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IgG neutralization potential of COVISHIELD™ vaccinated individual's sera after booster vaccination: Longitudinal and prospective cohort study

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ABSTRACT

The novel SARS-CoV-2 (COVID-19) has caused widespread human turmoil by posing challenges concerning infection prevention, disease diagnosis, and treatment. Several approved vaccines including Sinovac (CoronaVac), COVISHIELD™ (Oxford/AstraZeneca formulation), Janssen (Johnson & Johnson), Sputnik V (Gamaleya), Covaxin (Bharat Biotech), Pfizer (BNT162b2), and others are being used to combat COVID-19. It is crucial to evaluate the kinetics of SARS-CoV-2 antibodies to predict the possibility of reinfection and the longevity of vaccination protection. There is a lack of data on longitudinal humoral antibody dynamics following two and three doses of the SARS-CoV-2 vaccination ChAdOx1-nCOV (COVISHIELD™) in Indians. Thus, concerns about the efficacy of current vaccines have been raised by a sharp rise in coronavirus disease 2019 (Covid-19) cases caused by sub-variants of SARS-CoV-2 (Severe acute respiratory syndrome corona virus 2) in communities that have received massive vaccinations. The relative immunogenicity and safety of various COVID-19 immunizations administered as a third (booster) dose are not well known. We examined the reactogenicity and immunogenicity of the COVID-19 vaccine as a third dose after two doses of COVISHIELD™ to produce data to optimize the selection of booster vaccinations. After three doses of the COVID-19 vaccine, we evaluated the sera of COVISHIELD™ vaccine recipients for their ability to neutralize the virus. Primary immunization with two doses of COVISHIELD™ vaccine recipients provided significant protection against symptomatic disease caused by the SARS-CoV-2 variants. A COVISHIELD™ vaccine booster vaccine recipient substantially increased protection. The immunization findings showed a significant difference ($p \geq 0.001$) between the COVID-19 naive vaccine (n = 438) and the sera of COVID-19-positive recovered subjects (n = 371) who received three doses of COVISHIELD™. Our findings reveal that anti-RBD antibodies persist over time, which may reduce the probability of reinfection. A three-dose vaccination (n = 53) increases defense against variations by noticeably increasing cross-neutralizing antibody titers. Particularly against variants with antibody escape mutations.

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1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-induced coronavirus disease 2019 (COVID-19) pandemic has wreaked unimaginable havoc on the global economy and health [1]. Vaccination is one of the most effective treatments to greatly reduce the severity of disease and mortality caused by SARS-CoV-2 infection. Vaccination campaigns are being carried out worldwide. The Global Rise of Education, which was made available online at https://ourworldindata.org/ [2], provided information on the COVID-19 vaccine administration statistics to the present day (Figure 1). Worldwide, based on geographic variability across the 20 different regions of the world, the global COVID-19 vaccine administration doses were found to be 13.32 billion doses and 69.7% of the world's population and 759,319 are now administered each day. The majority of the vaccination coverage in India consists of COVISHIELD™. Therefore, understanding the potential of vaccinations to neutralize new strains, which are considered the cause of the increase in incidence in India, is crucial. As of September 1, 2022, 1,696.19 million doses of COVISHIELD™ COVID-19 vaccination had been delivered in India [3].

To assess the overall dynamics of the COVID-19 epidemic and post-pandemic dynamics, evidence of the immune responses endurance and long-term efficacy is required, especially in light of the recent emergence of novel SARS-CoV-2 variants. Due to ongoing genetic alterations in the SARS-CoV-2 genome, the COVID-19 pandemic is still a global threat today [4]. The most effective method for suppressing the COVID-19 pandemic may be vaccination. The first two vaccine administrations were considered the primary immunization, and the third booster dose was considered a secondary immunization [5].

