Acta Scientific MICROBIOLOGY (ISSN: 2581-3226)

Volume 5 Issue 8 August 2022

Research Article

Estimation of COVID-19 Antibody (IgG) Titer Among Fully Vaccinated, Partially Vaccinated and Non-vaccinated Individuals in a Tertiary Care Hospital in Eastern India

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DOI: 10.31080/ASMI.2022.05.1117

Received: July 04, 2022

Published: July 19, 2022

Abstract

Coronavirus disease 2019 (COVID19) is predominantly a respiratory tract infection sometimes presenting as a viral fever and sometimes having more severe presentation involving other systems also. This is a condition caused by a novel coronavirus named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2; called 2019-nCoV). It was first identified when an outbreak of respiratory illness transgressed throughout the Wuhan City, Hubei Province, China. Similar to many other infectious diseases, not only the humoral immunity but also the T-cell mediated immunity in the form of acquired immunity, is important in the elimination of pathogens, including SARS-CoV-2. Viral surface glycoproteins like the spike glycoprotein and the nucleocapsid protein plays important role in the generation of humoral immune responses to SARS-CoV-2, as they stimulate the production of antibodies. The current study was conducted to evaluate the immune response of the people by the development of virus specific IgG antibodies. 78 subjects were enrolled in the study and estimation of IgG antibody of COVID-19 among fully vaccinated, partially vaccinated, triple vaccinated and non-vaccinated individuals was done by ELISA. The inclusion criteria was the patients seeking admission in the hospital, undergoing RT-PCR test and further work up; and the exclusion criteria was the patients undergoing RT-PCR test for travel or other purposes, not seeking hospital admission. Our findings were that after COVID-19 vaccination sero-positivity to nucleocapsid and spike protein antigens developed in 92% of the individuals participating in the study. It was also observed that 26% vaccinated people developed mildly positive IgG antibody titer (1.1 - 3.0 U/ml); 56.5% vaccinated people developed moderately positive (3.0 - 10.0 U/ml) and 17% vaccinated people developed strongly positive (> 10.0 U/ml) COVID 19 IgG antibody titer. The vaccines are an efficacious tool to combat the deadly disease where they have reduced the chance of hospitalization and complications caused by the disease.

Keywords: SARS-CoV-2; IgG antibody; Spike Glycoprotein; Nucleocapsid Protein; ELISA

Introduction

The novel coronavirus SARS-CoV-2 is a newly emerging virus. The recent disease outbreak caused by the new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a global health emergency. Though the large cluster of infection first appeared in Wuhan, China in December 2019, initially the virus first emerged on a small scale in November 2019. It was initially thought that one of Wuhan, China's open-air "wet markets" was the place where human was first infected by SARS-CoV-2 [1]. In India the first cases of COVID-19 were reported on 30 January 2020 in the state of Kerala. Three Indian medical students, after returning from Wuhan, China, were infected with COVID- 19. On 23 March 2022, there have been 472,816,657 confirmed cases of COVID-19, including 6,099,380 deaths, reported to WHO. As of 17 March 2022, a total of 10,925,055,390 vaccine doses have been administered. Many new transmissible coronavirus (CoV) strains have emerged and the current COVID-19 pandemic is the result of their vast transgression throughout the world. The devastating effect of the current pandemic emphasizes the urgency in the field of research and innovation. Currently the world is in need of large-scale prophylactic and therapeutic intervention and promising steps have already been executed from different parts of the world [2,3].

After Edward Jenner's first demonstration of the beneficial effect of vaccine against smallpox in 1796, vaccines have gradually emerged as an important preventive measure in public health for decades to help prevent many diseases like measles, mumps, polio, rubella, tetanus, rabies, hepatitis-B and yellow fever. After the Emergency Use Approval (EUA), vaccination with two candidate vaccines namely Covishield™ and Covaxin™ in India has been started from January 16, 2021. Covishield™ (ChAdOx1-nCOV or AZD1222, acquired from Oxford University and AstraZeneca, manufactured by Serum Institute of India, Pune) is a recombinant replication-deficient chimpanzee adenovirus-vectored vaccine encoding SARS-CoV-2 spike antigen produced in genetically modified human embryonic kidney (HEL) 293 cells. CovaxinTM (BBV-152,) manufactured by Bharat Biotech, Hyderabad in collaboration with Indian Council of Medical Research [ICMR], India, is a ß-propiolactone inactivated whole virion vaccine having all structural SARS-CoV-2 antigens adjuvanted by imidazoquinoline Toll-Like Receptor 7/8 (TLR 7/8) agonist to boost cell-mediated immunity [4-6].

