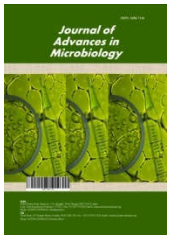

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Serological Profile of Convalescent COVID-19 Patients at an Infectious Diseases Hospital in Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AF and TOA conceptualized the study. Authors TOF and ECO analyzed the samples. Authors AF and TOA interpreted the laboratory data and the statistical analysis. Author AF drafted the initial manuscript and all authors contributed equally to its development. All authors read and approved the final manuscript.

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ABSTRACT

Background: IgG antibodies may serve as promising targets to detect and evaluate immune responses against the SARS-CoV-2 virus. Both IgA and IgM antibodies target the spike protein's receptor binding domain and are rapidly decayed, while IgG antibodies remain relatively stable for longer periods in COVID-19 patients.
Objectives: The current study was designed to detect the presence of SARS-CoV-2 antibodies among convalescent COVID-19 patients and to evaluate the relationship between these antibodies, the symptom grade and their baseline Cycle Threshold (CT) by RT-PCR.

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Methods: Eighty-nine convalescent COVID-19 patients on admission were recruited and tested until negative by RT-PCR. Sera obtained from participants were screened for SARS-CoV-2 IgM and IgG antibodies using rapid lateral flow assays.
Results: It was observed that 93,3% and 77,5% respectively had IgM and IgG antibodies against the S1 protein of SARS-CoV-2. Majority (74,0%) presented with mild COVID-19 symptoms with a mean RT-PCR Ct value of 31,4.
Conclusion: Convalescent COVID-19 patients develop a fairly good level of IgG antibodies. The antibody status is not dependent on CT value or symptom grade. However, there was a significant correlation between baseline CT and time taken to test negative by RT-PCR.

Keywords: COVID-19; cycle threshold; antibody; convalescent.

1. INTRODUCTION

Coronavirus Disease 2019 (COVID-19), caused by the novel Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), emerged in December 2019 in Wuhan, Hubei province of China and currently affects almost 213 countries and territories [1,2].

Evidence from a number of studies reveals that convalescent persons who have recovered from COVID-19 develop antibodies to the virus. The level and duration of protection conferred by such antibodies is largely controvertible as very low levels of neutralizing antibodies have been reported in COVID-19 convalescent sera [3]. As the World Health Organization (WHO) continues to review the evidence on antibody responses to SARS-CoV-2 infection, a better understanding is being developed on the dynamics of immunological responses evoked by the SARS-CoV-2 virus [3-5].

COVID-19 Serological Detection Tests have been developed using varied platforms including; Rapid Immunochromatographic Assay, Enzyme-linked Immunosorbent Assay (ELISA) and Virus Neutralization Assays [6]. Studies indicate that most COVID-19 patients seroconvert 2 weeks after the onset of symptoms with less than 40% having antibodies within a week and 94,3% (IgM), 79,8% (IgG) observed at about 2 weeks from onset of symptoms [6,7]. Decline in SARS-CoV-2 IgM level begins at about 4 weeks after onset of symptoms while IgG levels remain high 7 weeks post infection [7].

Immunological kinetics of COVID-19 antibodies is an important element in developing and implementing strategies to combat the pandemic. Two separate studies have established the persistence of antibodies that target SARS-CoV-2 in hundreds of patients with COVID-19 at least 3 months after symptom onset [8,9]. As knowledge on Immunity to COVID-19 increases, there is a need to evaluate the immunological profile of convalescent COVID-19 cohort, with a view to understanding the important clinical and virological factors.

2. METHODOLOGY

2.1 Participant Selection and Sample Collection

The current study was carried out among consenting convalescent COVID-19 patients recruited between 1st of April to 1st of May 2020 at the Infectious Diseases Hospital, Olodo, Ibadan, Nigeria. Eighty-nine consecutively recruited participants whose clinical symptoms had completely resolved were re-tested for SARS-COV-2 by RT-PCR. Clinical details were entered into pre-designed forms. Nasopharyngeal and oropharyngeal swabs were obtained into 2 mL Viral Transport Medium for testing by RT-PCR. Blood samples were collected from each consecutively recruited participant at the Infectious Diseases Hospital. Eligible patients are those whose nasopharyngeal swabs have tested negative by RT-PCR. A volume of 3 mL of blood was collected into Ethylene Diethyl Tetra Acetic acid (EDTA) bottles. Serum was separated by centrifugation at 3000 g for 15 min within 24 h of collection, and then stored at – 20°C until use.

