Article

Durability of ChAdOx1 nCoV-19 (Covishield®) Vaccine Induced Antibody Response in Health Care Workers

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Abstract: (i) Background: ChAdOx1 nCoV-19 (Covishield®) vaccine is widely used in India. We studied the Covishield® induced antibody response and its durability among health care workers (HCWs) (ii) Method: HCWs received two doses (0.5 mL) four weeks apart. Blood specimens, collected before each dose, day (D) 60, D150 and D270 after second dose, were tested for anti-spike antibody (ASAb) titre and neutralising antibody (%) (NAb) using Elecsys Anti-SARS-CoV-2 S (Roche) and SARS-CoV-2 NAb ELISA Kit (Invitrogen), respectively. Data are expressed as proportions and median (interquartile range) and compared using non-parametric (iii) Result: Among 135 HCWs (83 males; age 45 (37–53); 36 had pre-existing ASAb), 29 (21.5%) acquired COVID-19 after 60 (39–68) days of vaccination. ASAb titre before second dose and at D60, D150, D270 were 77.2 (19.4–329.4), 512 (114.5–9212), 149 (51.6–2283) and 2079 (433.9–8644) U/mL, respectively. Compared to those without pre-existing ASAb, titres were significantly higher before second dose (5929 vs. 41, $p < 0.001$), D60 (3395 vs. 234, $p = 0.007$) and D150 (1805 vs. 103, $p < 0.001$) in participants with pre-existing ASAb; NAb were also higher (80 vs. 18, $p < 0.001$) before second dose. Between those who acquired infection or not after vaccination, ASAb titres were comparable before second dose (77 vs. 78, $p = 0.362$) but significantly higher at D60 (14,019 vs. 317, $p < 0.001$) and D150 (2062 vs. 121, $p = 0.002$) in the former group, though NAb percentage were higher at D60 (87 vs. 27, $p < 0.001$) and D150 (79 vs. 25, $p = 0.007$) only (iv) Conclusions: Covishield® induces a higher antibody titre in those with pre-existing ASAb. The vaccine induced antibody starts falling 5 months after vaccination.

Keywords: COVID-19; coronavirus; Covishield; COVID vaccine; Anti-SARS-CoV-2 antibody; neutralising antibody

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiological agent of COVID-19 illness, has caused over 0.6 billion virologically confirmed cases and over 6.5 million deaths [1] The pandemic has posed an unprecedent professional risk for health

care workers (HCWs). Estimate suggests ~10% of the total burden of SARS-CoV-2 infection was borne by HCWs [2]. As compared to the community, HCWs had 11.6 times higher risk of testing positive for COVID-19 nucleic acid test [3]. Further, HCW had a 7.4 times higher risk of developing severe Covid as compared to non-essential workers [4]. According to the World Health Organization (WHO), 135 million HCWs were deployed between January 2020 and May 2021 to combat the pandemic and we lost 80,000–180,000 due to COVID-19 [5].

Coherent and joint efforts of the scientific community, internal health organizations, government agencies and the pharmaceutical industry led to the discovery of several vaccines against corona virus in a very short time period. After completion of necessary regulatory trials, front-line workers, including HCWs, were given corona vaccine as a priority. The ChAdOx1 nCoV-19 vaccine (AZD1222), developed at Oxford University, consists of a replication-deficient chimpanzee adenoviral vector ChAdOx1. It contain SARS-CoV-2 spike protein gene and has shown promising protective efficacy in large trials [6–8]. In India, this vaccine is manufactured by Serum Institute of India (Covishield®) and over 1.75 billion doses have been administered to date. The real-life data of Covishield® shows promising results among HCWs, patients with liver cirrhosis [9], renal transplant recipients [10] and those on maintenance hemodialysis [11].

At present, data are limited on the persistence of protective antibodies in vaccine recipients. This study was conducted to study the serological immune response and temporal pattern of vaccine induced antibody response among HCWs who had received two doses of Covishield® vaccine.

2. Methods

This prospective, observational, cohort study was conducted between January 2021 and November 2021 in the Covid vaccination facility of Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India. The HCWs of the institute, who were planned for vaccination were invited through electronic media (email, WhatsApp, text message) to participate in our study. Those who voluntarily agreed to participate were asked to contact our study center at the vaccination site. After obtaining written informed consent, they were requested to complete a data collection form followed by donation of their blood specimens. We enrolled all the participants from a single vaccination site. Participants were excluded if they were already vaccinated with any COVID-19 vaccine or had nucleic acid confirmed Covid infection in the preceding eight weeks.

Two intramuscular 0.5 mL doses of Covishield® were given four weeks apart. All the doses were administered in the vaccination facility established and manned by the state government. The vaccine dose and dosing interval were as dictated in the guidelines laid by the Government of India. The guidelines were in coherence with the contemporary literature [7]. Following each dose, recipients were observed for 30 min followed by telephonic contact for the next 48 h to report any significant adverse event.

Specimen collection: We collected a 5.0 mL blood specimen at five-time points from each of the participants (a) within 48 h before administering the first dose, (b) within 48 h prior to the administration of second dose, (c) at 60 ± 7 days after second dose, (d) at 150 ± 14 days after second dose and (e) at 270 ± 14 days after second dose (Figure 1). Serum was separated, within one hour of its collection, by centrifugation at $4000 \times g$ for 10 min at 4 °C and stored in multiple aliquots at -80 °C temperature for serological testing at the end of the study.