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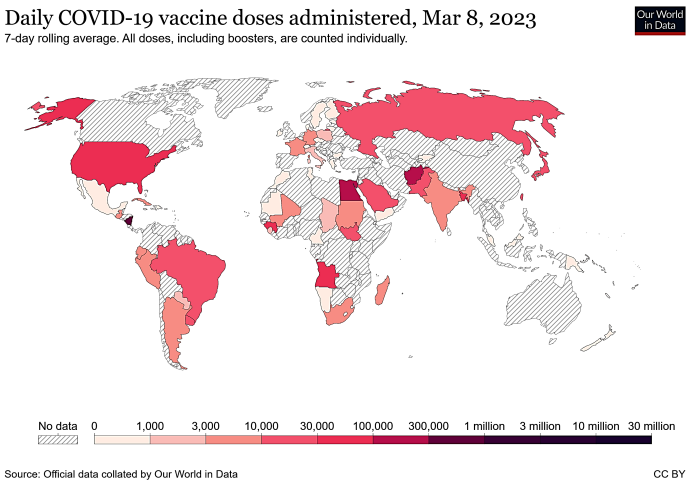

Figure 1. COVID-19 vaccine doses administrated in men and women, all ages worldwide [2].

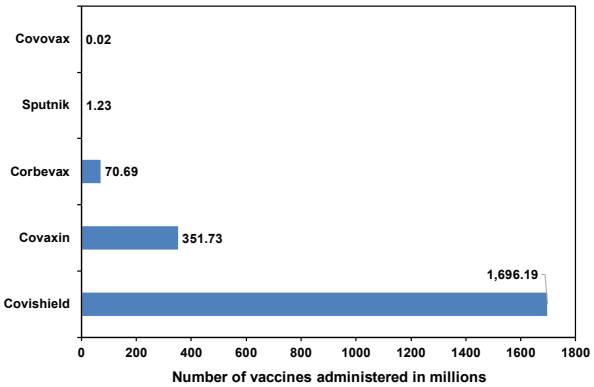

Figure 2. Number of anti-COVID-19 vaccine (COVISHIELD™) doses administered in men and women all ages in India. (Data retrieved from https://www.statista.com/statistics/1248301/india-covid-19-vaccines-administered-by-vaccine-type/) [12].

The US Food and Drug Administration authorized the use of a third booster dose for all individuals after the completion of primary vaccination with approved COVID-19 vaccines to protect against the ongoing resurgence of the COVID-19 outbreak [6]. Because early research indicates that three doses of the Pfizer Biotech mRNA vaccine can neutralize the omicron variant of the virus with a roughly 40-fold decrease in viral titres [7]. For several reasons, serological techniques for detecting SARS-CoV-2 antibodies are important [8]. Serological assays help to better understand the host immune response triggered by viruses [9]. Additionally, it is crucial to evaluate innovative vaccines by identifying the quantities and variety of immunoglobulins that are created in response to various vaccines, in a dose-dependent and time-dependent manner. Due to decreased immunity and a significant increase in breakthrough infections caused by viral diversity, one more booster vaccination dose is recommended, according to our previous study on neutralizing antibodies [10]. Data from the Centers for Disease Control and Prevention suggest that individuals who have not received the COVID-19 vaccine have a higher risk of testing positive for the virus. Therefore, it is essential to continuously assess how well COVID-19 vaccinations protect against increasingly virulent variants [11]. As of September 1, 2022, 1,696.19 million doses of the COVISHIELD™ vaccination had been administered in India. In particular, compared to Covaxin (351.73 million) and Corbevax (70.69 million) vaccine doses, it is the type of vaccination that has been administered the most frequently (Figure 2) [12]. In light of this, the focus of research has been on the mechanism by which a vector-encoded recombinant anti-SARS-CoV-2 vaccine, COVISHIELD™, induces a strong specific IgG antibody response. We conducted a prospective cohort study to longitudinally assess the dynamic response of anti-SARS-CoV-2 IgG antibodies in order to gain more knowledge about IgG in vaccine-elicited immunity, particularly over longer periods of time after three doses of immunization.

2. Methods

2.1. Study population and characteristics

A total of 371 healthcare workers(HCW), working at the Sri Jayadeva Institute of Cardiovascular Sciences and Research, including doctors, nurses, paramedics, technologists, female community health volunteers, and sanitation workers and office assistants, had received the first, second, and third doses of the COVISHIELD™ vaccine. Blood samples were taken from 371 healthy adults twelve months after the third dose of immunization (Table 1). Samples were examined for SARS-CoV-2 specific neutralization antibody (Nab) responses using the enzyme-linked immunoassay (ELISA). After immunization, sequential blood samples were taken from each individual to track and analyze variability in virus-specific Nab titers. This study was approved by the Institutional Ethics Committee and adhered to the ethical guidelines. The ethical study approval code was SJICR/EC/2023-24/003. Demographics, history of SARS-CoV-2 infection, and vaccination details were noted at the time of sample collection from the patient's medical record.