In infected patients, the immune response followed by the antibody production and the clinical value of antibody testing has not been fully delineated and antibody response still remains largely unknown. It has been found that the antibody levels progressively decrease following SARS-CoV-2 infection, but the immune memory persists for months. Thus, individuals who have been naturally infected by SARS-CoV-2 are expected to develop a more rapid and sustained response after COVID-19 vaccination than uninfected individuals. The vaccine was well tolerated by most of the vaccinated and partially vaccinated individual, with no significant difference in the frequency of vaccine-associated side effects. However, local pain, mild fever and body ache was more common in previously infected subjects [5]. Antibody testing against SARS-CoV-2 is helpful in many respects. It can predict the prevalence of COVID-19 in the community and promptly diagnose asymptomatic patients. Antibody testing also helps in evaluation of patients with COVID-19 after treatment. In the vaccination era of the COVID-19 pandemic, antibody testing is also very important in monitoring the presence and persistence of antibodies against SARS-CoV-2 after vaccination. The antibody test is broadly classified into two groups, like binding antibody assays and neutralizing antibody assays [6,7]. Binding antibody assays can detect IgG, IgM or total antibodies that has been developed against the spike protein receptor-binding domain (RBD) or the antibodies against partial spike protein (S1 subunit, S2 subunit), or viral nucleocapsid protein (N). To identify the presence of functional antibodies to prevent SARS-CoV-2 infection, the neutralizing antibody assay is required. It has been found that in plasma of SARS CoV2 patients, the SARS CoV 2 IgG binding antibody assay and neutralizing antibody assay, both were highly correlated [7-9]. Aim of the current study was to estimate the antibody (IgG) of COVID 19 in vaccinated, partially vaccinated, triple vaccinated and non-vaccinated individuals in a tertiary care hospital.

Material and Methods

The present study was a hospital based observational study, carried out over a period six months from December, 2021 to May, 2022. A total of 78 subjects from the Kali Pradip Chaudhuri Medical College and Hospital, who were seeking admission in the hospital, undergoing RT-PCR test and further work up, were enrolled for this study. The subjects undergoing RT-PCR test for travel or other purposes, not seeking hospital admission, were not included in the study. The demographic profile like the age, gender and vaccination history of the patients were recorded (non-vaccinated, single dose vaccinated, fully vaccinated and booster dose taken). The patients were given either Covishield™ or Covaxin™. Out of these, 55

participants in the study were admitted for surgery and medical purposes. In this study triple vaccinated health care workers and PG students in the Microbiology department were also involved.

About 5 ml of venous blood was collected from each patient aseptically in a sterile vial and the blood was allowed to clot for separation of serum. In the Microbiology laboratory the serum was completely separated by centrifuging the sample vials in a centrifuge machine at 3000 revolutions per minute (rpm) for 5 min. The separated serums were then transferred to sterile vials, labeled properly with serial numbers and stored at −80°C till the assay was done. The antibody assay was carried out using the DIA.PRO Diagnostic Bioprobes Srl kit for COVID 19 IgG. Statistical softwares GraphPad Prism (GraphPad Inc, San Diego, USA) and microsoft office Excel 2007 (Microsoft Corporation, USA) were used to prepare graphs and analyse the data.

Principle of the test

Microplates are coated with recombinant nucleocapsid and spike antigens specific to COVID 19. The direct addition of the samples and the reagents of the assay are followed up visually by a step wise change of colours. A cut off value let optical densities to be interpreted into COVID 19 IgG negative and positive results. For internal quality control a check is carried out on the controls. Any time the kit is used in order to verify whether their OD450nm/620nm values are as expected and for that, reported in the table below.

Check	Requirements
Blank well	< 0.100 OD450 nm value
Negative control (NC)	< 0.150 mean OD450 nm value after blanking
Positive Control	> 0.500 OD450 nm value

Table a

The Tests Results are calculated on by means of a cut off value determined with the following formula on the mean od450nm/620-6630n, value of the negative (NC).

