease (COVID-19) globally among front line health workers has been a challenging task, it encompasses working lengthy hours at the isolation Centre's, intensive care units, emergency departments and molecular laboratories, and also been exposed to pathogens, fatigue, and psychological distress. This has led to high morbidity and mortality rate among front line health workers all over the globe.

Aim: This study aimed at determining the distribution of SARS CoV -2 IgG and IgM among frontline health workers in Eleme Local Government of Rivers State, Nigeria.

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Materials and Methods: A total of 100 frontline Covid-19 health workers were recruited for this study comprising 48 males and 52 females with ages between 23 and 49 years and included janitors, hygienist, nurses, data Officers, logistics and ambulance drivers, physicians, and medical laboratory scientists, working at the molecular laboratories, sample collection booths and isolation center. The bio-data of the subjects were obtained using a well-structured questionnaire. Only subjects who gave informed consent were recruited for this study. 60ul of capillary blood was collected from each participant using an aseptic technique and immediately followed by the analysis of SAR-CoV-2 IgM and IgG using a lateral flow immunochromatographic assay technique.

Results: Results from this study showed a total of forty-two (42) 42% subjects were reactive to IgG antibodies while fifty-eight (58) 58% subjects were non-reactive to IgG antibodies also a total of Twenty-One (21) 21% subject were reactive to IgM antibodies while a total of seventy-nine (79) 79% subjects were none reactive to IgM antibodies. A total of nine (9) subjects who were exposed to SAR-CoV-2 for more than one year were reactive to SAR-Cov-2 IgM and IgG antibodies, while a total of two (2) subjects who were exposed to SAR-CoV-2 for less than one year were reactive to SAR-Cov-2 IgM and IgG antibodies and the difference ($p = 0.013$) was statistically significant. Also, a higher number of subjects within the 20-29 age bracket were reactive to SAR-CoV-2 IgM antibodies while subjects with in the age bracket of 30-39 were more reactive to IgG antibodies.

Conclusion: This study reveals that serological testing is an ideal approach in assessing the proportion of frontline health workers who might have been exposed to SARS-CoV-2 as part of effort in combating COVID-19 disease globally.

Keywords: *Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2); Corona Virus Disease (COVID-19); frontline health workers; immunoglobulin.*

1. INTRODUCTION

The fight against Corona Virus Disease (COVID-19) globally among frontline healthcare workers has been a challenging task, it encompasses working lengthy hours at the isolation Centres, intensive care units, emergency departments and molecular laboratories, and also been exposed to pathogens, fatigue, and psychological distress [1,2].

“Coronaviruses are large, single-stranded, enveloped RNA viruses found in humans and other mammals with a genome ranging from 26 to 32 kilobases in length” [3]. “They belong to the coronaviridae family and Coronavirinae subfamily, which has four genera: Alphacoronavirus, betacoronavirus, gammacoronavirus, and deltacoronavirus” [4].

“Phylogenetic study of the entire genome of Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2) shows that it belongs to the subgenus Sarbecovirus of the genus Beta coronavirus where it is clustered with other SARS-CoV and SARS-related coronaviruses. Structurally, severe acute respiratory syndrome corona virus-2 (SARS-CoV-2) possess a capsid outside the genome which was created by the nucleocapsid protein (N), and the genome is further packed by an envelope linked to three structural proteins namely Spike (S), membrane (M), and envelope (E) proteins” [5].

“Antibodies, also known as immunoglobulins, are produced in response to an invasion of a foreign molecules in the body. Immunoglobulins are a collection of structurally and functionally related glycoproteins which provide humoral immunity. Together with B and T cells, antibodies comprise the most important part of the adaptive immune system” [6].

“In mammals, immunoglobulins are classified into five main isotypes namely IgA, IgD, IgE, IgG and IgM and their classification is based on the heavy chain they possess – alpha, delta, epsilon, gamma, or mu, respectively. These immunoglobulins basically are made up of about of 82-96% protein and 4-18% carbohydrate, they also have different effector functions” [7].

