

A Prospective Study of Seroconversion Post Covishield Vaccination in COVID-19 Warriors

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ABSTRACT

Introduction: During the Coronavirus Disease 2019 (COVID-19) pandemic in India, two vaccinees were predominantly administered to prevent the spread of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The first vaccine introduced in India was ChAdOx1-nCOV (Covishield), followed by BBV-152 (Covaxin). In the first phase, Healthcare Workers (HCW) were prioritised for vaccination, given their crucial role in the healthcare system.

Aim: To assess the antibody response post Covishield vaccination at specific time intervals in HCWs and to determine the correlation of antibody response with age, gender, co-morbidities and blood group.

Materials and Methods: This prospective study was conducted at Dr. Balasaheb Vikhe Patil Rural Medical College, Pravara Institute of Medical Sciences, Loni, Maharashtra, India, over a duration of six months from January 2021 to June 2021. A total of 110 vaccinated HCWs who volunteered were included in this study. SARS-CoV-2 antibodies at specific time intervals were assessed using Ortho Clinical Diagnostic's VITROS 3600 based on the principle of Chemiluminescent Immunosorbent Assay (CLIA). Assessment of anti-SARS-CoV-2 total and IgG antibodies was performed at 15 days, one month, one and a half months, two months, three months, and four months postfirst dose of vaccination, or in other words, 15 days and one month

after the 1st dose, and 15 days, one month, two months, and three months post second dose of vaccination. For analysis, Pearson's correlation and a regression model were performed using GraphPad Prism 8.0.2 version.

Results: Fifteen days post second dose, 110 HCWs (100%) and 109 (99.09%) HCWs turned seropositive for total antibodies and IgG antibodies, respectively. It was observed that the majority of participants (33, 30.27%) with peak IgG levels in the medium range were from the age group of 31-40 years. Overall, there was a negative correlation between age and IgG antibody levels for peak IgG values (r -value=-0.224, p -value=0.019). The peak values were achieved in the majority of participants 15 days post second dose (53.6%). The difference in antibody levels based on gender was not significant (Chi-square value=3.387, p -value=0.184). No significant difference in SARS-CoV-2 IgG levels was observed between participants with co-morbidities and those without co-morbidities. Participants who developed SARS-CoV-2 infection during the study period exhibited robust antibody responses after vaccination.

Conclusion: These findings help elucidate Covishield vaccine-specific antibody responses in vaccinees of different age groups, genders, blood groups, and with co-morbid conditions. The vaccine has substantially reduced the burden of disease by preventing serious illness in vaccinated HCWs during the second wave of the COVID-19 pandemic.

Keywords: Chemiluminescent Immunosorbent Assay, Covishield vaccine, Healthcare workers

INTRODUCTION

The SARS-CoV-2 pandemic has affected more than 692 million people and caused seven million deaths globally as of August 2023 [1]. To address this, the first vaccine introduced in India was ChAdOx1-nCOV (Covishield), followed by BBV-152 (Covaxin) [2]. In the first phase, India commenced the administration of COVID-19 vaccines on January 16, 2021, with HCWs being prioritised [3]. Two doses of the Covishield vaccine were administered at an interval of one month, following guidelines from the Ministry of Health and Family Welfare of the Government of India [3].

The vaccine candidate ChAdOx1 nCoV-19 vaccine (AZD1222) was developed by Oxford-AstraZeneca under the name Vaxzevria. It was licensed in India as 'Covishield' and manufactured by the Serum Institute of India, Pune. Covishield is a monovalent vaccine composed of a single recombinant, replication-deficient chimpanzee adenovirus (ChAdOx1) as a vector. One dose (0.5 mL) of the Covishield vaccine contains 5×10^{10} viral particles of the adenovirus vector encoding the SARS-CoV-2 spike glycoprotein.

After administration, the SARS-CoV-2 spike glycoprotein is expressed locally, eliciting neutralising antibody and cellular immune

responses [4]. The S-protein binds to the Angiotensin Converting Enzyme 2 (ACE2) receptor on the host cell's surface. The virus enters the cell through the Receptor Binding Protein (RBD) on the S-protein structure. Since the S-protein plays a crucial role in viral cell entry, it is a key target for virus inactivation and postvaccine immune response [5].

The COVID-19 pandemic presented unprecedented professional risks for HCWs, estimated to bear approximately 10% of the total burden. HCWs faced an 11% higher risk of testing positive on Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and a seven times higher risk of severe COVID-19 compared to the general community [6]. Antibodies are valuable for assessing an individual's immune response during viral infections. SARS-CoV-2-specific IgG antibodies are considered vital indicators of immunity in COVID-19. Therefore, testing for SARS-CoV-2 antibodies in HCWs who received either the Covishield or Covaxin vaccinations can be beneficial in understanding vaccine efficacy and determining immunisation strategies [7].

