ORIGINAL RESEARCH

Six Months Longitudinal Follow up of Antibody Response against SARS-CoV-2 after ChAdOx1-nCOV (Covishield™®) Vaccination amongst Health Care Workers

[1]Dr. Nitu Chauhan, [2]Dr. Yatendra Mohan, [3]Dr. Hiba Sami, [4]Dr. Satyam Arora, [5]Dr. Ajeet Singh Chahar, [6]Dr. Seema Dua, [7]Dr. Virendra Singh Saini, [8]Dr. Garima

[1]Associate Professor and Head, [2]Assistant Professor, Department of Immunohematology and Blood Transfusion, S.N. Medical College, Agra, UP, India
[3]Assistant Professor, Department of Microbiology, JNMCH, AMU, Aligarh, UP, India
[4]Associate Professor, [6]Additional Professor and Head, Department of Transfusion Medicine, Postgraduate Institute of Child Health, Noida, UP, India
[5]Associate Professor, [8]Junior Resident, Department of Medicine, S.N. Medical College, Agra, UP, India
[7]Associate Professor, Department of Medicine, S.M.M.H. Medical College, Saharanpur, UP, India

Correspondence:
Dr. Satyam Arora
Associate Professor, Department of Transfusion Medicine, Postgraduate Institute of Child Health, Noida, UP, India
Email: satyamarora83@gmail.com

Received: 17 November, 2022 Accepted: 22 December, 2022

ABSTRACT

Background & Objective: Active immunization (vaccination) is the main strategy adopted for the prevention of COVID-19 across the world. Vaccination is known to stimulate the human body to produce protective molecules and prepare for a possible encounter with the actual virus in the future. Antibody formation against the virus is one of the types of response to vaccination. The objective of the study was to analyze the antibody response of ChAdOx1-nCOV (Covishield™®) vaccine administration among health care workers (HCWs).

Methods: The transfusion medicine department of SNMC, Agra enrolled health care workers who received ChAdOx1-nCOV (Covishield™) vaccine and obtained their informed consent. The enrollment for the study was done in January and February2021 (6 months follow-up was done till August 2021). The sampling was done on five points: day 0 (prior to the first dose), day 28 (prior to the second dose), day 56, day 120, and day 180 (from the first dose). Antibody against SARS-CoV-2 spike protein (mainly IgG) quantification was done by Roche (using Elecsys Anti-SARS-CoV-2 S kit) based on Electrochemiluminescence Immunoassay (ECLIA) at the department of microbiology, AMU, UP.

Results: A total of 117 HCWs were enrolled in the study who received the vaccine between 16thJanuary to 18thFebruary 2021. 57 health care workers out of 117 (48.8%) had pre-existing antibodies on day 0 (group A) and 60 did not have any pre-existing antibodies (naïve participants, group B). The mean gap between the first and second dose of the vaccine was 29.8 days. The baseline (day 0) antibody levels in group A were 150.8±332.2 U/mL compared to 0.47 ±0.14 U/mL in group B. The antibody response due to vaccination was significantly higher in group A than group B on the 28thday and 56thdays sampling. A total of

26 out of 117 enrolled HCWs (22.2%) reported a breakthrough infection of COVID-19 after 56thdays sampling. The breakthrough infection resulted in a significant rise in the antibody levels compared to participants who were never exposed toSARS-CoV-2 in group B (39 out of 60; naïve participants).

Interpretation and Conclusion: We concluded that the antibody production in response to vaccination, was significantly higher in a group of participants who had previous exposure to the virus before vaccination. Exposure to the virus after a complete schedule of vaccination also induced a significant rise in antibody levels. The antibody response in naïve participants who hadboth doses of the vaccines (never exposed to the virus) had significantly lower levels at 180 days compared to their antibody peak at 56 days.

Key Words: COVID-19, Vaccination, Covishield Vaccine

KEY POINTS

Question: Quantification of antibody against spike protein of SARS-CoV-2 up to 6 months of ChAdOx1-nCOV (Covishield$^{TM®}$) Vaccination amongst Health Care Workers in India

Finding: Our study showed that there was as high as 98.9% responder rate of antibody (against "spike protein" RBD domain) production after ChAdOx1-nCOV (Covishield$^{TM®}$) vaccination in previously naïve (unexposed) participants. The antibody production was significantly higher in group of participants who had preexisting antibodies. The antibody response in naïve participants who had both dose of the vaccines (never exposed to the virus) had significantly lower levels at 180 days compared to antibody peak at 56 days.

Meaning: An individual who has vaccination and have been exposed with the infection produces high quantity of antibodies as well as for longer duration as compared to an individual who only receives vaccination. These antibodies due to vaccination only reduces over the period.