Immunoglobulin M (IgM) has been described as the most primitive antibody class [8]. “Macro-immunoglobulin, IgM, is initially produced as a surface bound molecule and is expressed in early B cell differentiation. Later in immune response, IgM is produced by plasma cells and secreted as soluble pentamers that contain 10 antigen binding sites and the joining (J) chain, or as hexamers containing 12 antigen binding sites and no joining chain” [7]. “Immunoglobulin M (IgM) has a molecular weight of approximately 900 or 1050 kDa for the pentamer or hexamer respectively. Due to the polyvalent nature of IgMs, they may exhibit higher avidity for antigen

than the bivalent IgG. In addition to neutralizing pathogens, IgM antibodies are highly effective at engaging complement to target lysis of cells and pathogens” [9].

“Immunoglobulin G (IgG) is the most abundant antibody in normal human serum, accounting for 70-85% of the total immunoglobulin pool” [10]. “It is monomeric with a molecular weight of approximately 150 kDa. Depending on the size of the hinge region, the position of disulfide bonds, and the molecular weight of the antibody, IgG can be further divided into 4 subclasses: IgG1, IgG2, IgG3, and IgG4” (10). However, the IgG is by far the most extensively studied class of immunoglobulins, due to its extremely important role in immune therapeutics as well as in viral immunity [11].

“After an infection with viruses, various classes of antibody appear sequentially. For example, during primary infection or immunization, most antigens first elicit IgM (early antibody) responses; IgA and IgG responses follow within a few days. Reinfection, in contrast, stimulates production mainly of IgG, although some IgM and IgA are generated. When the primary antigenic stimulation is in the respiratory or gastrointestinal tract, IgA antibody is predominant, accompanied by some IgM. These antibodies are secreted locally at mucosal surfaces and are important in protecting the host against localized surface viral infections such as the common cold, influenza, and enteric viral infections” [7].

Owing to the rapid global spread of COVID-19 among healthcare workers, it became pertinent among various health authorities in the world to expand their testing capacity beyond antigen-based or traditional quantitative real-time polymerase chain reaction (qRT-PCR) assays, this was conceptualized to have a better understanding of the disease progression [12]. This study seeks to determine the distribution of SARS CoV -2 IgG and IgM antibodies among frontline health workers in Eleme Local Government of Rivers State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Design

This was a cross-sectional study comprising frontline healthcare workers who were working at the COVID-19 isolation centers, and molecular

laboratory in Eleme local government Area of Rivers State, Nigeria.

2.2 Study Area

This study was carried out in Eleme local government area of Rivers State, Nigeria. Eleme local government area of Rivers State has a population of 190,884 as at the 2006 National Census [13] and is made up of 10 major communities namely Akpajo, Aletto, Alesa, Alode, Agbonchia, Ogale, Egbubu, Ekporo, Eteo and Onne. They generally speak Eleme language. Eleme is bounded in the north by Obio Akpor and Oyigbo, in the South by Okrika and Ogu Bolo, in the east by Tai and the West by Okrika and Port Harcourt City local government area. It covers an area of 138 km² with Global Positioning System (GPS) coordinates Latitude: 5.08333, Longitude: 6.65 5° 4' 60" North, 6° 39' 0" East. It serves as a host to numerous multinational companies and two refineries and has one of the biggest ports in Nigeria. The people of Eleme local government area of Rivers State are predominantly farmers and traders.

2.3 Study Population

A total of 100 frontline COVID-19 healthcare workers were recruited for this study comprising of 48 males and 52 females, aged between 23 and 49 years. They include Janitors, hygienist, Nurses, Drivers, Physicians, Data officers and Medical Laboratory Scientists. The demographic information of the subjects was obtained using a well-structured questionnaire.

2.4 Sample Size

Sample size was determined using Gpower version 3.1.9.2 power 0.08 at 95% confidence interval.

