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ORIGINAL ARTICLE

Long-Lasting Antibody Responses in Covid-19: Natural Infection Versus Vaccination

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Abstract:

Introduction: The COVID-19 pandemic caused by the SARS-CoV-2 virus was a devastating global occurrence that has prompted extensive efforts to diagnose and control its spread. Vaccination eventually emerged as a key strategy to mitigate the impact of the virus, yet several questions emerged on the efficacy of the vaccination in sustaining immunity in infected and non-infected subjects. There were also controversies that vaccination does not offer any advantage over herd immunity. This study was aimed at detecting the presence of SARS-CoV 2 Nucleocapsid IgM and IgG in vaccinated and unvaccinated subjects to bridge this knowledge gap.

Materials and Methods: This case-control study involved 80 subjects, including COVID-19-positive subjects, COVID-19-vaccinated subjects, and non-vaccinated COVID-19-negative subjects. Four milliliters (4ml) of blood were collected from each subject by standard venipuncture to assess SARS-CoV 2 Nucleocapsid IgM and IgG using an ELISA procedure. The data generated from this study were represented as percentages and mean ± SD where necessary. Inferential statistical analyses were performed using chi-square and unpaired t-tests with the aid of GraphPad Prism Software Version 9. Statistical significance was defined as a p-value of less than 0.05 at a 95% confidence interval.

Results: Using the calculated cut-off values from the test and controls, nucleocapsid IgG antibodies were detected in 11(50%) of the unvaccinated COVID-19 positive subjects and 3(7.9%) in the vaccinated positive COVID-19 subjects. Eleven (11) (50%) of those who tested negative for SARS-CoV-2 had the IgG antibodies detected in their serum as well giving a 50/50 situation, while surprisingly, 35(92.1%) of the vaccinated subjects tested negative for IgG antibodies ($\chi 2$ =13.81, P=0.002). Nucleocapsid IgM antibodies were detected in 19(86.4%) of COVID-19 positives and only 3(13.6%) among COVID-19 negatives. The vaccinated IgM antibodies were detected in 38(100%) COVID-19-positive subjects while zero percent

was detected among those who were COVID-19-negative ($\chi 2=5.455$, P=0.01). Overall, IgG antibodies were detected in 14 (23.3%) COVID-19-positive subjects and IgM in 57 (95%) COVID-19 subjects.

Conclusion: Distinct antibody response patterns were observed, with natural infection resulting in a more balanced distribution of IgG positivity compared to a pronounced skew towards negativity in the vaccinated group. The high prevalence of IgM in vaccinated individuals indicates a robust early immune response post-vaccination, suggesting the efficacy of vaccines in priming the immune system.

Keywords: IgG antibodies, IgM antibodies, vaccinated subjects, COVID-19 subjects, SARS-CoV-2

INTRODUCTION

The novel coronavirus disease of 2019 (COVID-19), caused by SARS-CoV-2, is a viral respiratory disease belonging to the Coronavirus family and causing severe acute respiratory syndrome (1).

Antibodies play a very crucial role in the host defense mechanism (humoral immunity) against infectious diseases by neutralizing the viruses and conferring protection to the host against viral re-infection. Detection of these antibodies to SARS-CoV-2 serves several purposes including: (i) confirming present or past infection, (ii) evaluating patients with negative Nucleic Acid Amplification Tests (NAATs) who show characteristic COVID-19 symptoms, (iii) sero-epidemiological studies on COVID-19, (iv) assessing the development of antibody-mediated protective immunity, and (v) investigating immune response and immunopathology in COVID-19 (2).

Vaccination against SARS-CoV-2 has been considered the most effective way to prevent the spread of the virus by reducing the severity of the disease and hospitalization, especially among vulnerable subpopulations. Controversies abound concerning the nature, stability, and durability of antibody responses over time in COVID-19 patients. Several studies have reported stable antibody persistent immunity (3) while others have reported rapid antibody waning immunity, or late appearance with low antibody levels and/or complete lack of long-lasting antibodies (4,5).