This study aimed to evaluate the antibody immune response post Covishield vaccination in HCWs and to explore the correlation with age, gender, co-morbidities, and blood group.

MATERIALS AND METHODS

This prospective study was conducted at Dr. Balasaheb Vikhe Patil Rural Medical College (DBVPRMC), Pravara Institute of Medical Sciences (PIMS), Loni, Maharashtra, India, for a duration of six months, consisting of four months for sample collection and processing and two months for analysis from January 2021 to June 2021. This study protocol was reviewed and approved by the Institutional Ethics Committee (PIMS/DR/RMC/2021/454).

At the time of conducting this study, the national policy was to extend immunisation to HCW with two doses of both vaccines, at intervals of four weeks. The study was designed to assess SARS-CoV-2 antispikes binding antibodies qualitatively at various time intervals. This study was conducted on the HCW of the institute who accepted and volunteered to investigate the antibody response following Covishield vaccination. A total of 110 HCW volunteered to participate in this study, which was conducted from January 2021 to June 2021. Being a time-bound study, participants available during the study duration were included in the study.

Participants were provided with a questionnaire, and information was gathered regarding age, sex, prior COVID-19 infection, blood group, and co-morbidities. Informed written consent was obtained from all participants.

Prior to vaccination, all participants underwent a baseline antibody test to determine the baseline antibody status against SARS-CoV-2 (total antispikes Ab).

Inclusion criteria: Baseline value of SARS-CoV-2 total antibody <1, vaccinees willing to participate. When baseline values are more than one before vaccination, it indicates immune responses present due to previous infection. The inclusion of such participants in the study will affect the outcomes of the study. Hence, only participants with a value <1 were included in the study.

Exclusion criteria: Baseline value of SARS-CoV-2 total antibody ≥1, vaccinees not willing to participate.

In subsequent samples, both total antibody (Ab) and Immunoglobulin G (IgG) antibodies to spike proteins of SARS-CoV-2 were measured, and these antibody responses were followed longitudinally in participants, examining for differences in responses based on age, gender, blood group, and co-morbidities.

Specimen collection: 2 mL venous whole blood samples were collected by antecubital phlebotomy of eligible participants by expert technicians following all aseptic precautions [8]. All samples were collected in plain vials and analysed at the Central Clinical Laboratory of Dr. BVPRMC, PIMS (DU).

The blood collected was allowed to clot and later centrifuged to separate the sera. The samples were run on the same day of collection. If a delay was expected, such serum samples were stored in a -20°C deep freezer until the antibody studies were performed [9].

Blood samples were collected from enrolled participants at seven different time intervals: The first sample at day 0 (n=165, before the first dose of the Covishield vaccine). A total of 37 out of 165 participants were positive for antibodies at baseline and were therefore excluded from the study. During the study period, 15 participants developed clinically and laboratory-proven COVID-19. These participants were followed-up separately to ensure that their readings did not influence the actual study. Additionally, three participants left the study. Hence, the final sample size was 110 participants.

In these 110 HCW, subsequent samples were collected as follows. [Table/Fig-1] describes the sample codes for samples collected at different intervals.

The serum sample was subjected to anti-SARS CoV-2 total Ab and IgG assays on VITROS 3600 by Ortho Clinical Diagnostics. The total Ab assay evaluated IgA, IgM, and IgG antibodies together [10].

Sample collection	Time point	Sample code
1 st sample	15 days post 1 st dose	1.15
2 nd sample	30 days post 1 st dose	1.30
3 rd sample	15 days post 2 nd dose	2.15
4 th sample	30 days post 2 nd dose	2.30
5 th sample	60 days post 2 nd dose	2.60
6 th sample	90 days post 2 nd dose	2.90

[Table/Fig-1]: Time points for sample collection.

SARS-CoV-2-specific Chemiluminescence Immunoassay

Both the VITROS Anti-SARS-CoV-2 Total and IgG assays [8,10] (Ortho Clinical Diagnostics, New Jersey, US) are based on CLIA using luminol-Horseradish Peroxidase (HRP)-mediated chemiluminescence. Both assays were performed on the VITROS 3600 automated immunoassay analyser using the Anti-SARS-CoV-2 Total and IgG reagent pack according to the manufacturer's instructions [8,10]. In these assays, specific antibodies against the S protein of SARS-CoV-2 were automatically analysed. Results are reported as signal/cut-off (S/C) values and as qualitative results indicating non reactive (S/C <1.0; negative) or reactive (S/C ≥1.0; positive). The anti-SARS-CoV-2 total assay and IgG combinedly require a minimum of 100 µL serum per assay [11]. The sensitivity for total Ab and IgG Ab is 100% and 90%, respectively, while the specificity for both total Ab and IgG Ab is 100% [8].