The sample size obtained was 64 but this study adopted a sample size of 100.

2.5 Eligibility Criteria

2.5.1 Inclusion criteria

Apparently healthy subjects who gave informed written consent within the age bracket of 23 to 49 years, who were front liners in the fight against COVID-19, working at the molecular laboratories, sample collection booths and isolation center were used for this study.

2.5.2 Exclusion criteria

Subjects who were not frontline healthcare workers in the fight against COVID-19 and those who did not give their consent were excluded from this study.

2.6 Sample Collection

Using a swab pad saturated with 70% isopropyl alcohol, the subject's left thumb is disinfected and allowed to air dry, a puncture sterile needle was used to prick the thumb of each subject and 60ul of capillary blood was aspirated using an aseptic technique and immediately followed by the analysis of SARS-CoV-2 IgM and IgG antibodies.

2.7 Sample Analysis

2.7.1 Detection of IgG and IgM antibodies using Egens diagnostic kit (Lot Number 20200402; expiry date: 04/2022)

Method: A lateral flow immunochromatographic method was used according to manufacturer's instruction.

The principle of rapid SARS-CoV-2 IgM and IgG antibodies detection kit is based on lateral flow immunochromatographic assay.

Procedure for IgG and IgM: A Test cassette, buffer and control sample was allowed to equilibrate to room temperature, there after the cassette was placed on a plan surface and the covering foil was removed. Using a sterile manual mini pipette, 10ul of capillary blood is added into the sample pad of the cassette well labeled A. Furthermore, a 60ul (2 drops) of sample buffer is added to well B. Result was read after 15 minutes.

It uses anti- human IgM antibodies (Test line IgM), anti- human IgG antibodies (Test line IgG) and goat anti -rabbit IgG (Control line C) immobilized on a nitrocellulose strip. The burgundy-coloured conjugate pad contains colloid gold conjugates to recombinant COVID-19 antigen conjugate with colloid (Covid-19 conjugate) and rabbit IgG gold conjugate. When specimen is added followed by an assay buffer, IgM and/or IgG antibodies if present will bind to COVID -19 conjugate thereby forming an antigen-antibody complex. This complex migrates through intracellular membrane by capillary action and meets the line of its

corresponding immobilized (anti-human IgM and/or IgG antibodies) hence, the complex is trapped forming a burgundy-coloured band which confirms a reactive test. Absence of a coloured band in the test region indicates a non-reactive test. Also, the test contains an internal control (C band) which exhibits a burgundy-coloured band of the immunocomplex goat anti rabbit IgG/rabbit IgG-gold conjugate regardless of the colour development on any of the test band.

2.8 Data Analysis

The data generated from the analysis were analyzed using the Statistical Package for Social Sciences (SPSS) version 23. Results obtained were presented in tables. Comparisons of values was done using the Chi-Square, and p-values less than or equal to 0.05 were considered statistically significant.

3. RESULTS

3.1 Demographics Characteristics of Study Subjects

Table 1 shows the demographic information of the study subjects.

Table 1. Demographics characteristics of study subjects

Variable	Frequency (%)
Sex	
Male	48 (48)
Female	52 (52)
Marital Status	
Married	51 (51)
Single	49 (49)
Educational Qualification	
Primary	0 (0)
Secondary	26 (26)
Tertiary	74 (74)
Profession	
MLS	38 (38)
Nurses	16 (16)
Physicians	18 (18)
EHO	3 (3)
Janitors	13 (13)
Ambulance/ logistics drivers	10 (10)
Data Officers	2 (2)

3.2 Distribution of SARS-COV-2 Antibody among Study Subjects

Table 2 shows the distribution of the subjects into reactive and non-reactive status. The number of

reactive subjects were less than the non-reactive subjects for both IgG and IgM infections.