Contrastingly, infected individuals have been reported to have some humoral protection against COVID-19 (6), with the possibility of reinfection (7,8). The rapid spread of the disease coupled with the unequal distribution of vaccines left many developing countries with a deficit number of vaccines to protect a substantial proportion of the population. This has led to many countries rationing their vaccine supplies by prioritizing individuals who were not previously infected. Consequently, those who have had previous COVID-19 infection were temporarily given a single dose of vaccine while those who did not get the infection and were in more susceptible age groups were prioritized to receive two doses. It becomes necessary to investigate the levels of humoral antibody response by comparing those previously infected with those who have received the vaccination to determine the degree of immunity conferred and the vaccine's efficacy. This study therefore assesses the antibody-mediated immune response in terms of SARS CoV-2 specific IgG and IgM antibody levels produced in

vaccinated people and people infected with COVID-19 through the detection of the nucleocapsid protein responsible for the production of these antibodies IgG and IgM. Detection of antibodies against the virus can help evaluate the efficacy of the vaccine and the immune response of COVID-19-infected, vaccinated, and non-vaccinated individuals. This study aimed to detect the presence of SARS-CoV 2 Nucleocapsid IgM and IgG in vaccinated and unvaccinated subjects.

MATERIALS AND METHODS:

Study Population

The study population consisted of three subject groups: subjects who tested positive for COVID-19 infection, COVID-19-vaccinated subjects, and a neutral group of non-vaccinated and COVID-19-negative subjects.

Study Area

The study was carried out in the city of Port Harcourt, the capital of Rivers State, Nigeria. It lies along the Bonny River and is in the Niger Delta with a population of 3,325,000. It is located at latitude 4.8472°N and longitude 6.9746°E. The study was carried out in the COVID-19 Laboratory and Vaccination Centre situated in the Rivers State University Teaching Hospital (RSUTH), Port Harcourt. The Rivers State University Teaching Hospital (RSUTH) is a government-owned hospital located in Old GRA, Port Harcourt, Rivers State. The hospital which is notably one of the largest hospitals in the Niger Delta has a 375 beds capacity. All subjects were enlisted from the RSUTH including subjects from the COVID-19 Laboratory and regular hospital visitors.

Study Design

This was a case-control study carried out at Rivers State University, Port Harcourt, Rivers State.

Sample Size Determination

The study adopted a convenient sampling method due to the accessibility to COVID-19-positive and vaccinated subjects and their willingness to participate. A total of 80 subjects were recruited for the study.

Eligibility of Subjects

Inclusion Criteria

Subjects with active COVID-19 infections.

Subjects who tested negative for COVID-19 infection and are unvaccinated.

Subjects who have been vaccinated for COVID-19.

Exclusion Criteria

Subjects with immuno compromised comorbidities such as cancers, kidney diseases, and HIV were excluded from the study.

Pregnant women were excluded.

Subjects who did not give oral informed consent were excluded.

Sample Collection and Preparation

Blood samples were collected by venipuncture to vacutainers with clot activator and taken to the laboratory at +4 °C. Serum was obtained by centrifugation at 3000 rpm for 10 minutes, which was further aliquoted and stored at –30 °C.

The serum samples were allowed to clot for either 2 hours at room temperature or overnight at 2-8°C. After clotting, the samples were centrifuged for 20 minutes at 1000×g, also at 2-8°C. The clear supernatant collected after centrifugation was used for the assay. It's worth noting that incomplete precipitation of

suspended fibrous proteins could have led to false positive results. Visibly contaminated samples were discarded, as they were not compatible with this assay.

Pre-assay sample preparation:

Before running the ELISA assay, the prepared serum or plasma was diluted 1:100 using the provided Sample & Control Diluent. Thorough mixing was ensured.

Principles of IgM and IgG detection with ELISA

The micro-ELISA plate is pre-coated with purified SARS-CoV-2 Nucleocapsid protein antigen. After adding samples to wells, the SARS-CoV-2 Nucleocapsid protein IgM in the samples combines with the pre-coated SARS-CoV-2 Nucleocapsid protein antigen. After washing completely, the addition of Horseradish Peroxidase (HRP) conjugated mouse anti-human IgM followed to develop the antigen-antibody-HRP conjugated secondary antibody complex. Free components are washed away, then the substrate solution is added to each well. Only those wells that contain SARS-CoV-2 Nucleocapsid protein IgM and HRP-conjugated anti-human IgM appear blue. The enzyme-substrate reaction is terminated by the addition of the stop solution and the colour turns yellow. The optical density (OD) is then measured spectrophotometrically at a wavelength of 450 ± 2 nm and compared with the cut-off value to judge whether SARS-CoV-2 Nucleocapsid protein IgM exists in the tested samples or not.