Serological testing: Sera were tested for anti-spike antibody (ASAb) titre and proportion of neutralising antibody (NAb) using Elecsys Anti-SARS-CoV-2 S (Roche Diagnostics, GmbH, Campus Vienna Biocenter 2, Vienna, Austria) and SARS-CoV-2 Neutralising Antibody ELISA Kit (Invitrogen, Catalog no BMS2326, Thermo-Fisher Scientific, Third Avenue, Waltham, MA, USA), respectively. Elecsys Anti-SARS-CoV-2 S immunoassay is an in vitro quantitative assay for antibodies against spike (S) protein receptor-binding domain (RBD) in humans. The test principle is based on an automated system's double-antigen sand-

wich assay format. The assay had a sensitivity of 98.8% and specificity of 99.98%. The limit of quantitation for the assay is 0.40–250 U/mL. The specimens with antibody titre above 250 U/mL were serially diluted ×20, ×50 and ×100 fold to obtain results within the detection range. The titres that remained above the quantitation limit after 100-fold dilutions were reported as >25,000 U/mL. The antibody concentrations are expressed as U/mL and a value $\geq$ 0.80 U/mL is considered positive for the ASAb. The SARS-CoV-2 Neutralising Antibody ELISA Kit is a competitive ELISA assay. Specimens with calculated neutralisation $\geq$ 20% are considered positive. Both the assays were performed following the manufacturer's recommendations.
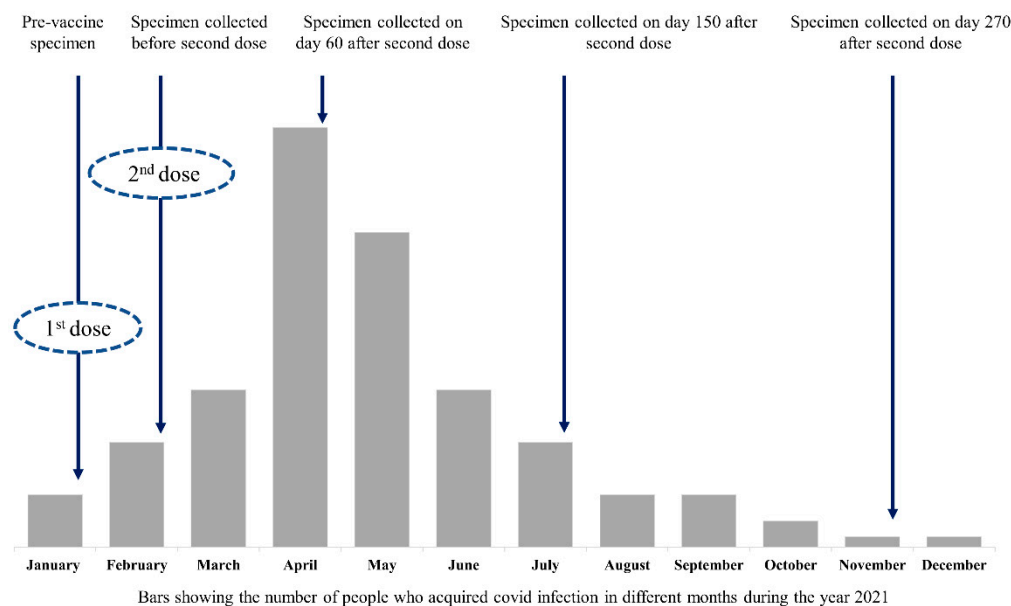

Figure 1. Temporal pattern of the Covid pandemic in the city, time of vaccination and time of blood specimen collection over the period of study.

The trial data from India, available at the time of the start of the study suggested that 79% of people develop anti-Covid antibody after one dose [12]. We expected 75% response in our study, 10% relative error in the assumed response (75% $\pm$ 7.5%), two-sided 95% confidence interval, and estimated minimum required sample size was 129. After including expected loss of follow up, we targeted to include 150 HCWs. We could enroll and follow 135 participants.

Qualitative and quantitative data are expressed as numbers, proportions and median (interquartile range). Paired numerical data are compared using a non-parametric Wilcoxon signed-rank test. The data between the groups are compared using the Mann-Whitney test. The analyses were carried out using STATA 16 software. The level of significance was kept at <0.05. Our institute ethics committee approved the study (2021-16-IMP-EXP-118) and the participants were enrolled after obtaining written informed consent.

3. Results

We included data from 135 participants. Clinical characteristics of the participants are summarized in Table 1. Among the 17 participants, (12.6%) who had nucleic acid test confirmed prior COVID-19 infection 163 (145–174) days before the first dose, 14 (82.4%) had detectable ASAb and antibody titre was 82 (55–376) U/mL, and 10 of 17 (58.8%) tested positive for Nab (30.2 (13.6–40)).

Of the 135 participants, 36 (26.7%) had detectable ASAb before vaccination and the titre was 114.5 (37.1–327) U/mL. Only 14 of 36 (38.9%) had symptomatic, nucleic acid test confirmed prior COVID-19 infection which indicate that 61.1% of infection remained asymptomatic. The ASAb titres of those with symptomatic (191.5 [70.3–520.6] U/mL)

or asymptomatic (70.4 (7.2–279.3) U/mL) prior COVID-19 infection were comparable (*p* = 0.105). None of the 135 participants acquired COVID-19 infection between the administration of the two doses of vaccine but 29 (21.5%) acquired infection 60 (39–68) days after receiving the second dose. None of those who acquired infection after vaccination had serological evidence of prior covid infection. The vaccine effectiveness against delta variant related clinical illness was 79.5%. The ASAb titre and NAb percentage in the overall cohort are summarized in Table 2. The ASAb titre and proportion of NAb in log (natural) scale at various time points are depicted in Figure 2.