Principles for SARS-CoV-2 IgG detection: An immunometric technique is used; this involves a two-stage reaction. In the first stage, antibodies to SARS-CoV-2 present in the sample bind with SARS-CoV-2 spike protein coated on wells. Unbound sample is removed by washing, and in the second stage, HRP-labeled murine monoclonal anti-human IgG antibodies are added in the conjugate reagent. Post that, the conjugate binds specifically to the antibody portion of the antigen-antibody complex. The unbound conjugate is removed by the subsequent wash step if the complexes are not present. The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent is added to the wells. The HRP in the bound conjugate catalyses the oxidation of the luminol derivative, producing light. The electron transfer agent (a substitute dacetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the system. The amount of SARS-CoV-2 IgG antibody present is indicated by the amount of HRP conjugate bound [8].

$$\text{Results [8]} = \frac{\text{Signal for test sample}}{\text{Signal at Cut-off (cut-off value)}}$$

Antibody values were categorised as low, medium, and high levels of protective antibodies when S/CO lay between 1-4.62, 4.62-18.45, and >18.45, respectively, as per Ortho Clinical Diagnostic's Kit Literature [12]. For a given participant, the highest value of IgG Ab from all six samples was considered as the peak IgG value for that participant. Collectively, a categorisation was done for all participants as low, medium, and high levels of protective antibodies [12].

STATISTICAL ANALYSIS

The data collection of the patients was done on a prestructured case record form and then compiled using Microsoft Excel 2013 to create the final master chart. Statistical analysis was performed using Pearson's correlation and regression models in GraphPad Prism 8.0.2 version.

RESULTS

The overall age distribution is depicted in [Table/Fig-2]. There were more male participants 61 (55.5%) than female participants 49 (44.5%). Among the participants, 22 (20%) had a history of

Age (years)	n (%)
<20	14 (12.72)
21-30	24 (21.81)
31-40	36 (32.72)
41-50	11 (10.00)
51-60	17 (15.45)
>60	08 (7.3)

[Table/Fig-2]: Age distribution.

co-morbidities, which included hypertension (6%), diabetes (7%), diabetes and hypertension combined (3%), and others (4%). The blood group distribution is provided in [Table/Fig-3].

Blood group	n (%)
A +	20 (18.18)
A -	02 (1.82)
B +	30 (27.27)
B -	0
O +	43 (39.09)
O -	04 (3.64)
AB +	10 (9.09)
AB -	1 (0.91)

[Table/Fig-3]: Distribution based on blood group.

Effect of vaccination on participants: Fifteen days after the first dose, 101 out of 110 (91.81%) participants became seropositive for total Ab, and 80 out of 110 (72.72%) participants became seropositive for IgG Ab. Thirty days after the first dose, 108 out of 110 (98.18%) participants attained total Ab levels, and 96 out of 110 (87.27%) participants attained IgG levels. Fifteen days after the second dose, all participants were reactive for total Ab, while 109 out of 110 (99.09%) were reactive for IgG Ab. One female participant was a non responder and did not achieve IgG antibody levels throughout the study period. Details are elaborated in [Table/Fig-4].

Effect of age on antibody response: It was observed that the maximum number of participants (33, 30.27%) with peak IgG levels in the medium range were from the age group 31-40 years. Overall, for peak IgG values, there was a negative correlation between age and IgG antibody levels ($r=-0.224$, $p=0.019$) [Table/Fig-5].

Test	Number of participants (n=110)	M	F	<20 years	21-30 years	31-40 years	41-50 years	51-60 years	>60 years
15 days post 1 st dose, Total Ab detectable	101	54	47	14	22	34	11	13	07
15 days post 1 st dose, IgG Ab detectable	80	37	43	12	20	26	09	08	05
30 days post 1 st dose, Total Ab detectable	108	59	49	14	24	36	11	16	07
30 days post 1 st dose, IgG Ab detectable	96	50	46	14	24	31	09	12	06
15 days post 2 nd dose, total Ab detectable	110	61	49	14	24	36	11	17	08
15 days post 2 nd dose, IgG Ab detectable	109	61	48	14	24	35	11	17	08
15 days post 1 st dose, total Ab and IgG both non-detectable	09	07	02	00	02	02	00	03	02
15 days post 1 st dose, total Ab detectable, IgG not detectable	21	17	04	02	02	08	02	05	02
30 days post 1 st dose, total Ab and IgG both non-detectable	02	02	00	00	00	00	00	00	02
30 days post 1 st dose, total Ab detectable, IgG not detectable	12	09	03	00	00	05	02	04	01

[Table/Fig-4]: Participants showing antibody response at different stages of sample collection.