3.3 Comparison of Duration of Exposure to SARS-CoV-2 by the Study Subjects

Table 3 shows the distribution of the subjects according to the duration of exposure for both IgM and IgG concurrently. There were statistically significant ($p=0.013$) more subjects who were reactive for more than one year than for less than one year.

3.4 Comparison of SARS-CoV-2 IgM and IgG Antibody Status Based on Sex of the Study Subjects

Table 4 shows the number of subjects who were reactive for IgM and IgG according to sex. There was no statistically significant difference ($p=0.166$) between male and female subjects studied.

3.5 SARS-CoV-2 IgM and IgG antibody status based on profession of the study subjects

Table 5 shows the number of subjects who were reactive for both IgG and IgM according

to professional status. There was no statistically significant difference ($p=0.538$) in the number of reactive subjects among various professions.

Table 2. Distribution of SARS-COV-2 antibody among study subjects

Antibody	Reactive	Non- Reactive	p value
IgG	42	58	0.032
IgM	21	79	<0.001
	<0.001	<0.001	

Table 3. Comparison of duration of exposure to SARS-CoV-2 by the study subjects

Duration/Status	Reactive
More than one year	9
Less than one year	2
p-value	0.013
$\chi^2 =$ value	6.231

3.6 Status of Subjects According to Age Brackets

Fig. 1 shows the status of subjects according to age brackets.

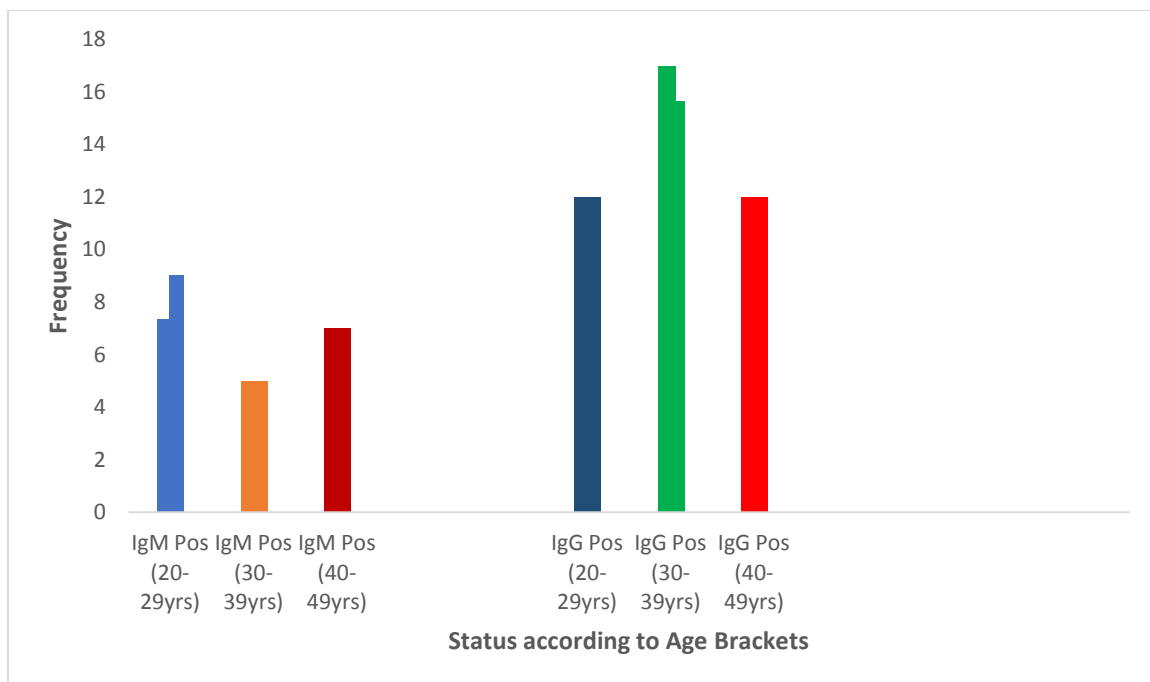


Fig. 1. Status of subjects according to age brackets

Table 4. Comparison of SARS-CoV-2 IgM and IgG antibody status based on sex of the study subjects