2.2 RNA Extraction and qRT-PCR

RNA was extracted from the nasopharyngeal and oropharyngeal swabs using QIAamp viral RNA mini kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. A commercial real-time RT-PCR kit (BGI, Europe) which detects the open reading frame 1ab (ORF1 ab) was used for the real time RT-PCR assay. The PCR was set up with a reaction volume of 30 µL according to

manufacturer's protocol. The reaction procedure was 50ºC for 20 min, 95ºC for 10 min, followed by 40 cycles of 95ºC for 15s and 60ºC for 30s. The RT-PCR Threshold cycle (CT value) less than 38 indicated a positive sample based on manufacturer's instructions.

2.3 Serological Test

Sera were tested for the presence of Immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibodies to SARS-CoV-2 virus using COVID-19 Innovita Rapid Test (Tangshan Biological Technology Co. Ltd) according to manufacturer's instructions. Briefly, the kit detects 2019-nCoV IgM and IgG antibodies by immuno-capture method. The nitrocellulose membrane has been precoated with mouse-anti human monoclonal IgM antibodies, mouse-anti human monoclonal IgG antibodies, and goat-anti-mouse IgG antibodies. The recombinant 2019-nCoV antigen and mouse IgG antibodies are labeled with colloidal gold as a tracer. After addition of the specimens, if 2019-nCoV IgM antibodies are present, the antibodies will bind to colloidal gold-coated 2019-nCoV antigens to form compounds, which are further captured by pre-coated mouse-anti human IgM antibodies to form new compounds, and generate purple Test line designated T. This is similar for the detection of IgG antibodies, which also give rise to purple line T. The colloidal gold-labeled mouse IgG antibodies bind with goat-anti-mouse IgG antibodies and presents with a purple line, which indicates the control line (C).

2.4 Data Analysis

All statistical analyses were conducted using IBM SPSS Statistics for Windows, version. 22.0 (IBM Corp, Armonk, NY, USA). Descriptive analysis was used to obtain the mean age and standard deviation of respondents, frequency distribution for the demographic features, CT range and the clinical characteristics of COVID-19 patients. Inferential statistics were finally carried out to establish relationships using the chi-square test, and the correlation between repeat test, baseline CT and symptoms was tested using the Pearson Moment Correlation Test.

3. RESULTS

Of the 89 participants, 63 (68,5%) were males giving a male to female ratio of 1.6:1. Majority (43,8%); of the participants were within the age category; 21-30 years.

Fever (28,1%), headache (24,1%), cough (16,3%) and anosmia (14,8%) were the most common symptoms (Fig. 1). A total of four patients (4,4%); had a critical course of illness with treatment in the Intensive Care Unit during their stay in hospital. The longest time to negative RT-PCR test was 23 days with an average duration of 7,3, days +/- Standard Deviation (SD) of 5,9. The average number of repeat RT-PCR tests done to test negative from an initial positive was 2,22 +/- SD of 3,627. The SARS-CoV-2 CT ranged from 16,37 to 36,5 with an average CT of 31,4.

A total of 93,3% and 77,5% respectively had only IgM and IgG antibodies to SARS-CoV-2 while 6,7% had neither IgG nor IgM antibodies to SARS-CoV-2. Age category was not significantly related to SARS-CoV-2 antibody profile of participants (Tables 1 and 2).

The COVID-19 symptoms of participants were classified as mild, moderate or severe. Mild or moderate cases were generally defined based on less severe clinical symptoms (low grade fever, cough, discomfort) with no evidence of pneumonia and not requiring admission to ICU. Although majority (74,0%) had mild COVID-19 symptoms and 71,1% had moderate CT values that ranged from 26-35, the CT values and the symptom grade were not significantly related to the presence of IgM and IgG antibodies to SARS-CoV-2 among participants (Tables 3 and 4).

The majority of patients (70,2%) turned negative by RT-PCR after less or equal to two follow-up RT-PCR tests done 5 days apart. Compared with the group that required more than two Tests, SARS-CoV-2 IgM 54(64,3%) and IgG 44 (52,4%) were higher among those who required two or less than two follow-up RT-PCR Tests. Although the CT and symptom grading had no significant effect on antibody status, there was a significant correlation between baseline CT and time taken to test negative by RT-PCR using the Pearson Product Moment Correlation (Table 5). The Pearson Product Moment Correlation analysis showed that there is a weak negative relationship between the number of times follow-up RT-PCR Test was done and the baseline CT value (r =-0,330). This implies that the lower the baseline CT values the more the number of follow-up tests required to be done to get a negative RT-PCR result and *vice-versa.* The antibody status is not dependent on CT value or symptom grade. However, there was a significant correlation

between baseline CT and time taken to test negative by RT-PCR (Tables 3 and 4). Additionally, the CT value and the symptom grade revealed an indirect or weak negative correlation (r =-0,074). Hence the lower the CT values, the higher the tendency towards a severe symptom grade. For the relationship between symptoms and number of follow-up tests required, there is a weak positive or direct correlation (r = 0,016). This suggests that the number of required follow-up tests increases, as the severity of the symptoms also increase, this relationship is however not statistically significant (Table 5).