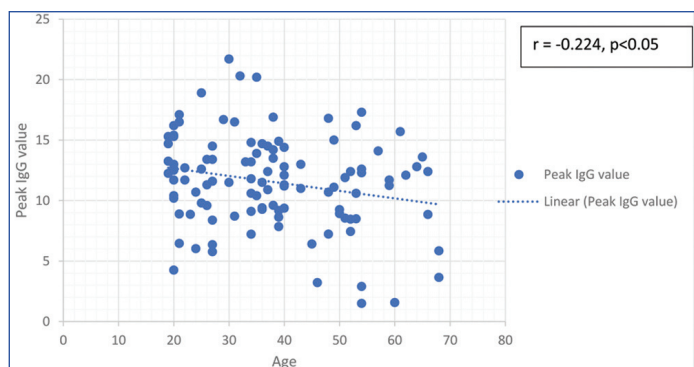
(As all the 110 participants showed seroconversion in the form of total Ab at 15 days post 2nd dose, the data of 30 days, 60 days and 90 days post 2nd dose won't be of significance)
(Also, 109 participants showed seroconversion in the form of IgG Ab at 15 days post 2nd dose. One participant was IgG negative throughout the study).

Peak antibody levels: Peak values were attained in the maximum number of participants at 15 days post-second dose (59, 53.6%), followed by 30 days post-second dose (38, 34.5%), and 30 days post-first dose (4, 3.6%). The detailed demarcation is listed in [Table/Fig-6]. As shown in [Table/Fig-7], the maximum number of participants (99 out of 110) attained a medium level of protective antibodies at their peak IgG levels.

Effect of gender on antibody response: Seroconversion was demonstrated in 87.75% (43 out of 49) of females and 60.65% (37 out of 61) of males at 15 days after the first dose. 93.87% (46 out of 49) of females and 81.96% (50 out of 61) of males demonstrated seroconversion at 30 days after the first dose. With the exception of one female, the rest of the participants, irrespective of gender, turned seropositive for IgG Ab after 15 days of the second dose. It was observed that one 39-year-old female was seronegative throughout the study. No significant association was found between gender and the category of the peak IgG level ($p=0.184$) [Table/Fig-8].

Impact of co-morbidity on levels of SARS-CoV-2 IgG antibodies: A total of 8 (7.27%) participants had a history of diabetes mellitus, while 7 (6.36%) had a history of hypertension. Furthermore, three people were suffering from both diabetes and hypertension. A history of other co-morbidities like hypothyroidism was seen in four participants. Regardless of the co-morbidity status, Covishield induced an increase in IgG antibodies in both groups at all time points after vaccination. Thus, no significant differences in SARS-CoV-2 IgG levels were observed between participants with co-morbidities and those without co-morbidities.

Effect of COVID-19 on antibody levels: During the study period, 15 participants developed clinically and laboratory-confirmed SARS-CoV-2 infection. Strong IgG antibody responses in these cases, with the majority of cases showing IgG cut-off values of more than 20, were observed, and they exhibited a maximum immune response with high protective antibodies after the development of COVID-19 infection. Only one of the cases did not develop a strong immune response even after getting naturally infected. The reason for this may be cellular immunity playing a major role in this person. Additionally, two of the participants who didn't develop COVID-19 infection during the study showed higher antibody levels. This might be due to subclinical infection which went unnoticed due to its asymptomatic nature or hyper antibody response.



[Table/Fig-5]: Scatter diagram based on Pearson's correlation (age with levels of peak IgG).

Sample code	Number of participants showing their peak IgG and % (n=110)	Male	Female	<20 years	21-30 years	31-40 years	41-50 years	51-60 years	>60 years
1.15	02 (1.8)	00	02	01	01	00	00	00	00
1.30	04 (3.6)	01	03	00	03	01	00	00	00
2.15	59 (53.6)	33	26	05	07	21	08	14	04
2.30	38 (34.5)	25	13	07	13	10	03	01	04
2.60	03 (2.7)	00	03	01	00	01	00	01	00
2.90	03 (2.7)	02	01	00	00	02	00	01	00
No Ab	01 (0.9)	00	01	-	-	01 (39 years)	-	-	-

[Table/Fig-6]: Peak IgG in different age groups and gender. (The maximum level of IgG that has been attained by individual participant overall in all the 6 samples. (2 after 1st dose and 4 after 2nd dose of vaccination)

Age group (years)	Categorisation of peak values of IgG		
	Low	Medium	High
<20	01	13	00
21-30	00	22	02
31-40	00	33	02
41-50	01	10	00
51-60	03	14	00
>60	01	07	00
Total* (n=109)	06	99	04

[Table/Fig-7]: Categorisation of peak values of IgG. *As one person was IgG negative throughout the study, the total is 109 instead of 110

Gender	Category of peak IgG			Total	Chi-square	p-value
	Low (1 to <4.62)	Medium (4.62 to 18.45)	High (>18.45)			
Male	5	55	1	61	3.387	0.184
Female	1	44	3	48		
Total	6	99	4	109		

[Table/Fig-8]: Effect of gender on antibody response.