INTRODUCTION

Severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) was a new member added to the *Coronaviridae* family, with its first report from China in December 2019. Within a few months the virus had reached almost all the countries of the world and Coronavirus disease (COVID-19) was declared a pandemic by March 2020. Since the declaration of the pandemic, there was an unprecedented speed of work done to develop an effective vaccine to prevent this disease. Soon many types of vaccines were developed and authorized by various countries for use. By December 2020, mRNA COVID-19 vaccine by Pfizer BioNTech and Moderna weas given emergency use authorization in the USA. AstraZeneca vaccine was approved by the Medicine and Healthcare Product Regulatory Agency (MHRA) of the UK on 30th December 2020. [1-3]

In Quick Succession, India gave emergency authorization to use Covishield$^{TM®}$ (ChAdOx1-nCOV or AZD1222, manufactured by Serum Institute, Pune, India) and Covaxin$^{TM®}$ (BBV-152, Bharat Biotech, Hyderabad, India) in January 2021 and the national vaccination drive was started on 16th January 2021. Both these vaccines are for intramuscular use. The initial regimen included two shots to be given in a gap of 4-6 weeks. Later the regimen was modified for Covishield$^{TM®}$ keeping a gap of 12-16 weeks between two doses. [4]

ChAdOx1-nCOV (Covishield$^{TM®}$) is a chimpanzee adenovirus vector-nonreplicating virus vaccine carrying recombinant spike protein of SARS-CoV-2. Active immunization or vaccination induces multiple immune responses (both cellular and humoral) in the recipient. The humoral immune response is in the form of antibodies, which provide rapid protection and immunity, produced by B cells. Memory B cells are also able to mount a rapid response in cases of re-exposure/ stimulation to provide protection. [5] Phase 2/3 trial from India shows the adequate immune response of ChAdOx1-nCOV (Covishield$^{TM®}$) vaccine in India.

[6]Our study is a longitudinal six-month follow-up to analyze and quantify the antibody response in health care workers who received ChAdOx1-nCOV (Covishield$^{TM®}$) vaccine.

MATERIAL AND METHOD

STUDY DESIGN AND PARTICIPANTS

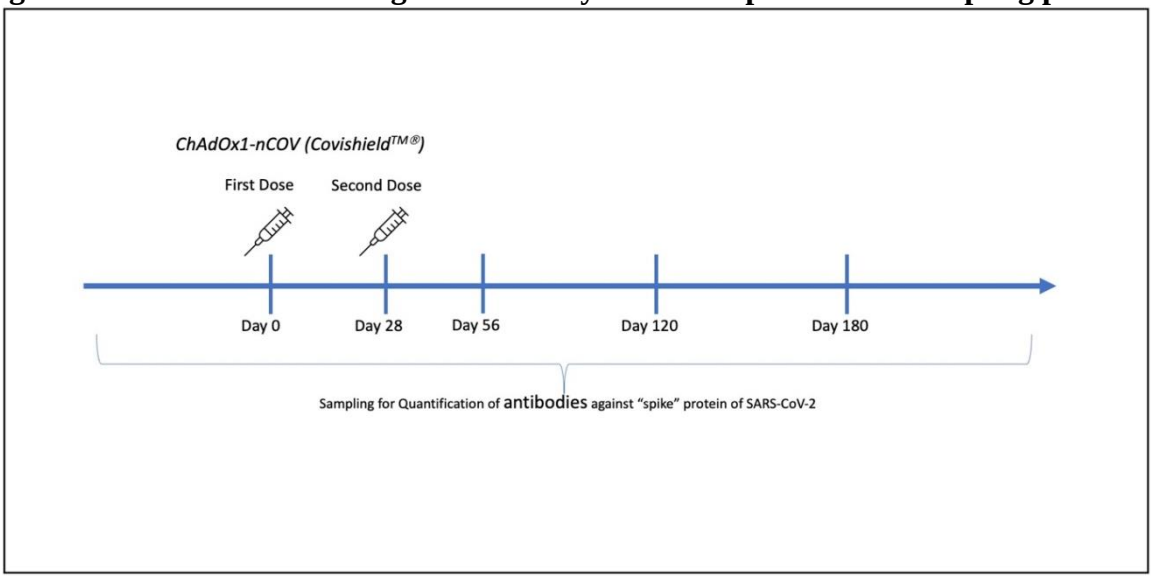
This was a cohort longitudinal prospective study on health care workers (HCWs) of more than 18 years of age. The study was done by the Department of Transfusion Medicine at S.N.Medical College (government) at Agra in the western region of the state of Uttar Pradesh in India and was approved by the Institutional Ethics Committee. Inclusion criteria for enrollment was the eligibility of HCWs to receive the Covishield$^{TM®}$ (ChAdOx1-nCOV) vaccination.

Exclusion criteria were HCWs with current confirmed SARS-CoV-2 infection (confirmed by RT PCR or Rapid testing) or HCWs who had SARS-CoV-2 infection in the last 4 weeks were excluded from the study. The minimum number of participants to be enrolled was 100. Voluntary participants who received their first dose of the Covishield$^{TM®}$ vaccine between the 16th January and 18th February 2021, were included in the study, and informed written consent was taken. Demographic details such as age, gender, prior history of Covid illnesses were recorded. Each participant was to be followed till 6 months from the first dose of the vaccination. [Figure 1]

ANTIBODY QUANTIFICATION

The antibody quantification was done on five points: day 0 (prior to the first dose), day 28 (prior to the second dose), day 56, day 120, and day 180 from the first dose of the vaccine. [Figure 1].