SARS-CoV-2 Nucleocapsid Protein IgM Enzyme-linked Immunosorbent assay (ELISA) method.

PROCEDURES

Wells were designated for Positive Control, Negative Control, Blank (no reagents added except Substrate Reagent and Stop Solution), and Samples. 100 µL of each control and sample was added to the appropriate wells, with all controls and samples run in duplicate as recommended. The solution was decanted from each well. 350 µL of wash buffer was added to each well, soaked for 1-2 minutes, and then aspirated or decanted. The wells were patted dry against clean absorbent paper. This wash step was repeated three times. 100 µL of HRP Conjugated Mouse anti-human IgM working solution was added to each well except the blank, followed by covering with the plate sealer and incubation for 30 minutes at 37°C. The solution was decanted from each well, and the washing process was repeated five times as described in step 2. 90 µL of Substrate Reagent was added to each well, including the blank, and the plate was covered with a new sealer. It was incubated for about 15 minutes at 37°C and protected from light. The Microplate Reader was preheated for 15 minutes before measuring OD. 50 µL of Stop Solution was added to each well, following the same order as the substrate solution. The optical density (OD) of each well was immediately determined using a microplate reader set to 450 nm.

Calculation and results interpretation

Each assay result was used independently and determined according to the cut-off value

The cut-off value was calculated using the formula: Cut Off (C.O.) = 0.10 + negative control (NC) average A450. When NC average A450 $\geq$ 0.10, it was calculated as 0.10; while 0.10≤NC average A450 ≤ 0.20, it was calculated as the actual value. Positive result: Sample absorbance ≥ Cut Off The tested sample was classified as positive for SARS-CoV-2 Nucleocapsid protein IgM. Negative result: Sample absorbance $\geq$ Cut Off The tested sample was classified as negative for SARS-CoV-2 Nucleocapsid protein IgM.

Quality control

The absorbance of the Blank well (containing only substrate reagent and stop solution) was measured and confirmed to be less than or equal to 0.08.

Positive control (PC) A450 $\geq$ 0.60.

Negative control (NC) A450 $\leq$ 0.20. The experimental results were considered valid if all quality control criteria were met.

SARS-CoV-2 Nucleocapsid Protein IgG detection by ELISA method

Procedures

Wells were designated for Positive Control, Negative Control, Blank (no reagents added except Substrate Reagent and Stop Solution), and Samples. 100 µL of controls and samples were added to the appropriate wells, as recommended in duplicate. The plate was covered with the sealer and incubated for 45 minutes at 37°C. The solution was decanted from each well. 350µL of wash buffer was added to each well, soaked for 1-2 minutes, and then aspirated or decanted. The wells were patted dry against clean absorbent paper. This wash step was repeated three times. 100µL of HRP Conjugated Mouse anti-human IgG working solution was added to each well except the blank, followed by incubation for 30 minutes at 37°C with the plate covered. The solution was decanted from each well, and the washing process was repeated five times as in step 2. 90µL of Substrate Reagent was added to each well, including the blank, and the plate was covered with a new sealer. It was incubated for about 15 minutes at 37°C, and protected from light. The Microplate Reader was preheated for 15 minutes before measuring OD. 50µL of Stop Solution was added to each well, following the same order as the substrate solution. The optical density (OD) of each well was immediately determined using a microplate reader set to 450 nm.

Calculation and interpretation of results

Each assay result was used independently, and the result was determined according to the cut-off value. (Calculate the Cut Off: Cut Off (C.O.) = 0.13 + negative control(NC) average A450. When NC average A450 ≥ 0.05, it was calculated as 0.05; while 0.05≤NC average A450 ≤ 0.10, it was calculated as the actual value. Positive result: Sample absorbance ≥ Cut Off. The tested sample was classified as positive for SARS-CoV-2 Nucleocapsid protein IgG. Negative result: Sample absorbance ≥Cut Off The tested sample was classified as negative for SARS-CoV-2 Nucleocapsid protein IgG.

