



ORIGINAL PAPER

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Serum levels of anti-corona virus specific-IgG and -IgM antibodies in COVID-19 patients at admission and at discharge

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ABSTRACT

Introduction. Clear understanding of duration of antibody based protective immunity following natural infection with SARS-CoV-2 will give idea about the efficacy of proposed prophylactic vaccines against SARS-CoV-2, establishment of herd immunity and use of convalescent plasma.

Aim. This study clarified the kinetics and magnitude of the initial antibody response against SARS-CoV-2 in a cohort of symptomatic COVID-19 patients from Ibadan, Nigeria.

Material and methods. This study quantified immunoglobulin M (IgM) and G (IgG) antibodies recognizing the SARS-CoV-2 Spike (S) protein in 35 symptomatic COVID-19 patients at admission and at discharge using ELISA.

Results. CovIgG was positive in none (0)% and 20% of COVID-19 patients at admission and at discharge respectively while CovIgM was positive in 54% and 69% of COVID-19 patients at admission and at discharged respectively. The level of CovIgG was significantly higher in COVID-19 patients at discharge compared with the level at admission while the level of CovIgM was insignificantly reduced in COVID-19 patients at discharge compared with the level at admission.

Conclusion. The data indicates increased anti-SARS-COV-2 IgG Spike antibody in symptomatic COVID-19 at discharge, thus providing basis for antibody-based therapies to treat COVID-19 patients.

Keywords. anti-SARS-CoV-2 specific antibodies, convalescence plasma, COVID-19, spike protein, vaccine

Introduction

The novel SARS-CoV-2 is a recently emerging virus causing a human pandemic having symptoms ranging from mild to severe, eventually leading to death in some cases.¹ Currently, the lockdown imposed by many governments controls the spread, but there is neither a sufficiently effective antiviral drug to treat COVID-19 cases nor an approved vaccine.² In order to guide future vaccine design and antibody-based therapies for

the management of SARS-CoV-2 disease, it is obligatory to understand duration of immunity against SARS-CoV-2 in infected individuals and whether antibodies produced in response to a natural infection provide protective immunity, which may prevent re-infection with SARS-CoV-2.³ Therefore, there is an urgent need to characterize viral-mediated antibody responses, in order to develop therapeutic tools to efficiently cure COVID-19 patients. In this study the dynamics of the

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anti-SARS-CoV-2 IgG and IgM immune response in COVID-19 patients were measured.

Coronaviruses are enveloped, single-stranded positive-sense RNA viruses having spike (S), envelope (E), membrane (M), and nucleocapsid (N) structural proteins.⁴ The SARS-CoV spike (S) protein is composed of two subunits (S1 and S2). The N-terminal S1 subunit contains a receptor-binding domain (RBD) binds the angiotensin-converting enzyme 2 receptor on human alveolar epithelial cells of the low respiratory tract while the C-terminal S2 subunit mediates fusion between the viral and host cell membranes. The S protein is highly immunogenic while M and E proteins are necessary for virus assembly.⁵ This necessitates the choice by the author to determine the levels of anti-SARS-CoV-2 IgM and IgG antibodies against S protein in COVID-19 patients.

Literatures reported that there are need to explore changes in anti-SARS-CoV-2 specific antibody response of follow-up COVID-19 patients, in order to guide vaccine design and antibody-based therapies for cheaper effective management of the disease.^{1-3,6,7} However, the dynamics of the antibody response against SARS-CoV-2 are still under investigation and previous studies showed that CovIgM was detected earlier than CovIgG.⁶⁻¹⁰ Xiao et al. reported that some SARS-CoV-2 laboratory confirmed cases were positive for IgM and IgG at week 3 post symptoms onset.⁶ Concomitantly to IgM decrease, IgG levels raised gradually from week 3 to week 7. Guo et al. showed that 90.4% and the 93.3% COVID-19 patients had plasma IgM and IgA, respectively, and the 77.9% of plasma samples were positive for IgG against nucleocapsid protein of SARS-CoV-2 at day 5 post symptom onset and day 14 post symptom onset for IgG.⁷ Higher numbers of COVID patients were positive for IgG than IgM at the moment of hospitalization and 5 days later; moreover, they had an earlier IgG than IgM seroconversion.⁸

As shown in short-term studies, a seroconversion of IgG and IgM occurred about two to three weeks after disease onset while IgM levels dropped significantly earlier than IgG titers.³ However, it is unclear which anti-SARS-CoV-2 specific antibody type (CovIgG or CovIgM) perform best in the epidemiologic identification of convalescent patients. Some authors favoured IgG while other proposed a higher positivity rate for IgM.⁹⁻¹¹ In addition, the reported peak of IgM response was assigned to different time points ranging from two to five weeks.^{9,11}

Aim

Thus, this study clarified the kinetics and magnitude of the initial antibody response against SARS-CoV-2 in a cohort of symptomatic COVID-19 patients from Ibadan, Nigeria. This might assist in crucial decision-making on vaccine development or antibody based therapy.