Figure 1: Flowchart of the longitudinal study and description of the sampling points



The above flowchart shows the study plan of a prospective study to study and analyze the immuneresponse due to ChAdOx1- nCOV (Covishield^{TM}) vaccination against SARS-CoV-2 in healthcare workers in India

A 3 ml whole blood sample was collected from all eligible enrolled participants (using EDTA) on each sampling point and plasma was separated for further testing. These samples were stored in a -80°C deep freezers for later batch testing for the antibodies.

Antibody quantification was done by FDA approved in vitro quantitative determination platform by Roche (using Elecsys Anti-SARS-CoV-2 S kit) based on Electrochemiluminescence Immunoassay (ECLIA). This assay uses a recombinant protein

representing the RBD of the S antigen, to detect the antibody levels in a double-antigen sandwich assay format. The assay quantifies the antibodies (including IgG) levels against the spike (S) protein receptor-binding domain (RBD) of SARS-CoV-2 with a sensitivity of 98.8%. The cutoff for a positive antibody was more than and equal to 0.80U/mL. The defined limit of quantitation and the maximum of the master curve is between 0.40-250 U/mL. Values more than 250 U/mL were diluted up to 1:100 dilution (using Universal Diluent) for reporting of the exact concentration of the antibody.

STATISTICAL ANALYSIS

Descriptive analysis was done in the present study. Data on a continuous scale was presented as Mean, Standard Deviation, and Median. An unpaired T-test was used to compare the antibody levels at various points of study and a two-sided P value of <0.05 was considered as statistically significant. For within the group analysis repeat measure ANOVA test was done. Entire statistical analysis, as well as graphs preparation, was carried out using Microsoft Word and Excel.

RESULTS

A total of 117 health care workers were enrolled in the study who received Covishield™®. Out of 117, 43 were females (36.7%) and 74 were males (63.3%). The mean age of the enrolled health care workers was 39.2 ± 11.6 years (median 39 years, range 22-70 years), 9 out of 117 were above the age of 60 years. [Table 1] The mean gap between the first and second dose of the vaccine was 29.83 days (median 28 days).

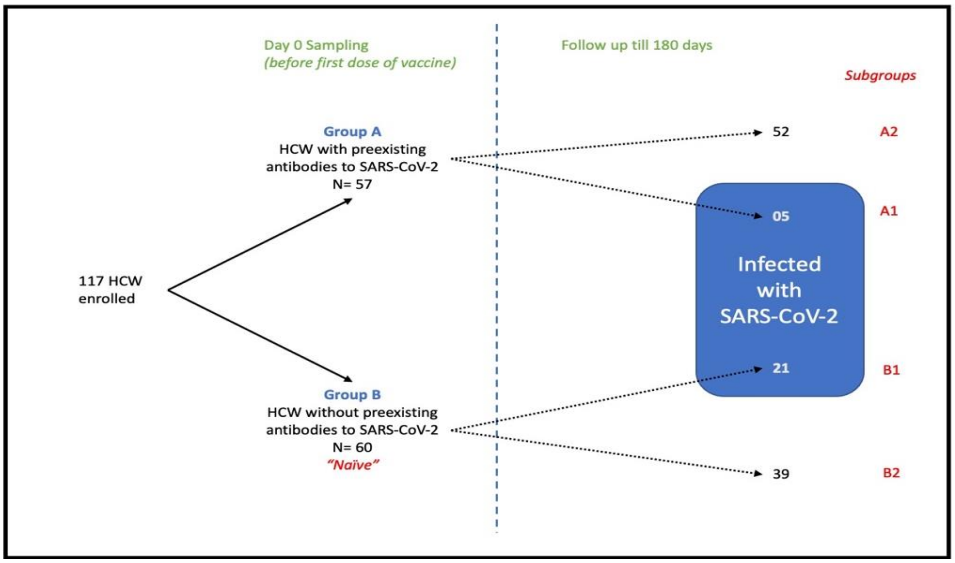
Table 1: Demography of health care workers enrolled in the study

Parameter	Total Enrolled (n=117)	Group A (n=57)	Group B (n=60)
Gender (M/F)	74 / 43	37 / 20	37 / 23
Age (mean ± SD) years	39.2 ± 11.6	37.6 ± 12.7	40.7 ± 12.5

BASELINE QUANTIFICATION OF SARS-COV-2 SPIKE ANTIBODY LEVELS AT THE TIME OF ENROLLMENT

57 health care workers out of 117 (48.8%) had preexisting antibodies against SARS-CoV-2 spike protein on the day of the first dose of vaccination (before vaccination), indicative of the previous history of SARS-CoV-2 exposure/ infection. [Figure 2]