We compared the ASAb and NAb response of the participants with or without detectable ASAb before vaccination (Table 3). As compared to those without pre-existing ASAb, significantly higher ASAb titres were achieved in those with pre-existing ASAb at day 28 (*p* < 0.001), day 60 (*p* = 0.007) and day 150 (*p* < 0.001) but their levels were comparable at day 270 (*p* = 0.333) after second dose (Figure 3a). The NAb percentages were significantly higher at day 28 (*p* < 0.001) but were comparable at day 60 (*p* = 0.255), day 150 (*p* = 0.155) and day 270 (*p* = 0.626) (Figure 3b).

We compared the ASAb and NAb between those who acquired COVID-19 infection (*n* = 29) or not (*n* = 106) after complete vaccination (Table 4). Their ASAb titres were comparable after first dose (*p* = 0.362) but were significantly higher at day 60 (*p* < 0.001) and day 150 (*p* = 0.002) in those who acquired infection. The ASAb titres again became comparable at day 270 (*p* = 0.306). Similarly, their NAb percentages were comparable at day 28 (*p* = 0.071) but significantly higher at day 60 and 150 in those who developed infection. The NAb again became comparable between the groups at day 270 (*p* = 0.206).

Among 99 participants without detectable ASAb before vaccination, 94 and 99 developed ASAb after first dose and second dose of vaccine and all had detectable ASAb in follow-up specimens collected at days 60, day 150 and day 270 after second doses (Table 5). The NAb was increased up to day 60 followed by a decrease in day 150 specimen. In follow-up at day 270, NAb proportion again increased from 54.5% to 73.4%.

Table 1. Characteristics of the study participants (*n* = 135).

Variable	Value
Males	83 (61.5)
Age (years)	45 (37–53)
Associated conditions	
Hypertension	15 (11)
Diabetes mellitus (DM)	7 (5)
Hypothyroidism	5 (4)
Bronchial asthma	3 (2)
Coronary artery disease	2 (2)
Rheumatoid arthritis	2 (2)
Osteoarthritis	1 (1)
Paroxysmal supraventricular tachycardia	1 (1)
Bronchial asthma + hypertension	1 (1)
Total	37 (27)
Body mass index (Kg/M^2)	25.2 (22.9–27.6)
Prior COVID-19 infection	17 (13)

Categorical data are presented as number and proportions; numerical data are expressed as median (interquartile range).

Table 2. Temporal pattern of vaccine induced COVID-19 antibody in overall cohort (*n* = 135).

COVID-19 Antibody	Time of Specimen Collection (Days)			
	After the First Dose	**60 Days after Second Dose**	**150 Days after Second Dose**	**270 Days after Second Dose**
Anti-Spike antibody	77.2 (19.4–329.4)	512 (114.5–9212)	149 (51.6–2283)	2079 (433.9–8644)
Neutralising antibody (%)	22.1 (8.5–54.2)	52.3 (7.7–92.7)	41.1 (3.8–86.4)	58.6 (17.6–85.1)

Anti-spike antibody titre is expressed as U/mL and neutralising antibody is expressed in percentage. The anti-spike antibody titre value ≥ 0.80 U/mL is considered positive. The specimens with calculated neutralisation ≥ 20% are considered positive for neutralising antibody. Data are expressed as median (inter quartile range).

Table 3. Comparison of COVID-19 antibody response between those with or without pre-existing anti-spike antibody before vaccination in overall cohort (*n* = 135).

Anti-COVID Antibody	COVID-19 Antibody	Time of Specimen Collection (Days)			
		Before Second Dose at 28 Days	**60 Days after Second Dose**	**150 Days after Second Dose**	**270 Days after Second Dose**
Anti-Spike antibody (U/mL)	Anti-spike antibody was absent before vaccination (*n* = 99)	40.9 (11.8–118.3)	234.3 (96.3–9268)	102.6 (41.4–1837)	1858 (215.8–6784)
		p < 0.001	*p* = 0.007	*p* < 0.001	*p* = 0.333
	Anti-spike antibody was present before vaccination (*n* = 36)	5929.5 (649.4–16,207.5)	3395 (800.1–8852)	1805 (201.9–4131)	2423.5 (707.1–9552)
Neutralising antibody (%)	Anti-spike antibody was absent before vaccination (*n* = 99)	18.3 (5.9–32)	36.6 (7.4–91.9)	26.7 (3.6–85.5)	63.4 (17.6–85.4)
		p < 0.001	*p* = 0.255	*p* = 0.155	*p* = 0.626
	Anti-spike antibody was present before vaccination (*n* = 36)	80 (24.1–95.8)	69.9 (12.6–94.2)	62.1 (13.2–89)	53.6 (17–84.1)

Anti-spike antibody titre is expressed as U/mL and neutralising antibody is expressed in percentage. The anti-spike antibody titre value ≥ 0.80 U/mL is considered positive. The specimens with calculated neutralisation ≥ 20% are considered positive for neutralising antibody. Data are expressed as median (interquartile range).

Table 4. Comparison of COVID-19 antibody response between the participants who acquired COVID-19 infection (*n* = 29) or not (*n* = 106) after the second dose of vaccine (*n* = 135) in overall cohort.