Effect of blood group on antibody response: Only B+ and O+ participants achieved IgG antibody peak at 15 days postfirst dose and 30 days postfirst dose [Table/Fig-9]. Similar findings were observed among different blood groups showing their peak at 15 days after the second dose of vaccination. As shown in [Table/Fig-10], at the end of the study, the maximum number of participants, 54 (49.0%), showed a decline in levels of antibodies and had a low level of protective antibodies, followed by 52 (47.2%) with a medium level and 3 (2.7%) with a high level of protective antibodies. Sixteen (14.5%) became non reactive for IgG Ab during the study period itself. Of these, one (0.9%), six (5.4%), and nine (8.1%) turned non reactive one month (2.30), two months (2.60), and three months (2.90) after the second dose, respectively. One person was non reactive throughout the study. The positive aspect was that all of these participants were atleast reactive for Total Ab. Thus, IgA and IgM antibodies provided support to ward off the virus.

DISCUSSION

The present study provided an analysis of the antibody response to Covishield, the Indian version of the AstraZeneca vaccine. Given that this vaccine was administered to approximately 80% of the Indian population, the findings of this study are of significant importance. The study was conducted during the initial phase of COVID vaccination, with HCWs being the priority group. It is worth emphasising that the vaccine efficacy was tested against the highly transmissible Delta (Δ) variant in a rural setting in western Maharashtra, India.

A total of 109 out of 110 participants tested seropositive for IgG 15 days after receiving the second dose, while all 110 participants were positive for total antibodies after 15 days of the second dose.

The peak (highest protection) was observed in 59 participants (53.6%) 15 days after the second dose and in 38 participants (34.5%) 30 days after the second dose. The summary of findings for the BNT162b2 mRNA (Pfizer) vaccine presented by the European Medicines Agency was used to identify relevant time periods for analysis: 1) 0-14 days after the first dose (no protection observed); 2) >14 days after the first dose and until the second dose (partial protection); 3) 0-7 days after the second dose (not previously evaluated); 4) >7 days after the second dose (highest protection) [13,14].

In the present study, authors observed that the maximum number of participants (n=33, 30.27%) with peak IgG levels in the medium

Blood group (ABO and Rh)	Time interval at which samples showed peak IgG values	Participant split up
A- (02)	2.15	02
A+ (20)	2.15	11
	2.30	06
	2.60	1
	2.90	02
B+ (30)	1.15	1
	1.30	2
	2.15	14
	2.30	12
	2.60	00
	NO AB	1
	B-(0)	-
AB-(01)	2.60	01

AB + (10)	2.15	5
	2.30	5
O-(04)	2.15	02
	2.30	01
	2.60	01
	2.90	00
O + (43)	1.15	01
	1.30	02
	2.15	25
	2.30	14
	2.60	00
	2.90	01
Total		110

[Table/Fig-9]: Immune response in different blood groups.

Age group (years)	Low IgG Ab	Medium IgG Ab	High IgG Ab
<20	06	08	00
21-30	14	10	00
31-40	12	20	03
41-50	08	03	00
51-60	10	07	00
>60	04	04	00
Total (n=109)	54	52	03

[Table/Fig-10]: Antibody levels at the end of study period (sample code- 2.90).

As one person was IgG negative throughout the study, the total is 109 instead of 110

range were from the age group 31-40 years. The peak levels of IgG antibodies were inversely proportional to age, indicating that lower values of peak IgG were recorded with increasing age (r -value=-0.224, p -value=0.019). Similar observations have been reported in many other studies, where postvaccination antibody response is inversely proportional to age [15-18].

A 39-year-old female tested reactive for total antibodies 15 days after the first dose, but during the study period, she consistently tested negative for IgG. She did not have any co-morbidities. Similarly, a 26-year-old female, despite having had COVID-19 during the study period, did not develop robust antibody levels. The predominant isotype induced by a COVID-19 vaccine is IgG, particularly the more protective IgG1 and IgG3 subclasses. However, IgA may also be important in reducing infection of mucosa, epithelial cells in the respiratory tract, and endothelial cells, which are widely targeted by the virus [19]. An alternative explanation could be the earlier innate cellular immune response for viral clearance before the acquired immune response reaches a significant level. In certain studies, patients who did not produce antibodies showed higher levels of neutrophils [20].