Material and methods

Study Population

Thirty-five symptomatic COVID-19 patients recruited from Infectious Diseases Isolation Center, Nigeria were enrolled into this study at admission and followed up till discharged. The clinical signs on admission were dry cough, high fever, sore throat and shortness of breath. The real-time reverse-transcriptase polymerase-chain reaction (RT-PCR) assay was used to confirm the status of all the study participants using nasal and pharyngeal swab specimens following WHO guideline.¹² COVID-19 patients were hospitalised until swab specimens were twice negative for SARS-CoV-2 which lasted between 4-19 days. The control subjects were COVID-19 free apparently healthy individuals recruited from staff and students of University of Ibadan, Nigeria. They were age and sex-matched with COVID-19 patients. None of the controls was on compulsory medication and without communicable or non-communicable diseases. Five milliliters (5 ml) of venous blood was obtained from each subject and was dispensed into plain sample bottles to obtain sera as appropriate. Blood samples were collected on the day of diagnosis when admitted into the isolation center and on the day of discharge when the swab specimens were negative for SARS-CoV-2. Enzyme Linked Immunosorbent Assay (ELISA) was used to determine levels of SARS-CoV-2 Spike protein IgM and IgG in the patients using optical density as specified by the kit manufacturer (Elabscience Biotechnology Inc, USA). Samples were analyzed in duplicates within 1 week of collection.

The test principle

This ELISA kit uses Indirect-ELISA as the method to qualitatively detect the level of anti-SARS-CoV-2 Spike protein -IgG or -IgM in the sample. The micro-ELISA plate is pre-coated with purified SARS-CoV-2 Spike protein antigen. On adding samples and controls to wells, the SARS-CoV-2 Spike protein -IgG or -IgM antibody in the samples bind the pre-coated SARS-CoV-2 Spike protein antigen in the wells of the plate. After washing, Horseradish Peroxidase (HRP) conjugated mouse anti-human antibody added will combine with SARS-CoV-2 Spike protein -IgG or -IgM antibody. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) which is directly proportional to the level of anti-SARS-CoV-2 Spike protein -IgG or -IgM antibody is measured spectrophotometrically at a wavelength of 450nm wavelength.

Assay procedures

One hundred μ L of controls (positive and negative) and samples were added into appropriate wells in duplicates. The plate was covered and incubated for 45 minutes at

37°C. After decanting, 350 µL of wash buffer was added to each well, washed 3 times and 100 µL of HRP Conjugated Mouse anti-human -IgG or -IgM was added which was incubate for 30 minutes at 37°C. The solution was decanted from each well, washed 5 times and 90 µL of Substrate Reagent was added to each well, incubated for 15 minutes at 37°C away from light. After which 50 µL of Stop Solution was added to each well and the OD value which was proportional to the level of CovIgG or CovIgM of each well was measured with a micro-plate reader set to 450 nm wavelength.

Calculation

Cut Off for SARS-CoV-2 Spike protein IgM was calculated thus.

Cut Off for CovIgM = 0.10 + negative control (NC) average A450. When NC average A450 < 0.10 = 0.10; while $0.10 \leq \text{NC average A450} \leq 0.20 = \text{actual value}$.

Positive result was taken as sample absorbance \geq Cut Off. Negative result was taken as sample absorbance < Cut Off.

Calculation of the Cut Off for SARS-CoV-2 Spike protein IgG was calculated thus:

Cut Off(C.O.) = 0.13 + NC average A450. When NC average A450 < 0.05 = 0.05; while $0.05 \leq \text{NC average A450} \leq 0.10 = \text{actual value}$.

Positive control (PC) A450 > 0.60. Negative control (NC) A450 \leq 0.10.

Negative result was taken as sample absorbance < Cut Off.

Statistical Analysis

The positivity and negativity of sera of COVID-19 patients were presented as frequencies (percentages) and were analyzed using X^2 . Optical density of all samples was presented as mean and Standard Deviation. Student *t*-test was used to analyze the differences between two mean values. *P*-value less than 0.05 was considered as statistically significant.