COVID-19 Antibody	Time of Specimen Collection (Days)							
	Before the Second Dose		**60 Days after Second Dose**		**150 Days after Second Dose**		**270 Days after Second Dose**	
	Acquired COVID-19 Infection	**Not Acquired COVID-19 Infection**	**Acquired COVID-19 Infection**	**Not Acquired COVID-19 Infection**	**Acquired COVID-19 Infection**	**Not Acquired COVID-19 Infection**	**Acquired COVID-19 Infection**	**Not Acquired COVID-19 Infection**
Anti-Spike antibody (U/mL)	76.8 (23.4–200.4)	78.3 (19.3–684.9)	14,019 (4546–25,000)	316.7 (102.3–3447)	2062 (139–4317)	120.7 (50.9–1584)	2185 (1414–4611)	2052 (215.8–10,177)
p value	0.362		<0.001		0.002		0.306	
Neutralising antibody (%)	18 (7.6–27.5)	24.5 (8.7–66.6)	86.9 (66.6–97)	27.3 (5.7–83.1)	79.3 (45–90.8)	24.6 (3.3–85)	67.7 (54–84.7)	50.3 (12.6–85.2)
p value	0.071		<0.001		0.007		0.206	

Anti-spike antibody titre is expressed as U/mL and neutralising antibody is expressed in percentage. The anti-spike antibody titre value ≥ 0.80 U/mL is considered positive. The specimens with calculated neutralisation ≥ 20% are considered positive for neutralising antibody. Data are expressed as median (inter quartile range).
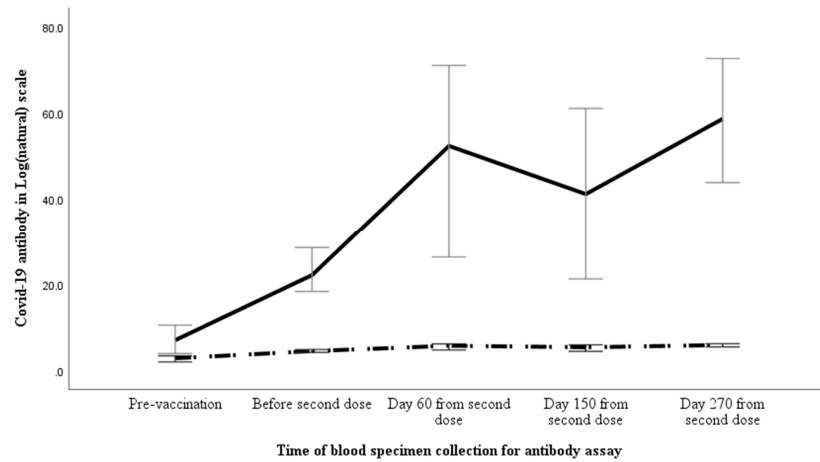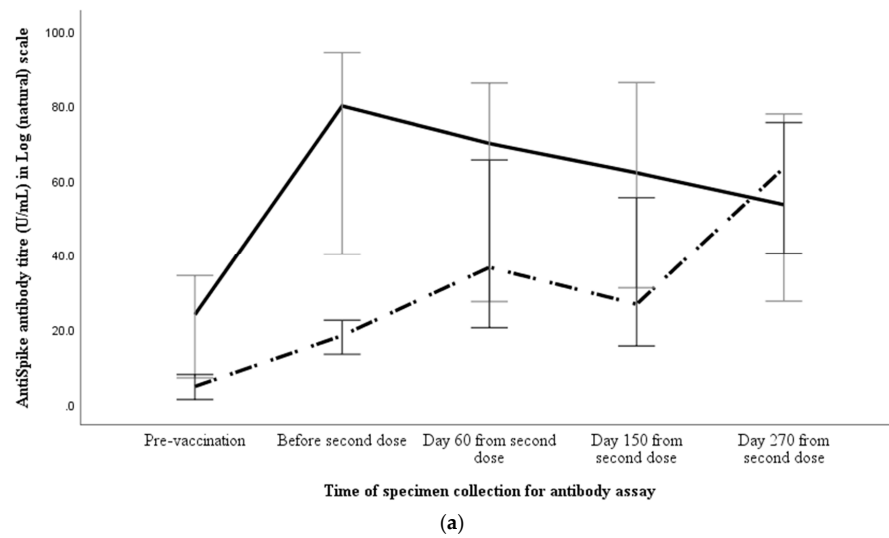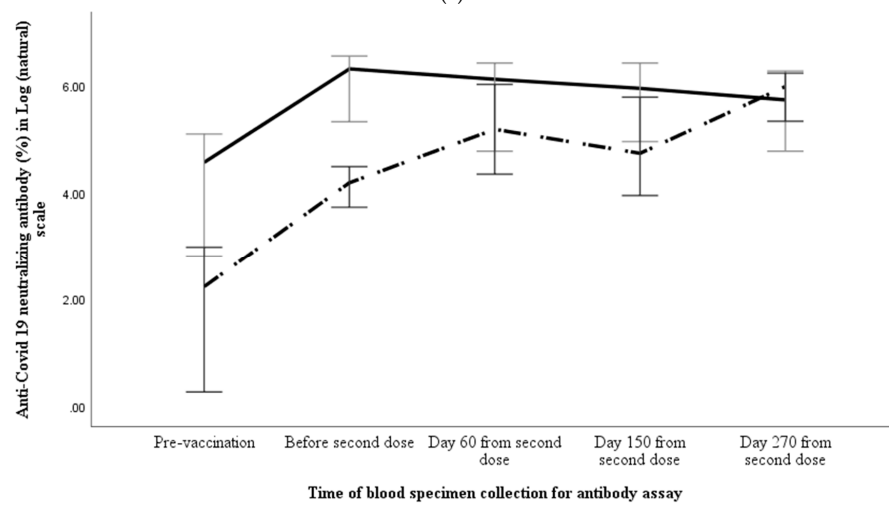
Figure 2. Temporal pattern of anti-spike antibody (solid line) and percentage of neutralising antibody (broken line) in study participants. The ASAb and NAb results along with 95% error bar are shown in Log (natural) scale along the *Y* axis, whereas *X* axis shows the time of specimen collection.

