Original Article

DOI: 10.7860/JCDR/2022/56189.16758

Neutralising Antibodies in Healthcare Workers after Two Doses of Covishield Vaccine at Three Months and Six Months: A Single-centre Observational Study

Microbiology Section

J NAVEENA[1], MP NANDHINI[2], K KAVITHA[3], CN MANJUNATH[4], AMULYA CHERUKUMUDI[5], N PRAPULLA[6]

ABSTRACT

Introduction: The emergence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) as a pandemic has put the global population at risk for its infection. It has also led to an accelerated effort to develop vaccines that can mitigate progression to severe infections at a minimum. The ambiguity about existence of antibodies in the human serum poses problem in formulating public health policies like suitable interval between doses of vaccines, appropriate time for vaccinating population, post natural infection, necessity of booster doses along with single dose.

Aim: To estimate neutralising antibody level following Covishield vaccination of Healthcare Workers (HCWs) after three months and six months, respectively.

Materials and Methods: This was a prospective observational study performed in Sri Jayadeva Institute of Cardiovascular Sciences and Research, Bengaluru, Karnataka, India after Institutional Ethics Committee (IEC) approval from January 2021 to February 2022. The study was conducted in 304 HCWs in the institute who had received two doses of Recombinant ChAdOx1 nCoV-19 Coronavirus Vaccine (Covishield). Forty one HCWs who were naturally infected with SARS-CoV-2 either before or after vaccination were also included. These participants were then subjected to IgG neutralising antibody titre estimation at three months and six months, postvaccination. The data was entered in MS excel spreadsheet 2016 and statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 21.0.

Results: The study included 304 eligible HCWs. Majority of the participants belonged to the age group of 31-40 years (35.9%). Majority of the study participants were females (51%). Of the 304 participants, 263 were uninfected and 41 participants had been infected before and after vaccination. At the six month follow-up, it was observed that all except one HCW had seroconverted with majority of the participants showing more than 60% antibody level. Participants in the age group of 31-40 years showed the highest level and this observation was found to be statistically significant.

Conclusion: Neutralising antibody response in HCWs is a key indicator of the efficacy of the vaccination program for Coronavirus Disease-2019 (COVID-19) in India.

Keywords: Coronavirus disease 2019, Immunoglobulin G, Recombinant ChAdOx1 nCoV-19 coronavirus vaccine

INTRODUCTION

A novel SARS-CoV-2 was detected in Wuhan, Hubei province in China in December 2019 which caused COVID-19. Following rapid and widespread transmission of this virus across the globe and absence of pre-existing immunity, World Health Organisation (WHO) declared COVID-19 a pandemic on 11th March 2020. HCWs who are the first line of defense, belong to high-risk population and can transmit the infection to patients and to other staff. Limited supply of vaccines led to prioritisation of vaccination of HCWs [1].

The pathology of COVID-19 involves immunological responses of the human body against the SARS CoV-2 virus which largely determines the disease evolution. Neutralising antibodies are those which block viral infection [2]. Development of neutralising antibodies especially against the receptor binding domain on the large trimeric glycoprotein, spike (S) protein confers immunity against reinfection by inhibiting the virus host interaction [3,4]. Animal and human studies have shown that Anti Receptor Binding Domain (RBD) IgG has strong correlation with virus neutralisation as it is these antibodies that counter the interaction of RBD with Angiotensin Converting Enzyme 2 (ACE2) receptors [5,6]. The kinetics of antibody development indicates mean to median time for seroconversion for IgM to be 4-14 days post-symptom onset, for IgA 4-24 days post- symptom onset and IgG 12-15 days post-symptom onset [4]. However inappropriate antibody response leading to cytokine storm, excessive neutrophil and macrophage activation causes lung damage and leads to disease progression to severe form with high morbidity and mortality [2]. Protective IgG antibody persistence has been studied in some patients till five months time and is believed to be instrumental in reducing the odds ratio for reinfection and attenuating disease in case of naïve patients [7]. However robust data in this regard is still lacking.