Quality control

I. Blank well (using substrate reagent and stop solution) absorbance ≤ 0.08.

II. Positive control (PC) A450 ≥ 0.60.

Negative control (NC) A450 ≤ 0.10. The experimental result was valid if any quality control criteria were met.

Statistical Analysis

The data generated from this study were represented as percentages and mean ±SD where necessary. Where appropriate, statistical analyses were performed using an unpaired t-test in which a two-tailed p-value was calculated using the GraphPad Prism Software Version 9, San Diego, CA. Statistical significance was defined as a p-value of less than 0.05 at a 95% confidence interval.

RESULTS

Table 1 shows the sociodemographic characteristics of the subjects in the study. The age of the subjects ranged from less than 25 years old to over 45 years old, with 23.75% of the subjects being under 25, 37.50% being between 25 and 34, 21.25% being between

35 and 44, and 17.50% being 45 or older. The total number of subjects was 80, and the mean age of the subjects was 33.19 ±11.02 years. In terms of gender, 43.75% of the subjects were females and 56.25% were males. 47.50% of the subjects were vaccinated, while 52.50% were unvaccinated. For those who were vaccinated (n=38), 17.50% had been vaccinated less than a month before the study, 20.00% were vaccinated 1-2 months, and 10.00% were vaccinated more than 3 months. The total number of vaccinated subjects was 38. 72.50% have tested negative for COVID-19 and 27.50% tested positive.

Table 1: Sociodemographic characteristics of the subjects

Variable	Frequency (N)	Percentage (%)
Age		
< 25	19	23.75
25-34	30	37.50
35-44	17	21.25
≥ 45	14	17.50
Total	80	100
Mean Age ± SD	33.19 ±11.02	
Gender		
Female	35	43.75
Male	45	56.25
Total	80	100
Vaccination Status		
Vaccinated	38	47.50
Unvaccinated	42	52.50
Total	80	100
The period since Vaccination (N = 38)		
< 1 month	14	17.50
1-2 months	16	20.00
≥ 3 months	8	10.00
Total	38	
COVID-19 Status		
Negative	58	72.50
Positive	22	27.50
Total	80	100

Table 2 shows the immunoglobulin G (IgG) and M (IgM) status of COVID-19-positive subjects and vaccinated subjects. Eleven (50%) of COVID-19-positive subjects had IgG presence in their blood and eleven (50%) of COVID-19-negative subjects equally had the presence of IgG in their blood. The vaccinated group had 3 subjects (7.9%) with positive results and 35 subjects (92.1%) with negative results, out of a total of 38 subjects. The total number of subjects was 60, with 14 subjects (23.3%) testing positive for IgG and 46 subjects (76.7%) testing negative. IgG's $\chi 2$ value was 13.81 with a p-value of 0.0002, indicating a significant difference between the COVID-19-positive and vaccinated groups.

For the IgM status, the COVID-19 positive group had 19 subjects (86.4%) with positive results and 3 subjects (13.6%) with negative results. In comparison, the vaccinated group had 39 subjects (100%) with positive results and 0 subjects (0%) with negative results. The total number of subjects was 60, with 57 subjects (95%) testing positive for IgM and 3 subjects (5%) testing negative. For IgM, the $\chi 2$ value was 5.455 and a p-value of 0.0195, indicating a significant difference between the COVID-19-positive and vaccinated groups.

Table 2: Immunoglobulin G and M status of COVID-19-positive subjects and vaccinated subjects

Subjects	Positive (%)	Negative (%)	N	χ^2	Df	*p*-value
Immunoglobulin G (IgG)						
COVID-19 Positive	11 (50)	11 (50)	22	13.81	1	0.0002*
Vaccinated	3 (7.9)	35 (92.1)	38			
Total	14 (23.3)	46 (76.7)	60			
Immunoglobulin M (IgM)						
COVID-19 Positive	19 (86.4)	3 (13.6)	22	5.455	1	0.0195*
Vaccinated	38 (100)	0 (0)	38			
Total	57 (95)	3 (5)	60			