(**a**)

(**b**)

Figure 3. Comparison of anti-spike antibody titre (**a**) and percentage of neutralising antibody (**b**) between those with (solid line) or without (broken line) detectable anti-spike antibody before vaccination. The anti-spike antibody and neutralising antibody results, along with 95% error bar, are shown in Log (natural) scale along the *Y* axis, whereas *X* axis shows the time of specimen collection.

Table 5. Proportion of participants, without anti-spike antibody before vaccination, who developed COVID-19 antibody after vaccination (*n* = 99).

COVID-19 Antibody	Time of Specimen Collection (Days)			
	After the First Dose	60 Days after Second Dose	150 Days after Second Dose	270 Days after Second Dose
Anti-Spike antibody (%)	94 (95)	99 (100)	99 (100)	99 (100)
Neutralising antibody (%)	46 (46.5)	61 (61.6)	54 (54.5)	73 (73.4)

The anti-spike antibody titre value ≥ 0.80 U/mL is considered positive. The specimens with neutralisation $\geq 20\%$ are considered positive.

Following vaccination, 29 of 99 (29.3%) acquired Covid infection. On further analysis between those who acquired (*n* = 29) or remained protected (*n* = 70), the ASAb titre was comparable between the groups after the first dose of vaccine but the levels were significantly higher at day 60 and day 150 among those who developed infection after vaccination, though the titre became comparable between the groups at day 270 (Table 6).

Table 6. Comparison of COVID-19 antibody response between those who acquired COVID-19 infection (*n* = 29) or not (*n* = 70) after the second dose of vaccine in the cohort of those without detectable anti-spike antibody before vaccination (*n* = 99).

COVID-19 Antibody	Time of Specimen Collection (Days)							
	Before the Second Dose		60 Days after Second Dose		150 Days after Second Dose		270 Days after Second Dose	
	Acquired COVID-19 Infection	Not Acquired COVID-19 Infection	Acquired COVID-19 Infection	Not Acquired COVID-19 Infection	Acquired COVID-19 Infection	Not Acquired COVID-19 Infection	Acquired COVID-19 Infection	Not Acquired COVID-19 Infection
Anti-Spike antibody (U/mL)	76.8 (23.4–200.4)	36.2 (10.2–98.6)	14,019 (4546–25,000)	147.5 (85.4–387.7)	2062 (139–4317)	64.3 (37.4–154.3)	2185 (1414–4611)	1450 (128–12,885)
p value	0.052		<0.001		<0.001		0.147	
Neutralising antibody (%)	18 (7.6–27.5)	18.4 (5.8–32.9)	86.9 (66.6–97)	21 (5.3–65.5)	79.3 (45–90.8)	16.1 (2.8–64.8)	67.7 (54–84.7)	48.7 (12.6–85.8)
p value	0.936		<0.001		0.001		0.277	

Anti-spike antibody titre is expressed as U/mL and neutralising antibody is expressed as a percentage. The anti-spike antibody titre value ≥ 0.80 U/mL is considered positive. The specimens with calculated neutralisation $\geq 20\%$ are considered positive for neutralising antibody. Data are expressed as median (interquartile range).

4. Discussion

Frontline workers such as HCWs and security personnel received Covid vaccine as a priority in January 2021 in our country. In accordance with the contemporary scientific evidence, two 0.5 mL doses of ChAdOx1 nCoV-19 vaccine were administered at an interval of four weeks. All the HCWs who participated in our study had received the two doses by the end of February 2021. Soon after the complete vaccination of study participants, the second wave of the Covid pandemic due to the 'Delta variant' started in the country.

We studied the temporal pattern of persistence of vaccine induced antibody up to 270 days after complete vaccination in 135 HCWs. We found that one fourth (26.7%) of HCWs had detectable ASAb before vaccination as evidence of prior COVID-19 infection, though only half had symptomatic Covid infection. Participants without pre-existing antibody developed detectable ASAb following a single dose of vaccine and these antibodies remained detectable at least for a period of nine months after complete vaccination. During the follow-up of nine months after complete vaccination, 21% of the subjects acquired symptomatic COVID-19 infection. This suggests a vaccine protection rate of 79%. We had not looked for asymptomatic Covid infection in vaccine recipients. In presence of pre-existing ASAb, the vaccine administration induced much higher antibody titres, though this booster effect waned after 9 months from the second dose of vaccine. In addition, data

suggest that a natural Covid infection, following complete vaccination, further enhances the antibody response, which is sustained for a few months.

Twenty percent of HCWs had detectable ASAb prior to vaccination suggesting prior infection. Several studies from India have shown high seroprevalence of antibodies to SARS-CoV-2 virus among HCW, though the seroprevalence varies from 2.5% in Kashmir [13] to 25% in New Delhi [14]. In India, the seroprevalence were higher in studies conducted in a sparsely populated hilly area [13,15] as compared to that reported from densely populated metropolitan cities [14,16,17]. High seroprevalence among HCWs is likely to be related to their inherent risk for repeated exposure and high-level of exposure during patient care.