The uncertainty around persistence of protective antibodies in the human serum poses problem in formulating key public health policies like appropriate interval between doses of vaccines, appropriate time for vaccinating population post natural infection, requirement of booster dose, single dose for naturally infected population etc. There is a gap in the knowledge of disease dynamics in the Indian population hindering appropriate public health decision-making. The objective of present study was to estimate the neutralising antibody level following vaccination of HCWs after three months and six months, respectively.

MATERIALS AND METHODS

This was a prospective observational study performed from January 2021 to February 2022 in Sri Jayadeva Institute of Cardiovascular Sciences and Research, Bengaluru, Karnataka,

India after IEC approval was obtained (IEC Approval Ref No. SJICR/EC/2021/051).

Inclusion criteria: The study included 304 HCWs in the institute who received two doses of Recombinant ChAdOx1 nCoV-19 Coronavirus Vaccine (Covishield).

Exclusion criteria: Those that received one dose of vaccination elsewhere.

The sample size was selected using convenience sampling and participants were included after signing an informed consent. These participants were then subjected to IgG neutralising antibody titre estimation at three months and six months, postvaccination. Development of COVID-19 infection before or after vaccination was documented.

Study Procedure

The test was performed on 2 mL of serum sample drawn from the study group after informed consent was obtained. Basic demographic details and COVID-19 infection status along with the antibody level at three months and six months was recorded in a semi-structured proforma. The serological test used to detect COVID-19 neutralising antibodies of all classes was "COVID-19 Neutralising antibody Microlisa" from J Mitra and Co. Pvt. Ltd., New Delhi, India, which is an enzyme immunoassay based on the principle of blocking Enzyme Linked Immunosorbent Assay (ELSIA). The test is designed for in-vitro semi-quantitative detection of neutralising antibodies developed against SARS-CoV-2 in human plasma/serum that prevent the interaction between receptor binding domain viral spike glycoprotein RBD and cell surface receptor ACE2. The kit instruction was followed for testing. The absorbance of the sample at the end of the procedure is inversely proportional to the titre of the neutralising antibodies present. Being a semiquantitative test, more than 30% inhibition is taken as positive and indicated presence of COVID-19 neutralising antibodies in the sample tested.

STATISTICAL ANALYSIS

The data was entered in MS excel spreadsheet 2016 and statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 21.0. The association between the age groups and antibody level was done with Chi-square test and p-value <0.05 was considered as significant.

RESULTS

The study included 304 eligible HCWs. Majority of the participants belonged to the age group of 31-40 years (n- 109, 35.9%) [Table/Fig-1]. Majority of the study participants were females (n-155, 51%) [Table/Fig-2].

Age (years)	Frequency	Percent
≤20	46	15.1
21-30	42	13.8
31-40	109	35.9
41-50	53	17.4
51-60	43	14.2
>60	11	3.6
Total	304	100.0

[Table/Fig-1]: Age of participants.

Sex	Frequency	Percent
Female	155	51.0
Male	149	49.0
Total	304	100.0

[Table/Fig-2]: Sex of participants.

Of the 304 participants, 263 were uninfected and 41 participants had been infected before and after vaccination [Table/Fig-3].

As there was difficulty in procuring the test kit, convenience sampling was included. At the three months follow-up, a total of 138 participants were evaluated and the antibody level was more than 30% in 82.1% (n- 96) of COVID-19 negative patients, while it was more than 30% in 85.7% (n-18) of those that were COVID-19 positive. At the six months follow-up, 304 participants were evaluated. The antibody level was more than 30% in 99.6% of uninfected HCWs, while it was more than 30% in 100% of those that were COVID-19 positive [Table/Fig-4].

COVID-19	Frequency	Percent
Positive	41	13.5
Negative	263	86.5
Total	304	100.0

[Table/Fig-3]: COVID-19 positivity in study participants.