NC+ 0.250 cut-off (Co)

Results

In the study population, 48 were males (62%) and 30 were females (38%) (Figure 1). Among the vaccinated patients, 17 (21.7%) were in the age group of 15 - 30 yrs., 17 (21.7%) were in the age group of 30 - 45 yrs., 26 (33%) were in the age group of 45 - 60 yrs., 9 (11.5%) were in the age group of 60 - 75 yrs. and 9 (11.5%) were in the age group of 75 - 90 yrs (Figure 2).

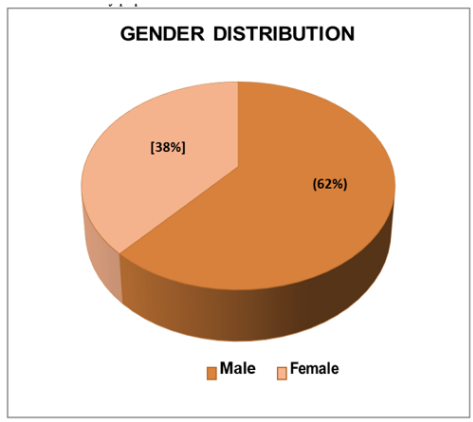

Figure 1: Gender distribution of the study population.

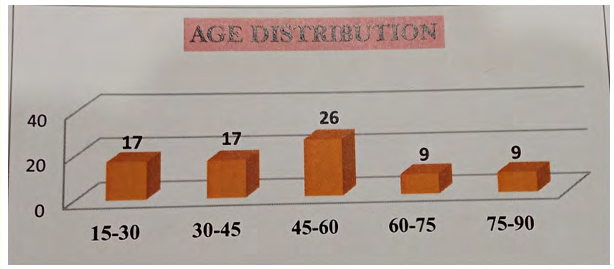

Figure 2: Age distribution of the vaccinated patients.

Regarding the vaccination status of the study population, 74% of the people were fully vaccinated, 17% were triple vaccinated, 5% were partially vaccinated and 4% of the people were not vaccinated (Figure 3).

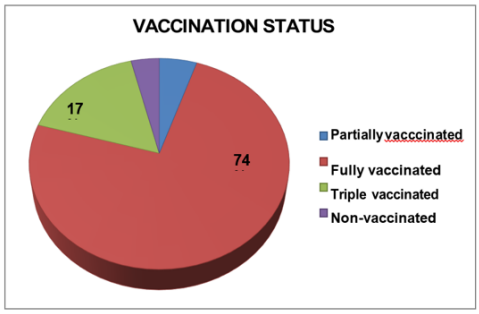

Figure 3: Vaccination status of the study population.

In the follow up of COVID 19 antibody titer estimation, the following interpretation applies.

S/Co	Interpretation
<0.9	Negative
0.9-1.1	Gray zone
>1.1	Positive

Table b

It indicates that a concentration of <0.90 U/ml considered negative and ≥1.1 U/ml considered positive. In the study population out of 78 people, 75 were vaccinated either fully or partially and non-vaccinated people were three. One non-vaccinated person was negative for COVID 19 IgG antibody titer. Other two non-vaccinated persons were positive for COVID 19 IgG antibody titer and they were ICU and ward admitted patients. Among the 75 fully or partially vaccinated persons, 69 persons were positive for COVID 19 IgG antibody titer and 6 were negative. Therefore, sero-positivity was 92% after full or partial vaccination. After vaccination, if the 69 sero-positive persons (92%) are divided into 3 groups of Mildly positive, Moderately positive and Strongly positive, it is found that 18 persons (26%) are Mildly positive; 39 persons (56.5%) are Moderately positive and 12 persons (17%) are Strongly positive for COVID 19 IgG antibody titer (Table 1).

Mildly Positive (1.1 - 3.0 U/ml) response	Moderately Positive (3.0 - 10.0 U/ml) response	Strongly Positive (> 10.0 U/ml) response	Total
18 vaccinated People (26%)	39 vaccinated People (56.5%)	12 vaccinated People (17%)	69 vaccinated people
2 doses of vaccine - 15 3 doses of vaccine - 03	1 dose of vaccine - 02 2 doses of vaccine - 23 3 doses of vaccine - 14	2 doses of vaccine - 07 3 doses of vaccine - 05	1 dose of vaccine - 02 people 2 doses of vaccine - 45 people 3 doses of vaccine - 22 people

Table 1: Distribution of Mildly positive, Moderately positive and Strongly positive IgG antibody titer among vaccinated people.

That roughly indicates that after vaccination, 26% of people show mild IgG antibody titer (1.1 - 3.0 U/ml).