Among those with pre-existing antibodies before vaccination, only half had symptomatic COVID-19 infection. A recent systematic review of 350 studies from across the world have estimated that 35.1% (95% CI: 30.7 to 39.9%) of infections were asymptomatic [EN_419]. A slightly higher proportion of asymptomatic infection in our study might be due to the fact that HCWs enrolled in our study might have ignored mild illness because of their professional responsibilities, according to the need of the hour everywhere.

Following vaccination, nearly all the participants seroconverted, similar to in other studies, conducted in HCWs, which reported a seroconversion rate of 99.5% [18] and 97.1% in the United Kingdom [19]. The high rates of seroconversion seen in our study might have been influenced by occurrence of asymptomatic natural infection following vaccination, because a second wave of corona virus started soon after the participants received their second dose of vaccine.

Second dose boosted the immune response by more than 6 fold (from 77 U/mL to 512 U/mL). The original AZD1222 vaccine in trial also showed a booster effect from the second dose [7]. Non-inferiority of Covishield® over original AZD1222 vaccine is proven in a good quality, double-blinded, randomized controlled trial [20]. This study also showed a 4 fold rise in ASAb (9786 AU to 38,576 AU) titre after second dose of vaccine. We have previously reported a 36-fold rise in antibody titre after two doses in immunosuppressed renal transplant recipients [10]. As compared to those who did not have ASAb (Table 3), the booster effect in participants with pre-existing ASAb showed peculiar features of marked booster effect after first dose without additional immune boosting after second dose. This suggests that the booster effect of vaccine is likely to be more intense in the presence of natural infection induced immunity as compared to vaccine induced immunity. Probably, in the presence of prior natural infection, a single dose of vaccine may be sufficient to achieve protective effect. Similar booster effects in the presence of prior natural infection have been reported by other groups [21,22]. Similarly, a natural infection had a booster effect even after the completion of a two dose schedule. Among those without evidence of prior natural infection, the ASAb titres were much higher on day 60, 150 and 270 in those who acquired natural infection as compared those who remained protected (Table 6).

The ASAb titre peaked at day 60 followed by rapidly cline. The serum ASAb titres were markedly reduced at day 150 (Table 2). Though, data on durability of vaccine induced antibodies are limited [23], the decline in natural infection induced antibody titre with time is reported [24]. In people on anticancer therapy, the ChAdOx1-nCoV-19 vaccine-induced antibody is shown to start decline soon after the second dose [23]. A large population-based cohort study from Brazil (*n* = 42,558,839) and Scotland (*n* = 1,972,454) showed that, after completion of a two dose schedule of ChAdOx1-nCoV-19 vaccine, its effectiveness against both severe COVID-19 and symptomatic infection diminished over time. This decline in effectiveness starts as soon as 5–7 weeks after the second dose. Such a decline in vaccine effectiveness is a surrogate marker of declining vaccine induced immunity [25]. We observed a rise in ASAb titre at day 270. This could be related to subclinical infection during the ongoing delta wave between day 150 and day 270. The delta wave was waning at that time but HCWs were still in contact with patients.

The vaccine effectiveness against delta variant to prevent clinical illness in overall cohort was 79.5%. All those who acquired covid infection after vaccination had no evidence of either clinical or serological prior covid infection. Among those who did not have ASAb

before vaccination, the vaccine effectiveness was 70.3%. Combined data from four large randomized trials, which used Astra Zeneca vaccine, showed a vaccine efficacy of 62.1% [8]. The studies from India showed that complete vaccination with Covishield® yield an efficacy of up to 88.6% [26]. Other studies from India have also shown that ChAdOx1-nCoV-19 vaccine led to reduced severity of illness [27] as well as risk of mortality during the second wave of the pandemic [28].

Our study had a few strengths. All the participants were recruited and vaccinated within a very short period. The blood specimens, from all participants, were also collected over a period of 2–3 days which reduced the chance for variable risk of natural infection among the participants. We studied the temporal pattern of vaccine-induced antibody response. We had data on pre-vaccination ASAb status which helped us to study the effect of vaccine on those with or without prior covid infection. Our participants, during and after vaccination, were actively involved in providing services to infected patients, which consistently posed a threat for breakthrough infection; hence the clinical effectiveness reflected in our data is an underestimation of vaccine effectiveness in the community.

The limitations of our study include lack of an unvaccinated control group, which limit the analysis of effectiveness of the vaccine; lack of genomic sequencing of the variant of the coronavirus which caused break through infection is another important limitation of our study.

Several questions remain unanswered and their answers will be needed in future. We will need to follow our cohort to know the long-term durability, antibody titre, neutralising capacity and effectiveness of vaccine-induced antibodies in either preventing new infections or reducing the severity of clinical illness following infection with novel variants, which could appear in future. Further, we also need to study the cell mediated immunity achieved with vaccination. Further data will be needed to study the effect of dosing interval on the durability of immune response.

5. Conclusions

ChAdOx1 nCoV-19 (Covishield®) vaccine induces detectable ASAb and NAb in all recipients after two doses. The antibody titre induced by vaccination is much higher in those who have had prior covid infection. The vaccine induced antibody persists for at least two months and starts falling 5 months after second dose.

References

1. WHO Board on COVID 19 Disease. Available online: https://www.who.int/emergencies/diseases/novel-coronavirus-2019 (accessed on 4 October 2022).