Table 1. Age distribution of SARS-CoV-2 IgM antibody

Age (Years)	Positive	Negative	Total	Chi-square	p-value
≤ 20	4(4,5%)	0(0%)	4(4,5%)		
21-30	39(43,8%)	1(1,1%)	40(44,9%)		
31-40	18(20,.2%)	3(3,4%)	21(23,6%)	6,08	0,29
41-50	10(11,2%)	2(2,2%)	12(13,5%)		
51-60	9(10,,%)	0(0%)	9(10,1%)		
≥ 61	3(3,4%)	0(0%)	3(3,4%)		
Total	**83(93,3%)**	**6(6,7%)**	**89(100,0%)**		

Table 2. Age distribution of SARS-CoV-2 IgG antibodies

Age (Years)	Positive	Negative	Total	Chi-square	p-value
≤ 20	3(3,4%)	1(1,1%)	4(4,5%)		
21-30	32(36,0%)	8(9,,%)	40(44,9%)		
31-40	15(16,9%)	6(6,7%)	21(23,6%)	2,32	0,80
41-50	10(11,2%)	2(2,2%)	12(13,5%)		
51-60	6(6,7%)	3(3,4%)	9(10,1%)		
≥ 61	3(3,4%)	0(0%)	3(3,4%)		
Total	**69(77,5%)**	**20(22,5%)**	**89(100,0%)**		

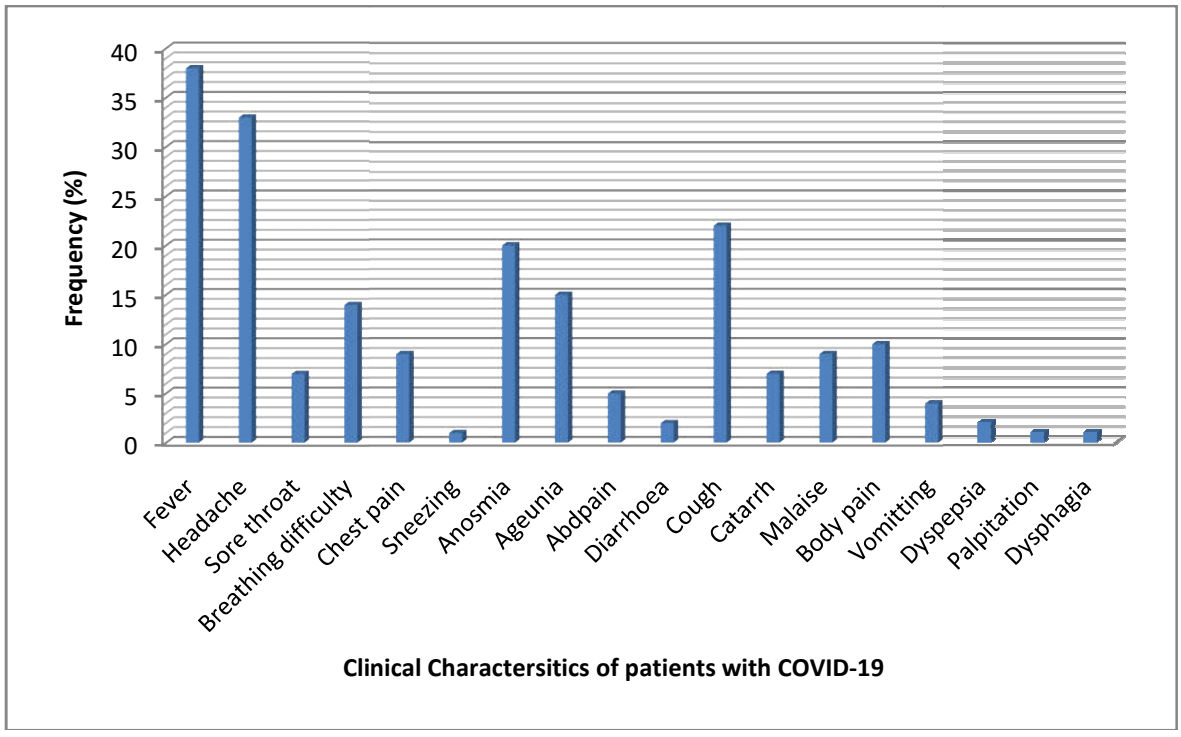

Fig. 1. Clinical characteristics of COVID-19 patients in Ibadan

Table 3. Symptom grading and antibody profile of participants

Variable	Status	Mild	Moderate	Severe	Total	Chi-square	P-value
IgM	Positive	63(70,8%)	17(19,1%)	3(3,4%)	83(93,3%)		
	Negative	3(3,4%)	2(2,2%)	1(1,1%)	6(6,7%)	3,06	0,27
	Total	**66(74,2%)**	**19(21,3%)**	**4(4,5%)**	**89(100,0%)**		
IgG	Positive	54(60,7%)	14(15,7%)	2(2,2%)	70(78,7%)		
	Negative	12(13,5%)	5(5,6%)	2(2,2%)	19(21,3%)	2,63	0,26
	Total	**66(74,2%)**	**19(21,3%)**	**4(4,4%)**	**89(100,0%)**		