Three months follow-up	COVID-19				Total	
	Negative		Positive			
	Count	%	Count	%	Count	%
<30%	21	17.9	3	14.3	24	17.4
≥30%	96	82.1	18	85.7	114	82.6
Total	117	100.0	21	100.0	138	100.0
p-value=0.482 (Chi-square test)						
Six months follow-up	**COVID-19**				**Total**	
	Negative		Positive			
	Count	%	Count	%	Count	%
<30%	1	0.4	0	0	1	0.3
≥30%	262	99.6	41	100.0	303	99.7
Total	263	100.0	41	100.0	304	100.0
p-value=0.865 (Chi-square test)						

[Table/Fig-4]: Neutralising antibodies at three and six months follow-up.

At the three month follow-up, when a comparison of age with the antibody level was made, it was observed that majority of the people with more than 30% antibody level belonged to the age group of 31-40 years who were uninfected. However, the association between the age groups and antibody level was not statistically significant.

At the six month follow-up, it was observed that all except one HCW had seroconverted with majority of the participants showing more than 30% antibody level. The participants in the age group of 31-40 years showed the highest level and this observation was found to be statistically significant [Table/Fig-5].

Age (years)	6 months				Total	
	≤30%		>30%			
	Count	%	Count	%	Count	%
≤20	0	0	46	18.3	46	15.1
21-30	0	0	42	21.4	42	13.8
31-40	1	100	108	77.2	109	35.9
41-50	0	0	53	41.4	53	17.4
51-60	0	0	43	32.7	43	14.2
>60	0	0	11	3.2	11	3.6
Total	1	0.32	303	99.68	304	100.0

[Table/Fig-5]: Neutralising antibody level across the age groups at six months.
p-value=0.037 (Chi-square test)

DISCUSSION

Present study was conducted to observe the persistence of neutralising antibodies after two doses of Covishield vaccine

in infected and unifected HCWs in the hospital. Present study findings showed that antibody response in naturally infected HCWs was higher when compared to infection naïve vaccinated HCW. It was in accordance with findings of the study by Anichini G et al., who demonstrated that neutralising antibody level in previously infected persons spiked higher after one dose when compared to uninfected vaccinated individuals after two doses although our numbers are not statistically significant [8]. The study by Sasikala M et al., showed that the memory B and T cells as well as the neutralising antibody titres were significantly higher in previously infected individuals after a single dose of vector based vaccine [9]. Morales-Núñez JJ et al., studied the effect of Pfizer-BioNTech (BNT162b2) also showed similar findings and suggested the use of single dose vaccination for naturally infected persons be considered in resource limited countries [10].

Covishield (Recombinant ChAdOx1 nCoV- 19) is a recombinant vaccine with replication-deficient chimpanzee adenovirus vector encoding the SARS-CoV-2 Spike (S) glycoprotein. The efficacy of this vaccine as measured by neutralising antibodies in present study indicates that good humoral antibody response was noted and maintained in the participants at six months of observation and surpasses the levels noted in vaccine efficacy trials conducted prior to its emergency use approval [11]. In infection naïve HCW, all except one participant had seroconverted at the end of six months and reiterates the efficacy of Covishield in Indian population.