This study demonstrated higher levels of protective antibodies in participants who developed COVID-19 during the study phase. Other studies have shown similar findings, indicating that a history of COVID-19 positivity impacts the magnitude and quality of the antibody response after COVID-19 vaccination [21,22]. Prior infection led to quicker and more robust immune responses compared to participants who were infection-naïve [21]. Similar results were observed in the study by Hammerman A et al., where the previously infected group showed a greater antibody response [22]. This suggests that repeated exposures to both vaccination and natural infection (e.g., hybrid immunity) help enhance the protection associated with vaccination. Additionally, none of these participants developed serious illness or required hospitalisation. A similar outcome was observed in the study by Thiruvengadam R et al., where fully vaccinated individuals

were highly protected against severe infection, hospitalisation, and death caused by the virus [23].

It is a well known fact that the antibody immune response to an acquired infection or vaccination brings about two major immune changes. Antibodies produced by the Antibody-Secreting Cells (ASC) provide rapid, protective immunity, and the generation of long-lived memory B cells which can mount recall responses whenever re-exposure occurs. Interestingly, if circulating antibodies fail to confer protection upon re-exposure, memory B cells initiate a recall response by producing new antibodies either through the formation of new ASC or by reinitiating germinal centre reactions to generate new, high-affinity B cell clones via an additional round of somatic hypermutation [24].

Various co-morbid conditions such as hypertension, cardiac disease, kidney disease, diabetes mellitus, and hypothyroidism were analysed to observe associations with differing responses

following COVID-19 vaccination. In this study, co-morbidities did not affect immune responses after Covishield vaccination. However, some studies reported lower antibody levels among individuals with co-morbidities after the second dose, although the difference in antibody levels was minimal [25].

Different blood groups showed their peak response at 15 days after the second dose of vaccination. No significant difference was observed in the response based on blood group. In this study, antibody levels decreased after five months postvaccination but were still detectable. A similar scenario was demonstrated in a study where Anti-S, anti-RBD, and neutralising antibodies remained detectable for at least 6-8 months following vaccination [26,27].

According to the Centers for Disease Control and Prevention (CDC), vaccines and natural infection both result in the early production of serum IgA, IgM, and IgG antibodies [28,29]. They also induce long-lasting memory B- and T-cell responses. Available studies indicate that fully vaccinated individuals and those previously infected with SARS-CoV-2 each have a low risk of subsequent infection for at least six months [30]. Although the antibody immune response is just a part of the immune response, it is easier to detect compared to others due to its widespread use and standardisation [31,32]. Notably, clinical trials have also shown a favourable T-cell response with Covishield up to eight weeks after a single dose [33]. However, for disease prevention, T cells alone are likely less potent than neutralising antibodies. IgG antibodies are produced slowly upon primary exposure to an antigen and then rapidly under secondary or subsequent exposure as part of an "adaptive" immune response, becoming the predominant antibody. The ability of the immune system to "adapt" to foreign substances and create memory against the same antigen is known as immunological memory. This memory enables the body to react more quickly and efficiently to the pathogen in the future [34].

This study helped us understand the immune response after centre and provided insight into the potential effects of age, gender, blood group, and co-morbidity on antibody response.

Limitation(s)

To answer a research question such as the antibody response rate, a community-based study in a larger population with multistage sampling would have been an ideal sampling method.

Authors here had only measured antispikes binding antibodies and were unable to assess neutralising antibodies as well as cell-mediated immune responses.

The mutant circulating during this study period was the Delta (Δ) variant. Other variants of concern, such as Omicron, were not present during the study period, so authors were unable to understand their impact on the antibody response.

CONCLUSION(S)

In the study, participants who developed COVID-19 during the study period experienced only minor symptoms and did not require hospitalisation. The pre-existing antibodies from COVID-19 vaccination may have been useful in combating the virus and causing minimal damage. Therefore, the findings of this study re-emphasise the role and usefulness of vaccination. It is expected that the present findings will facilitate understanding the immune response to the Covishield vaccine in different age groups, genders, and co-morbidities. A decline in seropositivity post-Covishield vaccination emphasises the importance of additional dose(s)/early booster.