Table 4. Baseline CT value in relation to antibody profile of participants

Variable	Status	≤ 25	26-35	≥ 36	Total	Chi- square	P-value
IgM	Positive	10(11,1%)	61(67,8%)	13(14,4%)	84(93.3%)		
	Negative	1(1,1%)	3(3,3%)	2(2,2%)	6(6,7%)	1,58	0,45
	Total	**11(12,2%)**	**64(71,1%)**	**15(16,7%)**	**90(100,0%)**		
IgG	Positive	10(11,1%)	49(54,4%)	11(12,2%)	70(77,8%)		
	Negative	1(1,1%)	15(16,7%)	4(4,4%)	20(22,2%)	1,32	0,52
	Total	**11(12,2%)**	**64(71,1%)**	**15(16,,%)**	**90(100,0%)**		

Table 5. Correlation between number of follow-up RT-PCR tests, baseline CT and symptom Grade

	Repeat test	Baseline CT	Symptom Grade
Repeat test	1		
Baseline CT	-0,330**	1	
Symptom Grade	0,016	-0,074	1

When correlation coefficient "r" lies within -0,1 and – 0,49, we have a weak negative correlation
When correlation coefficient "r" lies within -0,5 and – 0,99, we have a strong negative correlation
When correlation coefficient "r"= -1, we have a perfect negative correlation
When correlation coefficient "r"= 0, we have no correlation
When correlation coefficient "r" lies within 0,1 and 0,49, we have a weak positive correlation
When correlation coefficient "r" lies within 0,5 and 0,99, we have a strong positive correlation
When correlation coefficient "r"= 1, we have a perfect positive correlation

4. DISCUSSION

The knowledge of persistence of antibody response is useful for tracking the spread of 'hot spots' of the COVID-19 Disease. In the current study, majority of the convalescent patients had SARS-CoV-2 IgM (93,3%) and IgG (77,5%) antibodies at the point of testing negative by RT-PCR. This finding is similar to previous observations among COVID-19 patients from other parts of the world [6,7]. Lower IgG antibody (17%) against the S1 protein of SARS-CoV-2 was reported in a different study after an average period of 60 days post infection. The shorter convalescent period in the current study might account for the higher antibody observed among study participants. It has been observed that most persons display antibody response between day 10 and day 21 post infection [10]. The clinical manifestations of COVID-19 range from asymptomatic to Acute Respiratory Distress Syndrome (ARDS). The longevity of the antibody response in SARS-CoV-2 infections is still largely unknown, however antibodies to other Coronaviruses have been shown to wane over a period of 12-52 weeks from onset of symptoms and this observation has implications for potential re-infection.

The clinical manifestations of COVID-19 range from asymptomatic to ARDS. The majority (74,2%) of the COVID-19 patients in the study had mild forms of the disease. The reason for this is not clear and although a recent study had suggested cross protection from other coronaviruses as the significant reasons for lower COVID-19 severity in sub-Saharan Africa, [11] the currently available data shows that all of 6,7% had no antibodies at all (during the studies time scale). This data may support previous observations that have shown that mild cases take longer times to develop antibodies. The

most common symptoms at onset of COVID-19 among this cohort of convalescent COVID-19 patients, include fever, headache, cough and anosmia. Similar to our observation, fever has been reported as the most common symptom among mild to moderate cases [12-15] In the largest cohort study in Europe, fever was presented in 45,4% of the cases, [16] while in the two largest studies in China it went up to more than 80% [17,18].

The CT is defined as the number of cycles required for the fluorescent signal to cross the threshold and is inversely proportional to the amount of target nucleic acid in the sample. The predominantly mild infections observed among this studied cohort can be explained by the fact that the mean CT value was 31,4 low baseline CT values suggest a longer time to testing negative by RT-PCR and also correlate positively with the patients' symptom grade. This suggests that the SARS-CoV-2 CT was a useful independent predictor of patient symptom grade and duration of admission and consequent serological profile. However, further studies are required to evaluate the clinical utility of the CT value given the result variation due to specimen quality, phase of disease, and the limited discriminative ability of the test.

5. CONCLUSION

Sero-immunity of convalescent COVID-19 patients have broader implications in the possibility of re-infection. Longitudinal serological studies that follow up patients' Immunity over an extended period of time would be desirable to evaluate the duration of Immunity conferred by natural SARS-CoV-2 infection in humans.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

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

ETHICAL APPROVAL

Ethical approval was sought and obtained from the Oyo State, Ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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