2. Papoutsi, E.; Giannakoulis, V.G.; Ntella, V.; Pappa, S.; Katsaounou, P. Global burden of COVID-19 pandemic on healthcare workers. *ERJ Open Res.* **2020**, *6*, 00195-2020. [CrossRef] [PubMed]

3. Nguyen, L.H.; Drew, D.A.; Graham, M.S.; Joshi, A.D.; Guo, C.G.; Ma, W.; Mehta, R.S.; Warner, E.T.; Sikavi, D.R.; Lo, C.H.; et al. Risk of COVID-19 among front-line health-care workers and the general community: A prospective cohort study. *Lancet Public Health* **2020**, *5*, e475–e483. [CrossRef] [PubMed]

4. Mutambudzi, M.; Niedzwiedz, C.; Macdonald, E.B.; Leyland, A.; Mair, F.; Anderson, J.; Celis-Morales, C.; Cleland, J.; Forbes, J.; Gill, J.; et al. Occupation and risk of severe COVID-19: Prospective cohort study of 120 075 UK Biobank participants. *Occup. Environ. Med.* **2020**, *78*, 307–314. [CrossRef] [PubMed]

5. WHO. *The Impact of COVID-19 on Health and Care Workers: A Closer Look at Deaths*; WHO: Geneva, Switzerland, 2021.

6. Folegatti, P.M.; Ewer, K.J.; Aley, P.K.; Angus, B.; Becker, S.; Belij-Rammerstorfer, S.; Bellamy, D.; Bibi, S.; Bittaye, M.; Clutterbuck, E.A.; et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: A preliminary report of a phase 1/2, single-blind, randomised controlled trial. *Lancet* **2020**, *396*, 467–478. [CrossRef]

7. Ramasamy, M.N.; Minassian, A.M.; Ewer, K.J.; Flaxman, A.L.; Folegatti, P.M.; Owens, D.R.; Voysey, M.; Aley, P.K.; Angus, B.; Babbage, G.; et al. Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): A single-blind, randomised, controlled, phase 2/3 trial. *Lancet* **2021**, *396*, 1979–1993. [CrossRef]

8. Voysey, M.; Clemens, S.A.; Madhi, S.A.; Weckx, L.Y.; Folegatti, P.M.; Aley, P.K.; Angus, B.; Baillie, V.L.; Barnabas, S.L.; Bhorat, Q.E.; et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: An interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* **2021**, *397*, 99–111. [CrossRef] [PubMed]

9. Goel, A.; Verma, A.; Tiwari, P.; Katiyar, H.; Aggarwal, A.; Khetan, D.; Kishore, R.V.; Kumar, P.; Singh, T.P.; Sheikh, S.; et al. Serological Immune Response Following ChAdOx1 nCoV-19 Vaccine (Covishield®) in Patients with Liver Cirrhosis. *Vaccines* **2022**, *10*, 1837.

10. Bhadauria, D.S.; Katiyar, H.; Goel, A.; Tiwari, P.; Kishore, R.V.; Aggarwal, A.; Verma, A.; Khetan, D.; Kaul, A.; Yachha, M.; et al. Antibody Response to ChAdOx1 nCoV-19 (AZD1222) Vaccine in Kidney Transplant Recipients. *Vaccines* **2022**, *10*, 1693. [CrossRef]

11. Raja, N.; Rajagopalan, A.; Arunachalam, J.; Prasath, A.; Durai, R.; Rajendran, M. Humoral response to viral vector COVID-19 vaccine in hemodialysis patients. *Kidney Res. Clin. Pract.* **2022**, *41*, 342–350. [CrossRef]

12. Singh, A.K.; Phatak, S.R.; Singh, R.; Bhattacharjee, K.; Singh, N.K.; Gupta, A.; Sharma, A. Antibody response after first and second-dose of ChAdOx1-nCOV (Covishield™®) and BBV-152 (Covaxin™®) among health care workers in India: The final results of cross-sectional coronavirus vaccine-induced antibody titre (COVAT) study. *Vaccine* **2021**, *39*, 6492–6509. [CrossRef]

13. Khan, M.S.; Haq, I.; Qurieshi, M.A.; Majid, S.; Bhat, A.A.; Qazi, T.B.; Chowdri, I.N.; Sabah, I.; Kawoosa, M.F.; Lone, A.A.; et al. SARS-CoV-2 Seroprevalence Among Healthcare Workers by Workplace Exposure Risk in Kashmir, India. *J. Hosp. Med.* **2021**, *16*, 274–281. [CrossRef] [PubMed]

14. Wattal, C.; Oberoi, J.K.; Goel, N.; Datta, S.; Raveendran, R.; Rao, B.K.; Kumar, R. A cross-sectional study of SARS-CoV-2 seroprevalence among asymptomatic healthcare workers in a tertiary healthcare centre: Assessing the impact of PPE guidelines. *Indian J. Med. Microbiol.* **2021**, *39*, 528–533. [CrossRef] [PubMed]

15. Saini, A.K.; Panda, P.K.; Bahurupi, Y.; Omar, B.; Akhil, T.; Panwar, P.; Sharma, M. Seroprevalence of Antibodies Against SARS-CoV-2 Among Health Care Workers at a Tertiary Care Hospital in Uttarakhand: A Retrospective Study. *Cureus* **2022**, *14*, e24840. [CrossRef]

16. Goenka, M.K.; Afzalpurkar, S.; Goenka, U.; Das, S.S.; Mukherjee, M.; Jajodia, S.; Shah, B.B.; Patil, V.U.; Rodge, G.; Khan, U.; et al. Seroprevalence of COVID-19 Amongst Health Care Workers in a Tertiary Care Hospital of a Metropolitan City from India. *J. Assoc. Physicians India* **2020**, *68*, 14–19. [CrossRef] [PubMed]

17. Gupta, R.; Dwivedi, T.; Gajendra, S.; Sahoo, B.; Gupta, S.K.; Vikas, H.; Singh, A.R.; Mohan, A.; Bhatnagar, S.; Singh, S.; et al. Seroprevalence of antibodies to SARS-CoV-2 in healthcare workers & implications of infection control practice in India. *Indian J. Med. Res.* **2021**, *153*, 207–213.