The trajectory of decline in neutralising antibodies have demonstrated persistence till eight months postvaccination and present study findings show that even at six months humoral response persisted. Voysey M, et al., demonstrated that a longer prime boost interval for ChAdOx1 nCoV-19 (AZD1222) vaccine of four to twelve weeks produced strong humoral response that was consistent with similar vaccine studies on Malaria, Influenza and Ebola virus disease [11]. In present study, where participants had received two doses of covishield vaccine at interval of four weeks, 82.89% of participants with and without COVID-19 infection had neutralising antibody level above 60% at six months. One explanation for this sustained and strong humoral response could be that present study participants were HCWs in a busy cardiac super specialty referral hospital in a resource limited country and may have been exposed to multiple subclinical infections which may have boosted the response postvaccination. This may also possibly explain why COVID-19 pandemic may normalise as an endemic infection in the Indian subcontinent without huge waves of periodic surge in infections from time to time as noted in the developed world. Desai A et al., showed that a single dose of ChAdOx1 nCoV-19 vaccine gave 86% additional protection against mortality and reduced ICU stay to mean of 4.47±2.3 days for vaccinated patients compared to 6.29±2.19 days for non vaccinated patients and lesser requirement for mechanical ventilation [12]. Present study also correlates with the Singh AK et al., [13] which highlighted the higher seropositivity and antispike antibodies Geometric Mean Titre (GMT) of Covishield against Covaxin where 95% of uninfected recipients and COVID-19 recovered recipients of Covishield vaccine showed production of antispike antibody production after 3 weeks of receiving two doses [13]. In contrast to present findings, Choudhary HR et al., showed two fold decline in vaccine induced antispike IgG antibodies in Covishield recipients and four fold decline in Covaxin recipients at six months [14]. Singh AK et al., also has followed-up covishield and covaxin recipients for six months and showed 5.6 fold decline in GMT of antispike antibodies in both vaccine groups [15] which contrasts present study findings.

More analytical studies are needed to understand the natural decay of these antibodies both in general population and in HCWs to determine the ideal time for a booster dose and identify the groups which may require it. Understanding the dynamics of immune response both humoral and cell mediated will help us in formulating appropriate public health measures and present observation study serves as baseline in the Indian population in this direction. We intend to follow-up the neutralising antibody levels in our participants in the future as continuation of the study.

Limitation(s)

This was an observational study and the sample size was variable for three month and six month follow-up as we had taken convenience sampling. The kit used to estimate neutralising antibody levels was semiquantitative and provides levels of neutralising antibodies in terms of percentage of inhibition and not absolute values. The study did not perform tests to correlate the neutralising antibody levels with the antibodies against spike protein and memory B and T-cells.

CONCLUSION(S)

Neutralising antibodies can persist well upto six months and probably beyond, of receiving two doses of Covishield vaccine. Stronger antibody response seen in COVID-19 infected recipients is encouraging to implement single dose vaccination in resource limited countries in order to optimise limited vaccine supplies.

REFERENCES

[1] Cohort study to measure COVID-19 vaccine effectiveness among health workers in the WHO European Region. Available from: https://www.who.int/publications/i/item/WHO-EURO-2021-2141-41896-57484. Accessed, 2021.

[2] Post N, Eddy D, Huntley C, van Schalkwyk MCI, Shrotri M, Leeman D, et al. Antibody response to SARS-CoV-2 infection in humans: A systematic review. Mantis NJ, editor. PLoS One. 2020;15(12):e0244126.

[3] Isho B, Abe KT, Zuo M, Jamal AJ, Rathod B, Wang JH, et al. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. Science Immunology [Internet]. 2020;5(52). Available from: https://immunology.sciencemag.org/content/5/52/eabe5511/tab-pdf. [cited 2021 Mar 22].

[4] Tea F, Ospina Stella A, Aggarwal A, Ross Darley D, Pilli D, Vitale D, et al. SARS-CoV-2 neutralizing antibodies: Longevity, breadth, and evasion by emerging viral variants. PLoS Med. 2021;18(7):e1003656. https://Doi.org/10.1371/journal.pmed. 1003656.

[5] Rogers TF, Zhao F, Huang D, Beutler N, Burns A, He W, et al. Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. Science. 2020;eabc7520.

[6] Hingankar N, Deshpande S, Das P, Rizvi ZA, Wibmer CK, Mashilo P, et al. A combination of potently neutralizing monoclonal antibodies isolated from an Indian convalescent donor protects against the SARS-CoV-2 Delta variant. Rey FA, editor. PLOS Pathogens. 2022;18(4):e1010465.

[7] Wajnberg A, Amanat F, Firpo A, Altman DR, Bailey MJ, Mansour M, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. Science. 2020;370(6521):1227-30.