Results

None of the COVID-19 patients was positive for CovIgG at admission while 20% of the patients were positive for CovIgG at discharge. The difference was significant ($p < 0.01$). Fifty-four (54)% of the COVID-19 patients were positive for CovIgM at admission while 69% of the patients were positive for CovIgM at discharge. The difference was not significant (Table1). As shown in the Table 2, the mean CovIgG was significantly increased in COVID patients at discharge than at admission. However, mean CovIgM level was reduced though not significant at discharge compared with mean level at admission.

Table 1. Prevalence of anti-SARS-COV-2 specific -IgG or -IgM antibody in COVID patients at admission and at discharge

CovIgG		
Positive	Negative	
At admission = 0 (0%)	At admission = 35 (100%)	$X^2 = 7.79, p < 0.01$
At discharge = 7 (20%)	At discharge = 28 (80%)	
CovIgM		
Positive	Negative	
At admission = 19 (54%)	At admission = 16 (46%)	$X^2 = 1.56, p > 0.10$
At discharge = 24 (69%)	At discharge = 11 (31%)	

* significant at $p < 0.05$

Table 2. Mean Levels of anti-SARS-COV-2 specific -IgG or -IgM antibody in COVID patients at admission and at discharge

CovIgG			
At admission (n=35)	At discharge (n=35)	t-	p-value
0.042±0.022	0.090±0.087*	-2.793	0.009
CovIgM			
At admission (n=35)	At discharge(n=35)	t-	p-value
0.395±0.487	0.323±0.215	0.940	0.354

* significant at $p < 0.05$

Discussion

The recent COVID-19 pandemic caused by SARS-CoV-2 infection calls for urgent need for therapeutic interventions to manage the outcome of the disease.¹ The characterization of the humoral immune response of COVID-19 patients will elucidate the mechanism of natural protection and will guide through the use of SARS-CoV-2 specific antibodies as prophylactic and therapeutic options to manage the disease, which may contribute to the possibility of vaccine efficacy and herd immunity.^{2,3} To the best of author's knowledge, this is the first study demonstrating dynamism in the SARS-CoV-2-specific IgG and IgM recognizing S protein in Nigerian COVID-19 at the point of admission and at discharge.

In the present study, COVID-19 patients at admission had higher positivity and level of IgM recognizing S protein compared with patients at discharge. This might be related to the fact that COVID-19 patients at admission experienced higher virus replication leading to the expression of more virus antigens, eliciting strong primary humoral immune responses. Thus, suggesting that CovIgM antibodies are involved in immunopathology rather than antiviral effects. Contrary to this, the present study also reported higher positivity and level of CovIgG recognizing S protein in COVID 19 patients at discharge compared with at admission. This highlights the relevance of CovIgG against S protein as correlate of protection in humans as

previously elucidated, thus, suggesting sustained antiviral effects of CovIgG antibodies in COVID-19 patients.^{13,14} Previous study showed that IgG against receptor binding domain of S protein has neutralizing activity and that CoV specific IgG has been correlated with a neutralising function which persisted for 24 months, despite the declining titers.^{3,14-17} Another study showed the 74.2% and the 83.9% of the patients were positive for IgG and neutralizing antibodies 36 months post symptom onset.¹⁸ An observational cohort study including 16 COVID-19 patients whose serum samples were collected 14 days post symptom onset showed that the majority of patients harboured neutralizing IgG against both NP and receptor binding domain.¹⁹ Nucleocapsid protein (NP) is highly immunogenic, although smaller than S, lacks of glycosylation sites, and induces antibodies earlier than S during the infection, thus contributing to neutralization; therefore, anti-NP-specific antibodies might play a key role during the early stages of acute infection.²⁰

Neutralizing antibodies (NAbs) play critical roles in blocking viral infections, thus contributing to viral clearance during acute infection or controlling disease progression during chronic phase. These antibodies are, therefore, useful tools for the protection from viral infection and for the development of effective treatments. CovIgG which was found to be raised in COVID-19 patients considered for this study had been shown to have neutralizing activity.^{3,14-23} It was previously reported that NAbs in the plasma of convalescent COVID-19 patients were successfully employed in the passive antibody therapy to treat 10 severe cases of SARS-CoV-2 infection.²²

The present data strongly suggests that the deeper characterization of plasma from recovered patients might give important information for the development of effective antibody-based therapies to treat COVID-19 patients. However, the rapidly declining Cov specific antibodies from 6 months provoked doubts and anxiety about the long duration of COVID vaccine effectiveness and usefulness of antibody therapy.³ The present study therefore suggests collection of blood sample for the purpose of convalescent plasma therapy in selected COVID patients at discharge.

This study has some limitations, viz: small sample size and need for longer follow-up of COVID-19 patients to give opportunity for sub-grouping COVID-19 patients into days post-discharge.

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