Figure 2: Details of enrolled participants and their follow up



The above flowchart shows that 117 HCWs were enrolled in the study; among then 57 (48.7%) had preexisting antibodies to SARS-CoV-2. After 56th days of sampling, some of the 26 out of 117 HCWs reported breakthrough infections (5 from group A and 21 from group B) The mean baseline antibody levels in this group were 150.8 ± 332.2 U/mL. Rest 60 out of 117 (51.2%) health care workers enrolled did not have any detectable antibody (naïve participants) with mean antibody levels as 0.47 ± 0.14 U/mL. For further analysis, the enrolled HCW were categorized into two groups: group A (n=57), participants who had preexisting antibodies, and group B (n=60) naïve participants. [Table 1]

QUANTIFICATION OF SARS-COV-2 SPIKE ANTIBODY LEVELS ON 28TH DAY

The antibody response after the first dose (on the day of the second dose) of the vaccine was significantly higher ($p< 10^{-11}$) in group A (7530.46 ± 1789.6 U/mL) when compared to group B (87.6 ± 170.9 U/mL). [Table 2] There was one non-responder in group B who did not make any detectable antibody (1 out of 60) up till the 28th day after the first dose but showed immune response from day 56 onwards.

Table 2: Comparison of antibody response to ChAdOx1-nCOV (Covishield$^{TM®}$) vaccination against SARS-CoV-2

Group	Quantification of Antibody against Spike protein of SARS-CoV-2 (U/mL)				
	Day 0	**Day 28**	**Day 56**	**Day 120**	**Day 180**
A1* (n=5)	203.4 ± 256.4	11,510.3 ± 11,809	3,181.7 ± 1,926.1	10,082.4 ± 7,029.9	8,527 ± 9,571.5
A2 (n=52)	145.7 ± 135.1	7,147.7 ± 7,394.4	5,157.7 ± 5,828.8	3,516 ± 4,401.6	2,395.6 ± 3,143.2
A (n=57)	**150.8 ± 332.2**	**7,530.4 ± 7,829.9**	**4,984 ± 5,614.7**	**4,092 ± 4,968.5**	**2,933.4 ± 4,313.2**
B1* (n=21)	0.45 ± 0.12	42.7 ± 39.7	728.8 ± 1,568.7	9,665.9 ± 7,784.8	5,217 ± 4,011.2
B2 (n=39)	0.48 ± 0.16	111.7 ± 207	366.3 ± 380.9	268.7 ± 390.3	147.4 ± 169.5
B (n=60)	**0.47 ± 0.14**	**87.6 ± 170.9**	**493.2 ± 978.7**	**355.7 ± 6,408.7**	**1,921.8 ± 3,379.1**
**(***) Infected with the COVID-19 during the study period					

QUANTIFICATION OF SARS-COV-2 SPIKE ANTIBODY ON 56TH DAY

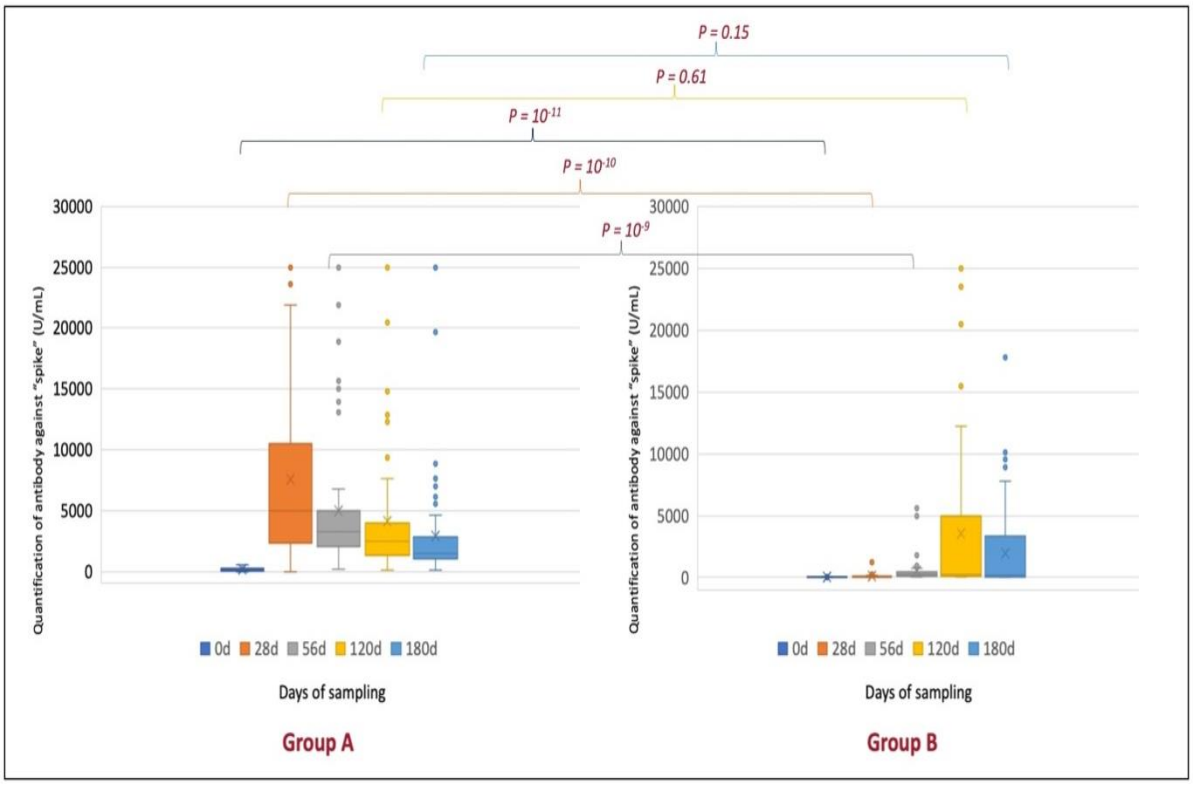
The quantification of antibody after 56 days (of the second dose) of the vaccine again showed significantly higher levels ($p< 10^{-8}$) in group A (4984.4±5614.7 U/mL) when compared to group B (493.2±978.7 U/mL). [Table 2]