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REFERENCES

- [1] Worldometers.info [Internet]. Dover, Delaware, USA. [updated 2024 January 09; cited 2024 January 10]. Available from: <https://www.worldometers.info/coronavirus/>.
- [2] Basta NE and Moodie EMM on behalf of the VIPER (Vaccines, Infectious disease Prevention, and Epidemiology Research) Group COVID-19 Vaccine Development and Approvals Tracker Team. COVID-19 Vaccine Development and Approvals Tracker. (2020). Available from: <https://covid19.trackvaccines.org/our-team/>.
- [3] Ministry of Health and Family Welfare [Internet]. Cowin. India: National health authority [updated 2024 January 09; cited 2024 January 10]. Available from: <https://www.cowin.gov.in/home>.
- [4] Serum Institute of India Pvt., Ltd., Cyrus Poonawala group [Internet]. Pune, India. [updated 2024 January 09; cited 2024 January 10]. Available from: https://www.seruminstitute.com/product_covishield.php.
- [5] Salazar E, Kuchipudi SV, Christensen PA, Eagar T, Yi X, Zhao P, et al. Convalescent plasma anti-SARS-CoV-2 spike protein ectodomain and receptor-binding domain IgG correlate with virus neutralization. *J Clin Invest*. 2020;130(12):6728-38.
- [6] Verma A, Goel A, Katiyar H, Tiwari P, Mayank, Sana A, et al. Durability of ChAdOx1 nCoV-19 (Covishield®) vaccine induced antibody response in healthcare workers. *Vaccines (Basel)*. 2022;11(1):84.
- [7] Venugopal U, Jilani N, Rabah S, Shariff MA, Jawed M, Batres AM, et al. SARS-CoV-2 seroprevalence among healthcare workers in a New York City hospital: A cross-sectional analysis during the COVID-19 pandemic. *Int J Infect Dis*. 2021;102:63-69.
- [8] Orthoclinical diagnostics [Internet], UK, Instructions for use CoV2G Vitros Immunodiagnostic Products Anti-SARS-CoV-2, Version 4.3 Pub. No. GEM1292_US_EN 1 of 11 [updated 2023 January; cited 2024 January]. Available from: https://imgcdn.mckesson.com/CumulusWeb/Click_and_learn/Vitros_Anti-SARS-CoV-2_IgG_510k_IFU.pdf.
- [9] Kanji JN, Bailey A, Fenton J, Robbin Lindsay L, Dibernardo A, Toledo NP, et al. Stability of SARS-CoV-2 IgG in multiple laboratory conditions and blood sample types. *J Clin Virol*. 2021;142(1):01-05.
- [10] Orthoclinical diagnostics [Internet], UK, Instructions for use CoV2T Vitros immunodiagnostic products Anti-SARS-CoV-2 total reagent pack, version 3.3 Pub. No. GEM1293_US_EN 1 of 11 [updated 2023 January; cited 2024 January]. Available from: https://imgcdn.mckesson.com/CumulusWeb_Click_and_learn/Vitros_Anti-SARS-CoV-2_Total_N_Antibody_Test_IFU.pdf.
- [11] Theel ES, Harring J, Hilgart H, Granger D. Performance characteristics of four high-throughput immunoassays for detection of IgG antibodies against SARS-CoV-2. *J Clin Microbiol*. 2020;58(8):01-11.
- [12] Joyner MJ, Senefeld JW, Klassen SA, Mills JR, Johnson PW, Theel ES, et al. Effect of convalescent plasma on mortality among hospitalized patients with COVID-19: Initial three-month experience, the US EAP COVID-19 Plasma Consortium; medRxiv. 2020.08.12.20169359.
- [13] Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine. *N Engl J Med*. 2020;383(27):2603-15.
- [14] Agency EM. Assessment report Comirnaty common name: COVID-19 mRNA vaccine (nucleoside modified) 2020. [Internet]. [cited 2021 Feb 22]. Available from: https://www.ema.europa.eu/en/documents/assessment-report/comirnatyepar-public-assessment-report_en.pdf.
- [15] Wang P, Liu L, Nair MS, Yin MT, Luo Y, Wang Q, et al. SARS-CoV-2 neutralizing antibody responses are more robust in patients with severe disease. *Emerg Microbes Infect*. 2020;9(1):2091-93.
- [16] Naaber P, Tserel L, Kangro K, Sepp E, Jürjenson V, Adamson A, et al. Antibody response after COVID-19 mRNA vaccination in relation to age, sex, and side-effects. medRxiv. 2021 May Available from: <https://www.medrxiv.org/content/10.1101/2021.04.19.21255714v2.article-info>.
- [17] Huang YP, Gauthey L, Michel M. The relationship between influenza vaccine-induced specific antibody responses and vaccine-induced nonspecific autoantibody responses in healthy older women. *J Gerontol*. 1992;47(2):M50-M55.