Sex/Status	Reactive
Male (n=48)	6
Female (n=52)	7
p-value	0.166
X ² = value	1.923

Table 5. SARS-CoV-2 IgM and IgG antibody status based on profession of the study subjects

Profession/Status	Reactive
Med. Lab. Scientists (n=38)	4 (30.7)
Physicians (n=18)	1 (7.7)
Nurses (n=16)	3 (23.1)
Env. Health Officers (n=3)	1 (7.7)
Drivers (n=10)	1 (7.7)
Janitors (n=13)	3(23.1)
Data Officers (n=2)	-
p-value	0.538
X ² = value	4.077

4. DISCUSSION

This study seeks to determine the distribution of SARS CoV -2 IgG and IgM antibodies among frontline health workers in Eleme Local Government of Rivers State. Findings from this study showed a higher number of subjects reacting to SARS- CoV -2 IgG antibody compared to SARS- CoV -2 IgM antibody. This finding may be attributed to IgG being the most abundant antibody in serum, its role in viral immunity, and also indicates previous exposure to SARS- CoV -2 [10,14]. In this study, duration of exposure to SAR-CoV-2 among study subjects showed subjects who had worked as COVID-19 frontline health worker for more than one year were significantly exposed compared to those that worked for less than one year ($p = 0.013$). This might be as a result of the working environment where subjects are daily in contact with infected patients and samples. This finding agrees with findings by Milazzo et al. [15] where longer-term exposure to SARS-CoV-2 as frontline health workers increased seroprevalence among health workers and this was attributed to extra hospital contacts over time. This finding also agrees with the findings by Vaezi et al. [16]; Chen et al. [17] where it was reported that health workers that spent more time with COVID-19 patients have higher

seroprevalence SARS-CoV-2 antibodies. Also in agreement is a systematic review and meta-analysis conducted by Hossain et al. [18] on seroprevalence of SARS-CoV-2 IgG antibodies among health care workers prior to vaccine administration in Europe, the USA and East Asia, results revealed an increase in seroprevalence from about 5% in February-April to about 10% in May-September. However, this is in disagreement with the study carried out by Saberian et al. [19] on Changes in COVID-19 IgM and IgG antibodies in emergency medical technicians, there findings revealed a significant reduction in COVID-19 antibody seropositivity over time.

Findings from this study also showed that there was no difference in exposure to SARS-Cov-2 among the various health professionals. The high number of IgM and IgG SAR- CoV -2 antibodies among the health professionals might be attributed to the frequent exposure to COVID-19 samples on the line of performing their professional duties such as during sample collection and testing, daily nursing care of COVID-19 patients and cleaning of isolation centers/molecular laboratories respectively.

This study also showed that a higher number of subjects within the 20-29 age bracket were reactive to SAR-CoV-2 IgM antibodies while subjects with in the age bracket of 30-39 were more reactive to IgG antibodies. The IgG antibody can increase with increasing age because individuals tend to expand their number of memory cells by way of accumulated immunological memory [20].

5. CONCLUSION

This study reveals that serological testing is an ideal approach in assessing the proportion of frontline health workers who might have been exposed to SARS-CoV-2 as part of effort in combating COVID-19 disease globally. Long term exposure to SARS-CoV-2 as a frontline health worker have shown to have a higher chance of been exposed to COVID-19 and this can be attributed to the working environment where frontline health workers are daily in contact with infected patients and patients' samples. It is recommended that further studies that will involve all frontline health workers in Rivers State and Nigeria to determine COVID-19 disease progression in Rivers State and Nigeria should be conducted.

CONSENT

As per international standard or university standard, Participants' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Ethical approval for this study was obtained from the Health Research Ethics Committee of the Ministry of Health, Rivers State, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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