QUANTIFICATION OF SARS-COV-2 SPIKE ANTIBODY ON 120TH AND 180TH DAY

Many HCWs enrolled in the study, reported SARS-CoV-2 infections after 56th day sampling, as there was a second wave with a high number of COVID-19 cases in India. A total of 26 out of 117 enrolled HCWs reported having breakthrough infections of COVID-19 (between April and May 2021) during the study period (22.2%). 5 out of 57 in group A and 21 out of 60 in group B reported being infected with the virus. [Figure 2]

Antibody levels in group A were significantly higher from group B for day 0, 28 and 56 but interestingly the antibody response on day 120 and day 180 among both the groups, did not show a significant difference. [Figure 3]

Figure 3: Comparison of quantification of the antibody (against "spike" protein) response on day 0, 28, 56, 120 and 180 to ChAdOx1-nCOV (Covishield™®) vaccination against SARS-CoV-2

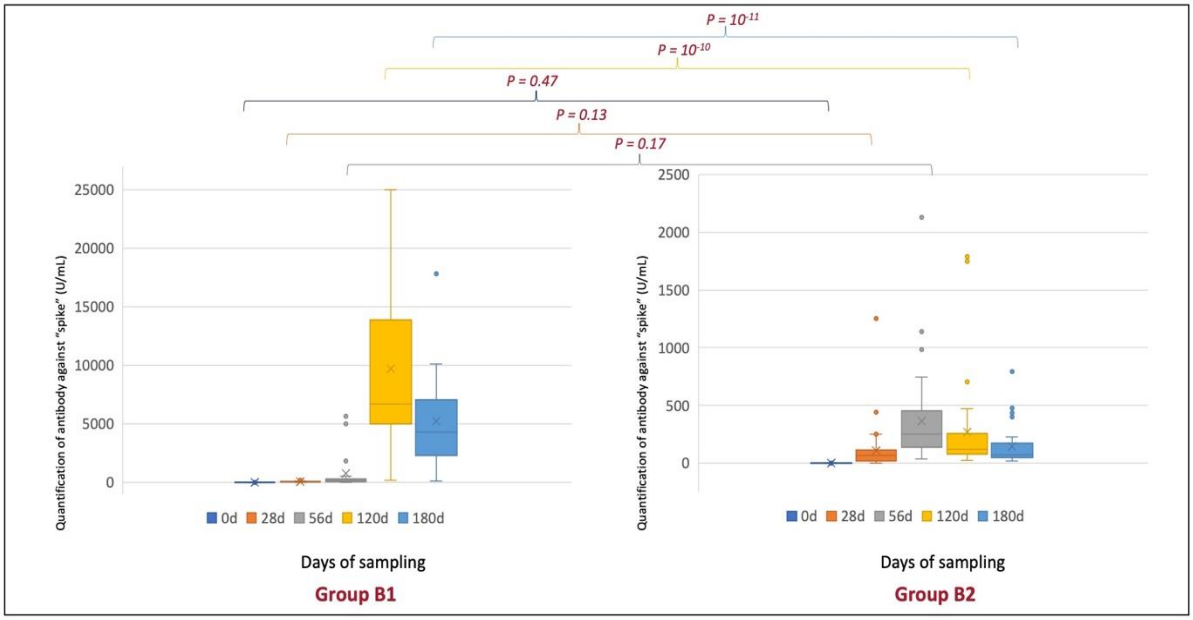


The antibody levels were higher in group A on the day 0 due to preexisting antibodies in that group of participants. The antibody response due to ChAdOx1- nCOV (Covishield™) vaccination against SARS-CoV-2 was significantly higher in group A on day 28th and 56th day of sampling after vaccination. The antibody levels did not show a significant difference on day 120th and day 180th sampling due to the breakthrough infections reported in both the groups

On day 120 sample, the mean antibody levels were 4092 ± 4968.5 U/mL in group A compared to 355.7 ± 6408.7 U/mL in group B (p=0.61). Similarly on day 180 sample, the mean antibody levels were 2933.4 ± 4313.2 U/mL in group A compared to 1921.8 ± 3379.1U/mL in group B (p=0.15). This comparative rise in antibody response in group B was due to the anamnestic response due to breakthrough infections in 21 out of 60 HCWs. The non-significant overall decline in both the groups was due to breakthrough infection in both the groups.