- [18] Goodwin K, Viboud C, Simonsen L. Antibody response to influenza vaccination in the elderly: A quantitative review. *Vaccine*. 2006;24(8):1159-69.
- [19] Speiser DE, Bachmann MF. COVID-19: Mechanisms of vaccination and immunity. *Vaccines*. 2020;8(3):404.
- [20] Newton AH, Cardani A, Braciale TJ. The host immune response in respiratory virus infection: Balancing virus clearance and immunopathology. *Semin Immunopathol*. 2016;38(4):71-82.
- [21] Tut G, Lancaster T, Krutikov M, Sylla P, Bone D, Kaur N, et al. Profile of humoral and cellular immune responses to single doses of BNT162b2 or ChAdOx1 nCoV-19 vaccines in residents and staff within residential care homes (IVALDI): An observational study. *Lancet Healthy Longev*. 2021;2:e544-53. Available from: <https://www.thelancet.com/action/showPdf?pii=S2666-7568%2821%2900168-9>.
- [22] Hammerman A, Sergienko R, Friger M, Beckenstein T, Peretz A, Netzer D, et al. Effectiveness of the BNT162b2 vaccine after recovery from COVID-19. *N Engl J Med*. 2022;386(13):1221-29.
- [23] Thiruvengadam R, Awasthi A, Medigeshe G, Bhattacharya S, Mani S, Sivasubbu S, et al. Effectiveness of ChAdOx1 nCoV-19 vaccine against SARS-CoV-2 infection during the delta (B.1.617.2) variant surge in India: A test-negative, case-control study and a mechanistic study of post-vaccination immune responses. *Lancet Infect Dis*. 2022;22(4):473-82.
- [24] Kurosaki T, Komatani K, Ise W. Memory B cells. *Nat Rev Immunol*. 2015;15(3):149-59.
- [25] Hoque A, Barshan AD, Chowdhury FUH, Fardous J, Hasan MJ, Khan MAS, et al. Antibody response to ChAdOx1-nCoV-19 vaccine among recipients in Bangladesh: A prospective observational study. *Infect Drug Resist*. 2021;14(1):5491-500.
- [26] Barouch DH, Stephenson KE, Sadoff J, Yu J, Chang A, Gebre M, et al. Durable humoral and cellular immune responses following Ad26.COV2.S vaccination for COVID-19. medRxiv, 2021: p. 2021.07.05.21259918. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8282116/pdf/nihpp-2021.07.05.21259918v1.pdf>.
- [27] Doria-Rose N, Suthar MS, Makowski M, O'Connell S, McDermott AB, Flach B, et al. Antibody persistence through 6 months after the second dose of mRNA-1273 vaccine for COVID-19. *N Eng J Med*. 2021;384(23):2259-61.
- [28] Wang Z, Schmidt F, Weisblum Y, Muecksch F, Barnes CO, Finkin S, et al., mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. *Nature*. 2021;592(7855):616-22.
- [29] Campillo-Luna J, Wisniewski AV, Redlich CA. Human IgG and IgA responses to COVID-19 mRNA vaccines. medRxiv. 2021;16(6):e0249499. Available from: <https://pubmed.ncbi.nlm.nih.gov/34133415/>.
- [30] National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases. CDC COVID-19 Science Briefs [Internet]. Atlanta (GA): Centers for Disease Control and Prevention (US); 2020-. Science Brief: SARS-CoV-2 Infection-induced and Vaccine-induced Immunity. 2021 Oct 29. PMID: 34748301. Available from: <https://pubmed.ncbi.nlm.nih.gov/34748301/>.
- [31] Gundlapalli AV, Salerno RM, Brooks JT, Averhoff F, Petersen LR, McDonald LC, et al. SARS-CoV-2 serologic assay needs for the next phase of the US COVID-19 pandemic response. *Open Forum Infect Dis*. 2020;8(1):ofaa555. Available from: <https://ncbi.nlm.nih.gov/pmc/articles/PMC7717402/pdf/ofaa555.pdf>.
- [32] Zimmermann P, Curtis N. Factors that influence the immune response to vaccination. *Clin Microbiol Rev*. 2019;32(2):1-50. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6431125/pdf/CMR.00084-18.pdf>.

- [33] Ewer KJ, Barrett JR, Belij-Rammerstorfer S, Sharpe H, Makinson R, Morder R, et al. T cell and antibody responses induced by a single dose of ChAdOx1 nCoV-19 (AZD1222) vaccine in a phase 1/2 clinical trial. *Nat Med.* 2021;27(6):270-78. Available from: <https://pubmed.ncbi.nlm.nih.gov/33335323/>.
- [34] Yang Z-Y, Kong W-P, Huang Y, Roberts A, Murphy BR, Subbarao K, et al. A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. *Nature.* 2004;428(6982):561-56.

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