56.5% of people show moderate IgG antibody titer (3.0 - 10.0 U/ml) and 17% people show strong IgG antibody response (> 10.0 U/ml).

Discussion

In the study population, 48 were males and 30 were females (Figure 1). Most of them had completed the second dose of COVID 19 vaccine with 22 participants had taken the booster dose (Tripple vaccinated), 4 had taken the first dose of COVID vaccine and 3 were non-vaccinated.

From the above table we can see that out of 75 vaccinated individuals, 69 people developed adequate COVID 19 IgG antibody titer. Therefore, sero-positivity was 92% after full or partial vaccination. Among the 6 vaccinated people who did not develop adequate IgG response, 2 persons had taken only one dose of vaccine and incomplete vaccination might be the cause of inadequate IgG response. However, 2 incompletely vaccinated (only 1st dose) patients developed moderate antibody titer (3.0 - 10.0 U/ml) which indicate even one dose might give some amount of protection. 4 patients did not show a positive result following 2 doses of the vaccine which could be due to the long time elapsed between the vaccination doses or poor sample collected for estimation of IgG titer or due to the associated co-morbidities. Of the 2 non-vaccinated patients who showed the mild elevation of IgG to COVID 19 titre (1.47 U/ml and 1.44 U/ml) which could be due to a previous episode of infection in the patients. Most of the fully vaccinated patients (2 doses of vaccine) and triple vaccinated (3 doses of vaccine) patients developed moderate antibody titer (3.0 - 10.0 U/ml). In a study conducted by Awadhesh Singh., *et al*. presence of co- morbidity, gender, type of vaccine, past history of COVID19 infection, might act as independent predictors of antibody titer [18].

To determine the prognosis of this disease, virus-specific immunity to SARS-CoV-2, plays an important role [10]. Both humoral and T cell- mediated adaptive immunity plays a critical role in the elimination of pathogens, including SARS-CoV-2. Cytotoxic lymphocytes mainly the cytotoxic CD8 + T cells can eliminate virus infected cells, and when the humoral immune response produces

specific antibodies against SARS-CoV-2, it has the potential not only to neutralize this virus, but also to help the cytotoxic T cells eliminate virus- infected cells to control disease progression [10-12]. Therefore, after vaccination, seropositivity to the range of 92% indicate that vaccination can be an effective measure in preventing infection. Ortega N., *et al*. have mentioned that antibody titer might be a good biomarkers for the protective efficacy of antibodies and successful humoral immune responses after SARS-CoV-2 exposure [12]. In the current study, 26% vaccinated people developed mildly positive IgG antibody titer (1.1 - 3.0 U/ml); 56.5% vaccinated people developed moderately positive (3.0 - 10.0 U/ml) and 17% vaccinated people developed strongly positive (> 10.0 U/ml) COVID 19 IgG antibody titer.

Reports on cellular immunity to SARS-CoV-2 have demonstrated that the proportion of many cells like CD38+, HLA-DR+ T cells (both CD4+ and CD8+) increases during the first week to 10 days of COVID-19 symptomatic stage and gradually begins to return to baseline around day 20. It has also been found that the SARS-CoV-2- specific T cells express perforin 1 and granzymes upon *in vitro* restimulation with viral antigens [13-15]. Therefore, although accumulating evidence suggests that IgG response is a correlate of disease protection, but cellular immunity cannot be ignored as it has also been suggested to play an important role in protecting against SARS-CoV-2. One limitation of the current study is that we are not performing cellular immunity testing and neutralizing antibody testing. Oxford University (Oxford, UK) and AstraZeneca, Pfizer and BioNtech, Moderna and the National Institutes of Health, Bharat Biotech, Hyderabad in collaboration with Indian Council of Medical Research [ICMR], India, have developed a series of COVID-19 vaccines. There are many other vaccines which are in the pipeline. However, more studies are required to learn adequately regarding the coronavirus immunity in general and SARS-CoV-2 immunity in particular, including the protective immunity induced by vaccines and the maintenance of immunity against this virus [15-17].