18. Varghese, S.M.; Mateethra, G.C.; George, G.; Chandran, V.S.; John, G.M.; Varghese, L.T.; Mammen, N.K.; Vinayak, V. A study on seroconversion following first & second doses of ChAdOx1 nCoV-19 vaccine in Central Kerala. *Indian J. Med. Res.* **2022**, *155*, 499–504.

19. Eyre, D.W.; Lumley, S.F.; Wei, J.; Cox, S.; James, T.; Justice, A.; Jesuthasan, G.; O'Donnell, D.; Howarth, A.; Hatch, S.B.; et al. Quantitative SARS-CoV-2 anti-spike responses to Pfizer-BioNTech and Oxford-AstraZeneca vaccines by previous infection status. *Clin. Microbiol. Infect.* **2021**, *27*, 1516.e7–1516.e14. [CrossRef]

20. Kulkarni, P.S.; Padmapriyadarsini, C.; Vekemans, J.; Bavdekar, A.; Gupta, M.; Kulkarni, P.; Garg, B.S.; Gogtay, N.J.; Tambe, M.; Lalwani, S.; 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. *EClinicalMedicine* **2021**, *42*, 101218. [CrossRef]

21. Deswal, V.; Phogat, R.; Sharma, P.; Kataria, S.; Soin, A. Is a single dose of ChAdOx1 nCoV-19 vaccine (AZD1222) enough for people with prior SARS-CoV-2 infection or baseline seropositive status? *Int. J. Infect. Dis.* **2022**, *123*, 143–144. [CrossRef]

22. Gelanew, T.; Mulu, A.; Abebe, M.; Bates, T.A.; Wassie, L.; Teferi, M.; Fentahun, D.; Alemu, A.; Tamiru, F.; Assefa, G.; et al. A Single Dose of ChAdOx1 nCoV-19 Vaccine Elicits High Antibody Responses in Individuals with Prior SARS-CoV-2 Infection

Comparable to That of Two-Dose-Vaccinated, SARS-CoV-2-Infection-Naive Individuals: A Longitudinal Study in Ethiopian Health Workers. *Vaccines* **2022**, *10*, 859. [CrossRef]

23. Wanchaijiraboon, P.; Teeyapun, N.; Pakvisal, N.; Sainamthip, P.; Susiriwatananont, T.; Zungsontiporn, N.; Suntronwong, N.; Vichaiwattana, P.; Klinsawat, W.; Wanlapakorn, N.; et al. Durability of Immune Response to ChAdOx1-nCoV-19 Vaccine in Solid Cancer Patients Undergoing Anticancer Treatment. *Vaccines* **2022**, *10*, 1662. [CrossRef] [PubMed]

24. Townsend, J.P.; Hassler, H.B.; Sah, P.; Galvani, A.P.; Dornburg, A. The durability of natural infection and vaccine-induced immunity against future infection by SARS-CoV-2. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2204336119. [CrossRef] [PubMed]

25. Katikireddi, S.V.; Cerqueira-Silva, T.; Vasileiou, E.; Robertson, C.; Amele, S.; Pan, J.; Taylor, B.; Boaventura, V.; Werneck, G.L.; Flores-Ortiz, R.; et al. Two-dose ChAdOx1 nCoV-19 vaccine protection against COVID-19 hospital admissions and deaths over time: A retrospective, population-based cohort study in Scotland and Brazil. *Lancet* **2022**, *399*, 25–35. [CrossRef] [PubMed]

26. Bobdey, S.; Kaushik, S.K.; Sahu, R.; Naithani, N.; Vaidya, R.; Sharma, M.; Vashishtha, K.; Yadav, A.K.; Sen, S.; Karade, S. Effectiveness of ChAdOx1 nCOV-19 Vaccine: Experience of a tertiary care institute. *Med. J. Armed Forces India* **2021**, *77* (Suppl. 2), S271–S277. [CrossRef] [PubMed]

27. Kaur, U.; Bala, S.; Ojha, B.; Jaiswal, S.; Kansal, S.; Chakrabarti, S.S. Occurrence of COVID-19 in priority groups receiving ChAdOx1 nCoV-19 coronavirus vaccine (recombinant): A preliminary analysis from north India. *J. Med. Virol.* **2022**, *94*, 407–412. [CrossRef] [PubMed]

28. Abhilash, K.P.; Mathiyalagan, P.; Krishnaraj, V.R.; Selvan, S.; Kanagarajan, R.; Reddy, N.P.; Rajendiran, N.; Hazra, D.; Gunasekaran, K.; Moorthy, M.; et al. Impact of prior vaccination with Covishield(TM) and Covaxin(R) on mortality among symptomatic COVID-19 patients during the second wave of the pandemic in South India during April and May 2021: A cohort study. *Vaccine* **2022**, *40*, 2107–2113. [CrossRef]