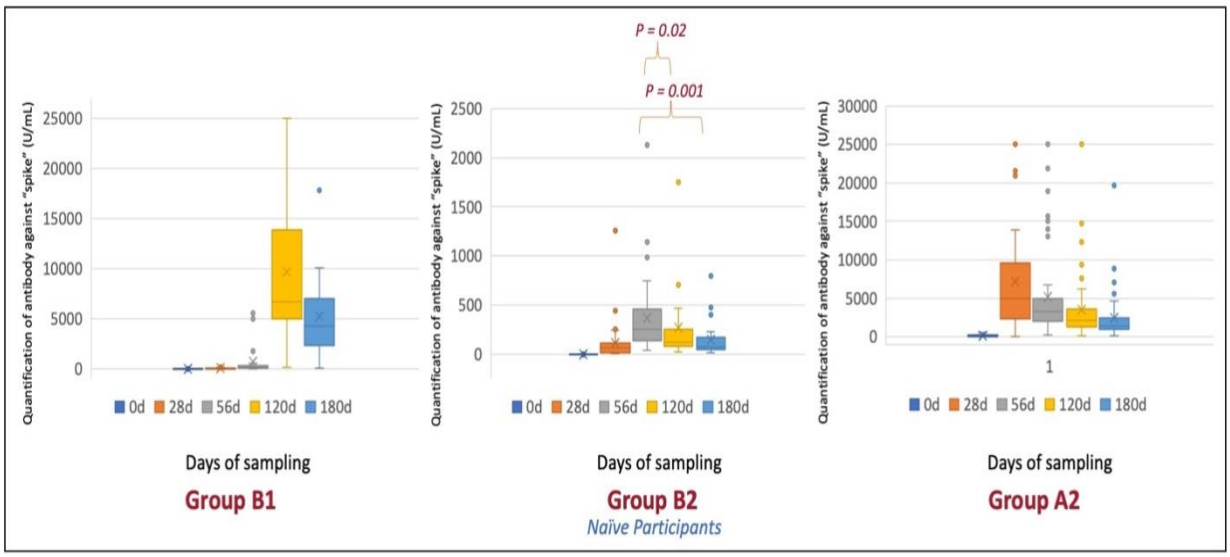
On subgroup analysis, within the group B (naïve participants), it was observed that the mean antibody response on day 120 and 180 was significantly higher ($p<10^{-10}$, $p<10^{-11}$) in individuals who had an exposure/ infection to the SARS-CoV-2 virus (group B1) when compared to HCWs who did not (group B2). [Figure 4] All the HCWs who reported COVID-19 infection within the study period, experienced a mild infection and did not require any hospital admission.

Figure 4: Comparison of quantification of the antibody (against "spike" protein) response on day 0, 28, 56, 120, and 180 to ChAdOx1-nCOV (Covishield™®) vaccination against SARS-CoV-2 in Group B HCWs (naïve participants)



In the naïve participants (group B), 21 individuals reported (group B1) breakthrough infection. The antibody levels between (B1 and B2) was not significantly different on day 0, day 28, and day 56 sampling. Whereas significant differences in antibody levels was observed on day 120 and day 180 samples in both groups. This was due to an anamnestic immune response due to breakthrough infection which produced high levels of antibodies.
Further subgroup analysis showed that the overall mean antibody levels, in response to vaccination, were significantly higher in group A2(HCW with preexisting antibodies but not exposed during the study) as compared to group B2(naïve HCWs without exposure exposed to the virus) during the study period. [Figure 5]

Figure 5: Comparison of quantification of the antibody (against "spike" protein) response on days 0, 28, 56, 120, and 180 to ChAdOx1-nCOV (Covishield™®) vaccination against SARS-CoV-2 in Group A2, B1, and B2



The antibody levels were significantly higher in the group A2 (HCWs who had preexisting antibodies and were not exposed to the virus during the study period) as compared to naïve group (group B1 and B2). As well within the naïve participants (B2; who never got exposed or infected with SARS-CoV-2 infection) had a significant reduction in their antibody levels by 120 days (p=0.02; 3 months) and by 180 days (p=0.001; 6 months) when compared to peak antibody levels achieved on 56 days after the first dose of vaccination.

This indicates that initiation of vaccination after the exposure to the virus generates a higher response compared to the immune response in naïve HCWs with vaccination.Inter-group analysis of both the breakthrough infection groups (A1 and B1) did not show significantly different levels of antibody levels after 180 days of follow-up (p=0.22), indicating that the antibody levels drop in both groups.

Naïve participants who did not report SARS-CoV-2 infection (group B2) had a significant (p=0.001) reduction in their antibody levels by 6 months when compared to 56 days sampling. [Figure 4] This reduction in antibody levels may make them a vulnerable group to be considered for a booster dose. Similar findings were reported from Italy which showed a drastic reduction of vaccine efficiency post 69 days of vaccination. [7] Within the group analysis by repeat measure ANOVA showed significant change in antibody levels at the end of 180 days of follow up when compared to their baseline levels [p value A1 p= 0.02; A2 $p<10^{-23}$; B1 $p<10^{-15}$ and B2 $p<10^{-12}$].