Table 1. Demographic characteristics of the participants who received the first, second, and third doses of the COVISHIELD™ vaccine (n = 371).

Characteristics	Value
Gender	
Female, N	214 (57.7%)
Male, N	157 (42.3%)
Previous positive SARS-CoV-2RT-PCR, N	161 (43.4%)
Previous negative SARS-CoV-2RT-PCR, N	210 (56.6%)
Age	
≤ 20 years	2 (0.5%)
21-30 years	62 (16.7%)
31-40 years	125 (33.7%)
41-50 years	96 (25.9%)
51-60 years	73 (19.7%)
> 60 years	13 (3.5%)

The vaccination certificate issued by the Ministry of Health and Family Welfare of India validated the type and dose of vaccinations. For the purpose of the analysis, participants were categorized into three groups. Group 1, those who had a history of SARS-CoV-2 infection and were vaccinated (n = 161). Group 2 were those who had no history of SARS-CoV-2 infection and received vaccines (n = 210). Group 3, those who had received 3 doses of the COVISHIELD™ vaccine at different time points (n = 114), Also, the primary objective of this study was to systematically review and analyze the longitudinal cohort study, including the measurement of IgG neutralization activity of the same individuals (53) after three doses of the vaccine and also involved in the previous [10] and the present study.

2.2. Sample collection and evaluation of SARS-CoV-2 specific neutralizing antibodies in serum and measurements

After completion of a consent form, venous blood samples were taken in 5 mL vacutainer tubes from all participants. Blood was spun at 4000 rpm for 10 minutes at room temperature to collect and test the serum. The serum samples were aliquoted and processed. According to the manufacturer's instructions, serum samples were tested for SARS-CoV-2-specific Nab using the COVID-19 neutralizing antibody Microlisa test kit supplied by J. Mitra & Co. Pvt. Ltd., New Delhi, India (Lot No. ECN010522) [13]. The assay kit was created to identify neutralizing antibodies made against SARS-CoV-2 in human serum/plasma in an *in vitro* semiquantitative manner to prevent the interaction between the viral spike glycoprotein receptor binding domain (RBD) and the cell surface receptor angiotensin converting enzyme-2 [14].

Following incubation, the unbound HRP-RBD neutralizing antibody complex was removed from the wells with 25× wash buffer by five washes of the working wash buffer solution. Using the Euphoria 4.1 Fully Automated ELISA Processor, the optical density (OD) was measured at a wavelength of 450 nm (Alto, Santacruz Bambolin Complex, Goa, India). By computing the inhibition rates for samples according to the suggested positive and negative cut-offs, the test findings were interpreted as follows.

$$\% \text{ Inhibition} = (1 - \text{Sample O.D.}/\text{Negative control O.D.}) \times 100 \quad (1)$$

Neutralizing antibody levels of more than 30% were considered positive, as per the manufacturer's recommend-dations (SARS-CoV-2 Neutralizing Antibody present). Serum samples from non-immunized and positively identified non-infected people were also used to validate the results under the manufacturer's negative cut-off control.

The assay kit was targeted to detect neutralizing antibodies generated against SARS-CoV-2 in human serum or plasma in an in vitro semiquantitative manner, preventing the interaction between the viral spike glycoprotein's receptor binding domain (RBD) and the cell surface receptor angiotensin converting enzyme-2 (ACE2). The detection protocols and routines adhered to the manufacturer's instructions (https://jmitra.co.in/wp-content/uploads/2021/10/Instruction-Manual-Covid-19-Neutralizing-Antibodies-Microlisa.pdf) [15].

The test evaluation was carried out following the recommended positive and negative cut-offs, and the test results were interpreted by calculating the inhibition rates for the samples as follows: According to the manufacturer's instructions, neutralizing antibody levels higher than >30% were considered as positive (SARS-CoV-2 Neutralizing Antibody present). In addition to the negative cutoff control provided by the manufacturer, serum samples from nonim-munized and confirmed noninfected subjects were also used to ratify the results.