Conclusion

To conclude the current study, it reveals that after completion of COVID 19 vaccination, seropositivity to nucleocapsid and spike protein antigens is observed in 92% ofthe individuals participating in the study. In the current study, 26% vaccinated people developed mildly positive IgG antibody titer (1.1 - 3.0 U/ml); 56.5% vaccinated people developed moderately positive (3.0 - 10.0 U/ml) and 17% vaccinated people developed strongly positive (> 10.0 U/ml) COVID 19 IgG antibody titer. The vaccines are an efficacious tool to combat the deadly disease where they have reduced the chances of hospitalization and complications caused by the disease. However, further studies are required to learn more accurately regarding the coronavirus immunity, specially the protective immunity induced by vaccines and the maintenance of immunity against this virus.

Acknowledgements

The authors would like to thank all the faculty members and other staffs of the Department of Microbiology of KPC Medical College and Hospital under West Bengal University of Health Science for helping them in writing the article.

Conflict of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Bibliography

1. Abdulrahman A., *et al*. "Association between RT-PCR Ct values and COVID-19 new daily cases: a multicenter cross-sectional Study". *medRxiv* (2020).

2. Yadav PD., *et al*. "Isolation and characterization of VUI-202012/01, a SARS-CoV-2 variant in travellers from the United Kingdom to India". *Journal of Travel Medicine* 88 (2021): 65.

3. Loconsole D., *et al*. "Genome sequence of aSARS-CoV-2 VUI 202012/01 strain identified from a patient returning from London, England, to the Apulia Region of Italy". *Microbiology Resource Announcements* 10.4 (2021): e01487-e1520.

4. van Oosterhout C., *et al*. "COVID-19 evolution during the pandemic— implications of new SARS-CoV-2 variants on disease control and public health policies". *Virulence* 12.1 (2021): 507-508.

5. Tang JW., *et al*. "Introduction of the South African SARS- CoV-2 variant 501Y.V2 into the UK". *Journal of Infection* (2021): S0163-4453 (21)00030-X.

6. Baglivo M., *et al*. "Natural small molecules as inhibitors of coronavirus lipid- dependent attachment to host cells: a possible strategy for reducing SARS-COV-2 infectivity?" *Acta Biomedicine* 91.1 (2020): 161-164.

7. Wang Q., *et al*. "A unique protease cleavage site predicted in the spike protein of the novel pneumonia coronavirus (2019-nCoV) potentially related to viral transmissibility". *Virologica Sinica* 5 (2020): 1-3.

8. Coutard B., *et al*. "The spike glycoprotein of the new coronavirus 2019- nCoV contains a furin like cleavage site absent in CoV of the same clade". *Antiviral Research* 176 (2020): 5.

9. Yu H., *et al*. "Genomic analysis of a 2019-nCoV strain in the first COVID-19 patient found in Hangzhou, Zhejiang, China". *Zhonghua yu Fang yi xue za zhi* 54 (2020): E026.

10. Zhang T., *et al*. "Probable pangolin origin of 2019-nCoV Associated with outbreak of COVID-19". *Current Biology* 20 (2020): 299.

11. Vankadari N and Wilce JA. "Emerging COVID-19 coronavirus: glycan shield and structure prediction of spike glycoprotein and its interaction with human CD26". *Emerging Microbes and Infections* 9.1 (2020): 601-604.

12. Ortega N., *et al*. "Seven-month kinetics of SARS-CoV-2 antibodies and role of pre-existing antibodies to human coronaviruses". *Nature Communication* 12 (2021): 4740.

13. Dugas M., *et al*. "Less severe course of COVID-19 is associated with elevated levels of antibodies against seasonal human coronaviruses OC43 and HKU1 (HCoV OC43, HCoV HKU1)". *The International Journal of Infectious Diseases* 105 (2020): 304-306.

14. Leisman DE., *et al*. "Cytokine elevation in severe and critical COVID-19: a rapid systematic review, meta-analysis, and comparison with other inflammatory syndromes". *Lancet Respiratory Medicine* 28 (2020): 1233-1244.

15. Pyrc K., *et al*. "Mosaic structure of human coronavirus NL63, one thousand years of evolution". *Journal of Molecular Biology* 364 (2006): 964-973.

16. Sagar M., *et al*. "Recent endemic coronavirus infection is associated with less-severe COVID-19". World Health Organization. 2020. Clinical management of COVID-19: interim guidance, 27 May (2020).

17. Sokal A., *et al*. "Maturation and persistence of the anti-SARS-CoV-2 memory B cell response". *Cell* 184 (2021): 1201-1213.

18. Awadhesh Singh., *et al*. "Is ethnicity linked to incidence or outcomes of covid-19?" *BMJ* 369 (2020): m1548.