DISCUSSION

The duration of protection provided by Covishield$^{TM®}$vaccination against COVID-19 disease is currently unknown. This prospective study was undertaken for the estimation of COVID-19 antibodies against the "spike protein" of SARS-CoV-2 up to 6 months after the vaccination. The study enrolled health care workers who received two doses of ChAdOx1-nCOV (Covishield$^{TM®}$) vaccination at a gap of 28 days. Many of the participants had preexisting antibodies to SARS-CoV-2 due to their previous exposure and subsequently, all of them showed a higher rise in antibody concentration following the first dose of the vaccine. 1 out of 60 previously naïve participants (1.6%) of group B did not seroconvert after the first dose. Similar findings were reported from Sri Lanka, where 2 out of 68 previously naïve individuals (2.9%) failed to mount a positive neutralizing antibody response to the first dose of AZD1222 vaccine. [8]

Antibody response after the vaccine showed a robust immune stimulation with an exponential rise in antibody levels in individuals who had a preexisting antibody to the virus. There was significantly higher antibody concentration on day 28 and day 56 in group A individuals when compared with antibody naïve participants. [Figure 3] Similarly, a single shot of AZD1222 vaccine has shown significant increase in ACE-2 blocking antibodies and RBD antibodies for variants such as B.1.1.7 and B.1.351. [8]

India reported a massive second wave of COVID-19 cases from April to June 2021. The daily positivity rate from 1.62% on 1st March 2021 rose to almost 20% on 13th May 2021.[9] India reported 29.27 million cases with a case fatality rate of 1.24% (363,079 deaths) till 11th June 2021. [10] Many of our participants (22.2%) also reported breakthrough infections with SARS-CoV-2 even after the complete vaccination. All these 26 out of 117, experienced a mild COVID-19 infection but did not require hospitalization. Subjects with no previous SARS-CoV-2 infection (group B), after the 28 days of second dose showed a significantly lower levels of circulating anti-spike-IgG-antibody levels compared with the samples from previously infected participants.

As previously reported, after full vaccination the odds of turning RT-PCR positive was 0.17, odds of hospitalization were 0.12 and ICU admission/ death was 0.07. [11] Recently reported from eastern India showed that individuals who required hospitalization after a breakthrough

infection had a low median antibody level when compared to individuals who were managed at home[12] thus considering a booster dose for such individuals may be beneficial. Although antibody levels are not an appropriate method to judge immune protection, it can still provide some guidance.Another study from India reported 16.9% of breakthrough COVID-19 mild infections after any dose of vaccine.[13]

Apart from the milder clinical course of the disease, the infected individuals showed higher antibody levels on the next sampling (120 days). There was no significant difference in the antibody levels among both the groups for days 120 and 180 as many in group B (21 out of 60 naïve participants) got infected. [Figure 3] This was an anamnestic antibody response due to re-exposure of the virus after the vaccination. These individuals had made memory cells against the SARS-CoV-2 (with vaccination) which got restimulated with a breakthrough infection and produced a high concentration of antibodies as well as provided protection from severe disease.

There are a few limitations of this study such as the study was done on a very small number of vaccine recipients. This was due to financial consideration and limited support provided to the study. Another limitation was that quantification of neutralizing antibodieswas not done which would have provided more clinical relevance to the results. Another aspect was that the group (naïve) which did not have any detectable antibody on the day of enrollment can also be due to weaning off the antibody of an old infection, only humoral immune response was studied in this study (B cell response) and only a single platform was used to quantify the antibody as the specificity and sensitivity may vary based on the platform used for antibody detection. [14]

Vaccines are the only effective tool for us to manage and prevent the spread of the pandemic. The vaccines used in India are new and not much data is available on their efficacy. Antibody level can provide a window in the immune stimulation which any type of vaccination may induce.

CONCLUSION

Our study showed that there was as high as 98.9% responder rate of antibody (against "spike protein" RBD domain) production after ChAdOx1-nCOV (Covishield$^{TM®}$) vaccination in previously unexposed participants. The antibody production was higher in a group of participants who had preexisting antibodies, in response to vaccination, due to a robust humoral immune response in them. Breakthrough infection (22.2% reported in our data) after vaccination furtherincreased the antibody production and contributed to milder severity of the disease. The antibody response in naïve participants who had both doses of the vaccines (never exposed to the virus) had lower levels at 180 days compared to its antibody peak at 56 days. This study further reinforces the concept that vaccine is an effectivetool to provide prevention from the severity of the disease.

AUTHORS CONTRIBUTION

NC, YM, ASC, VSS&GA motivated and recruited the participants for enrollment and sampling. HS conducted the antibody quantification. SA and SD prepared the manuscript. Final manuscripts were reviewed and approved by all the authors.