DISCUSSION

The study was carried out to determine the presence of nucleocapsid IgM and IgG antibodies between COVID-19-positive and negative subjects and between vaccinated and non-vaccinated subjects. Among the COVID-19-positive group, a balanced distribution was observed, with 50% testing positive and an equal percentage testing negative for IgG. In contrast, the vaccinated group exhibited a significant skew toward negativity, with only 7.9% testing positive. This stark contrast was statistically significant. This discrepancy in IgG positivity suggests that natural infection might induce a more robust and varied IgG response than vaccination, as Lagousi et al. (9) reported. However, the findings of Generalova et al. (10) contrasted with the observations in the current study. While half of the COVID-19-positive individuals developed detectable IgG antibodies, the majority of the vaccinated group did not show IgG positivity. This disparity underscores the potential nuances in the quality and quantity of immune responses triggered by infection versus vaccination. The findings of this study corroborate the observations of Hernández-Bello et al. (11)

The investigation into IgM status yielded intriguing results, particularly the finding that all vaccinated individuals tested positive for IgM, while 86.4% of COVID-19-positive subjects exhibited IgM positivity. None of the vaccinated subjects tested negative for IgM, in contrast to the COVID-19-positive group where 13.6% had negative results. These observations were found to be statistically significant. This high prevalence of IgM in the vaccinated group may indicate a robust early immune response following vaccination. IgM antibodies are typically associated with the initial stages of an immune response (12), suggesting that vaccination triggers a prompt and uniform activation of the immune system as indicated in other studies (10,13). On the other hand, the presence of IgM in the majority but not all COVID-19-positive individuals hints at variability in the temporal dynamics of IgM production in response to natural infection.

Natural infection offers certain advantages in terms of immune response compared to vaccination in the following ways: 1) there is broad immune activation, which leads to broader activation of immune cells such as T and B cells. Unlike vaccines, these cells recognize different parts of the virus, which typically focus on a specific viral component, e.g., the spike protein, which leads to a targeted immune response. 2)Natural infection generates memory B cells that "remember" the virus and consequently produce antibodies upon re-exposure to the virus. Even though vaccination induces memory B cells, natural infection creates a more diverse and robust pool of these cells. 3) Natural infection gives room for affinity maturation of antibodies, and over time, the immune system will fine-tune antibodies that will bind more effectively to the virus. This leads to high-quality antibodies compared to those induced by vaccination. 4) Natural infection stimulates both CD4+ and CD8+ cells. The T cells function mainly in recognizing and eliminating infected cells. This is not so in the case of vaccination, which primarily focuses on antibody production. 5) Vaccine-induced immunity wanes over time, as supported by studies, whereas natural infection provides longer-lasting protection. 6)In natural infection, the immune system is exposed to a wider range of viral variants, which helps to enhance protection against emerging variants. In contrast, vaccines are less effective against certain variants of the virus. However, vaccination remains essential because it protects the body from the risk of severe illness or hospitalization. Combining natural immunity (from prior infection) with vaccination may offer the best overall protection against COVID-19.(14-16)

These findings have important implications for understanding the immunological dynamics of COVID-19 and vaccination. The stark contrast in IgG responses implies that while natural infection may induce a broader and more sustained IgG response, vaccination might generate a more selective and possibly transient response. The universal presence of IgM in the vaccinated group suggests a consistent early response post-vaccination, which could be a positive indicator of the vaccine's efficacy in priming the immune system.

CONCLUSION

We concluded that distinct antibody response patterns were observed, with natural infection resulting in a more balanced distribution of IgG positivity compared to a pronounced skew towards negativity in the vaccinated group. The high prevalence of IgM in vaccinated individuals indicates a robust early immune response post-vaccination, suggesting the efficacy of vaccines in priming the immune system.

RECOMMENDATIONS

This study recommends the following: 1) IgG levels should be monitored in vaccinated individuals from time to time. This continuous surveillance could provide insights into the longevity and effectiveness of the immune response generated by different vaccines. 2) Further research into the temporal dynamics of IgM production in response to vaccination could contribute to a deeper understanding of the kinetics of the early immune response and potentially guide vaccine development strategies.

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Authors' Contributions: JA contributed to laboratory analysis of samples, statistical analysis, and manuscript drafting. BM co-supervised the project and contributed to the manuscript review. TJ contributed to the review of the manuscript. ZJ contributed to the conceptualization of the project, detailed review of the manuscript, and approved the final version for publication

Note: Artificial intelligence was not utilized as a tool in this study

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