[8] Anichini G, Terrosi C, Gandolfo C, Gori Savellini G, Fabrizi S, Miceli GB, et al. SARS-CoV-2 antibody response in persons with past natural infection. N Engl J Med. 2021;385(1):90-92.

[9] Sasikala M, Shashidhar J, Deepika G, Ravikanth V, Krishna VV, Sadhana Y, et al. Immunological memory and neutralizing activity to a single dose of COVID-19 vaccine in previously infected individuals. Int J Infect Dis. 2021;108:183-86.

[10] Morales-Núñez JJ, Muñoz-Valle JF, Meza-López C, Wang LF, Machado Sulbarán AC, Torres-Hernández PC, et al. Neutralizing antibodies titers and side effects in response to BNT162b2 vaccine in healthcare workers with and without Prior SARS-CoV-2 infection. Vaccines. 2021;9(7):742.

[11] Voysey M, Costa Clemens SA, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Single-dose administration and the influence of the timing of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine: A pooled analysis of four randomised trials. The Lancet. 2021;397(10277).

[12] Desai A, Desai P, Mehta J, Sachora W, Bharti N, Patel T, et al. Measuring the impact of a single dose of ChAdOx1 nCoV-19 (recombinant) coronavirus vaccine on hospital stay, ICU requirement, and mortality outcome in a tertiary care centre. Int J Infect Dis. 2021;113:282-87.

[13] Singh AK, Phatak SR, Singh R, Bhattacharjee K, Singh NK, Gupta A, et al. Antibody response after first and second-dose of ChAdOx1-nCOV (CovishieldTM®) and BBV-152 (CovaxinTM®) among health care workers in India: The final results of cross-sectional coronavirus vaccine-induced antibody titre (COVAT) study. Vaccine. 2021;39(44):6492-09.
[14] Choudhary HR, Parai D, Dash GC, Kshatri JS, Mishra N, Choudhary PK, et al. Persistence of antibodies against spike glycoprotein of SARS-CoV-2 in healthcare workers post double dose of BBV-152 and AZD1222 vaccines. Front Med (Lausanne). 2021;8:778129.
[15] Singh AK, Phatak SR, Singh R, Bhattacharjee K, Singh NK, Gupta A, et al. Humoral antibody kinetics with ChAdOx1-nCOV (CovishieldTM) and BBV-152 (CovaxinTM) vaccine among Indian Healthcare workers: A 6-month longitudinal cross-sectional Coronavirus Vaccine-induced antibody titre (COVAT) study. Diabetes Metab Syndr. 2022;16(2):102424.

PARTICULARS OF CONTRIBUTORS:
1. Head, Department of Microbiology, Sri Jayadeva Institute of Cardiovascular Sciences and Research, Bengaluru, Karnataka, India.
2. Associate Professor, Department of Microbiology, Sri Jayadeva Institute of Cardiovascular Sciences and Research, Bengaluru, Karnataka, India.
3. Assistant Professor, Department of Microbiology, Sri Jayadeva Institute of Cardiovascular Sciences and Research, Bengaluru, Karnataka, India.
4. Director, Department of Cardiology, Sri Jayadeva Institute of Cardiovascular Sciences and Research, Bengaluru, Karnataka, India.
5. Resident, Department of Cardiothoracic and Vascular Surgery, Sri Jayadeva Institute of Cardiovascular Sciences and Research, Bengaluru, Karnataka, India.
6. Staff, Department of Microbiology, Sri Jayadeva Institute of Cardiovascular Sciences and Research, Bengaluru, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Amulya Cherukumudi,
Resident, Department of Microbiology, Sri Jayadeva Institute of Cardiovascular Sciences and Research, Bengaluru, Karnataka, India.
E-mail: amulyac1@yahoo.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Mar 22, 2022
- Manual Googling: May 24, 2022
- iThenticate Software: Jul 20, 2022 (6%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Mar 13, 2022**
Date of Peer Review: **Apr 26, 2022**
Date of Acceptance: **May 28, 2022**
Date of Publishing: **Aug 01, 2022**