ACKNOWLEDGEMENT

The HCWs were enrolled, data collection,and analysis by the department of Transfusion Medicine at S.N. Medical College, Agra, UP, India, and the antibody testing was done at the department of Microbiology of Aligarh Muslim University (AMU), Aligarh, UP, India. The department of Transfusion Medicine, PGICH, Noida, India did a review of literature, data analysis, and manuscript preparation.

FUNDING SOURCE

The kits for antibody testing were supported by the National Health Mission for the state of Uttar Pradesh, India.

REFERENCES

1. Oliver, S. E. et al. The Advisory Committee on Immunization Practices'Interim Recommendation for Use of Pfizer-BioNTech COVID-19 Vaccine—United States, December 2020. MMWR Morb. Mortal. Wkly Rep. 69,1922–1924 (2020).
2. Ledford, H. Moderna COVID vaccine becomes second to get US authorization. Nature https://doi.org/10.1038/d41586-020-03593-7 (2020).
3. Agency, M. A. H. P. R. (Medicines and Healthcare products RegulatoryAgency. Vaccines and vaccine safety, 2020).
4. Basavaraja CK, Sebastian J, Ravi MD, John SB. Adverse events following COVID-19 vaccination: first 90 days of experience from a tertiary care teaching hospital in South India. Ther Adv Vaccines Immunother. 2021 Nov 22;9:25151355211055833. doi: 10.1177/25151355211055833. PMID: 34841193; PMCID: PMC8611289.
5. Chmielewska AM, Czarnota A, Bieńkowska-Szewczyk K, Grzyb K. Immune response against SARS-CoV-2 variants: the role of neutralization assays. NPJ Vaccines. 2021 Nov 29;6(1):142. doi: 10.1038/s41541-021-00404-6. PMID: 34845231; PMCID: PMC8630184.
6. P.S. Kulkarni et al., A phase 2/3, participant-blind, observer-blind, randomised, controlled study to assess the safety and immunogenicity of SII-ChAdOx1 nCoV-19 (COVID-19 vaccine) in adults in India, EC linical Medicine (2021), https://doi.org/10.1016/j.eclinm.2021.101218E Clinical Medicine 000 (2021) 101218Contents lists available at Science Direct E Clinical Medicine journal homepage: https://www.journals.elsevier.com/eclinical medicine
7. Bianchi FP, Tafuri S, Migliore G, Vimercati L, Martinelli A, Lobifaro A, Diella G, Stefanizzi P, Group OBOTCRW. BNT162b2 mRNA COVID-19 Vaccine Effectiveness in the Prevention of SARS-CoV-2 Infection and Symptomatic Disease in Five-Month Follow-Up: A Retrospective Cohort Study. Vaccines (Basel). 2021 Oct 7;9(10):1143. doi: 10.3390/vaccines9101143. PMID: 34696252; PMCID: PMC8538139.
8. Jeewandara, C., Kamaladasa, A., Pushpakumara, P.D. *et al.* Immune responses to a single dose of the AZD1222/Covishield vaccine in health care workers. *Nat Commun* **12,** 4617 (2021). https://doi.org/10.1038/s41467-021-24579-7
9. Kumar S. Second wave of COVID-19: Emergency situation in India. J Travel Med. 2021 May 25:taab082. doi: 10.1093/jtm/taab082. Epub ahead of print. PMID: 34037783; PMCID: PMC8244620.
10. Choudhary OP, Priyanka, Singh I, Rodriguez-Morales AJ. Second wave of COVID-19 in India: Dissection of the causes and lessons learnt. Travel Med Infect Dis. 2021 Jun 16;43:102126. doi: 10.1016/j.tmaid.2021.102126. Epub ahead of print. PMID: 34144178; PMCID: PMC8214078.
11. Bhattacharya A, Ranjan P, Ghosh T, et al. Evaluation of the dose-effect association between the number of doses and duration since the last dose of COVID-19 vaccine, and its efficacy in preventing the disease and reducing disease severity: A single centre, cross-sectional analytical study from India. Diabetes MetabSyndr. 2021 Jul 30;15(5):102238. doi: 10.1016/j.dsx.2021.102238. Epub ahead of print. PMID: 34364299; PMCID: PMC8321688

12. Dash GC, Subhadra S, Turuk J, et al. Breakthrough SARS-CoV-2 infections in an eastern state of India: A preliminary report. Research Square; 2021. DOI: 10.21203/rs.3.rs-649914/v2.
13. Tyagi K, Ghosh A, Nair D, et al. Breakthrough COVID19 infections after vaccinations in healthcare and other workers in a chronic care medical facility in New Delhi, India. Diabetes MetabSyndr. 2021 May-Jun;15(3):1007-1008. doi: 10.1016/j.dsx.2021.05.001. Epub 2021 May 3. PMID: 33991805; PMCID: PMC8091733.
14. Mangwana S, Gohel D, Singhal D, Mutta J, Yadav D. Comparative analysis of different assays in assessing COVID antibody titer after ChAdOx1 nCoV-19 corona virus vaccine (recombinant) COVISHIELD vaccination. Edorium J Infect Dis 2021;6:100012I03SM2021.