2.3. Statistical analysis

For categorical variables, descriptive statistics are presented as frequencies and percentages, and for continuous variables, such as means (standard deviation, SD), and medians (interquartile range, IQR). Here, we describe the dynamics of the humoral immune response of SARS-CoV-2 up to one year after vaccination and examined the IgG neutralization potential of the vaccine using validated serological assays on a substantial cohort of healthcare workers who have recovered from COVID-19 and received the vaccine and received the vaccine without infection. Three tests, chi-square, one-way ANOVA, and student t-tests were applied. When the third booster dose of the vaccine had been administered, we assessed the differences in NAb titer values after one year of vaccination. Furthermore, a two-tailed t-test was used to compare neutralization titers of the same person's serum variables related to anti-spike antibody titers following three doses of the vaccine ChAdOx1-nCOV (COVISHIELD™). Following the first dose of the vaccine (3 months after immunization) and the second dose of the vaccine (6 months after immunization), the correlation with our prior Nab titer was evaluated using generalized linear models, respectively. This set of analyses had a 2-tailed $p < 0.001$ level of significance with 98.69% inhibition. STATA 16 MP was used to perform all statistical analyzes (Stata Corp., College Station, TX). The GraphPad Prism (Version 9.5.1) tool was used to analyze and visualize all additional data.

3. Results

3.1. Characteristics of participants

Healthcare professionals including physicians, nurses, paramedics technicians, housekeeping personnel, and office assistants, were included in this study with their consent. As indicated in Table 1, 214 women (57.7%; median age 31-41 years) and 157 male (42%; median age 31-45 years) out of 371 participants who had given blood samples after 365 days of their vaccine administration were included in this study (Figure 3). A total of 161 (43.4%) participants had COVID-19 infection and recovered during the post vaccination analysis period, while 210 (56.6%) participants did not have COVID-19 infection.

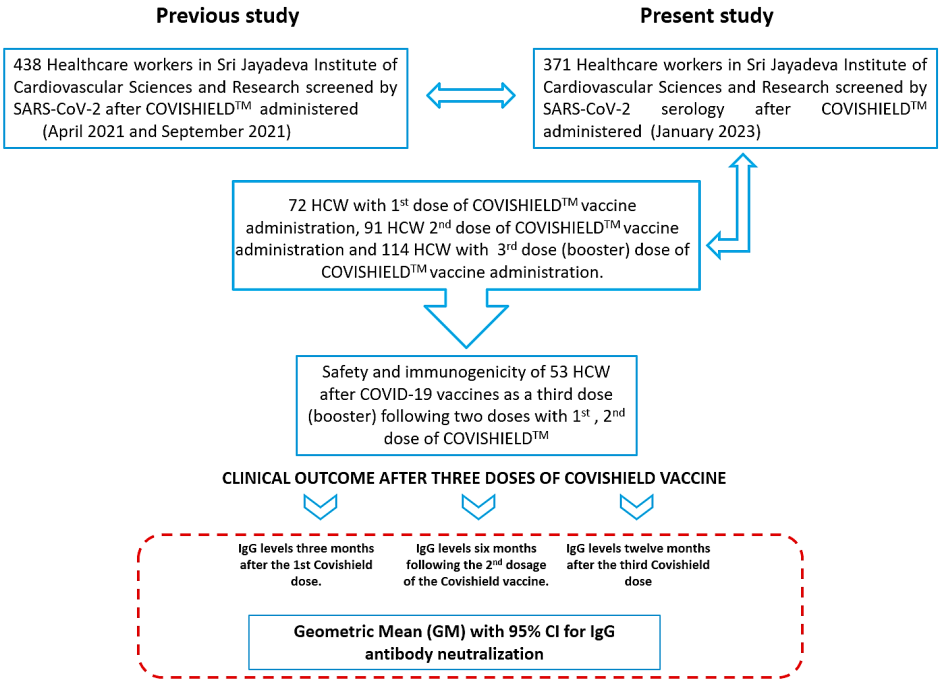

Figure 3. Flow diagram showing the selection process for studies to include.

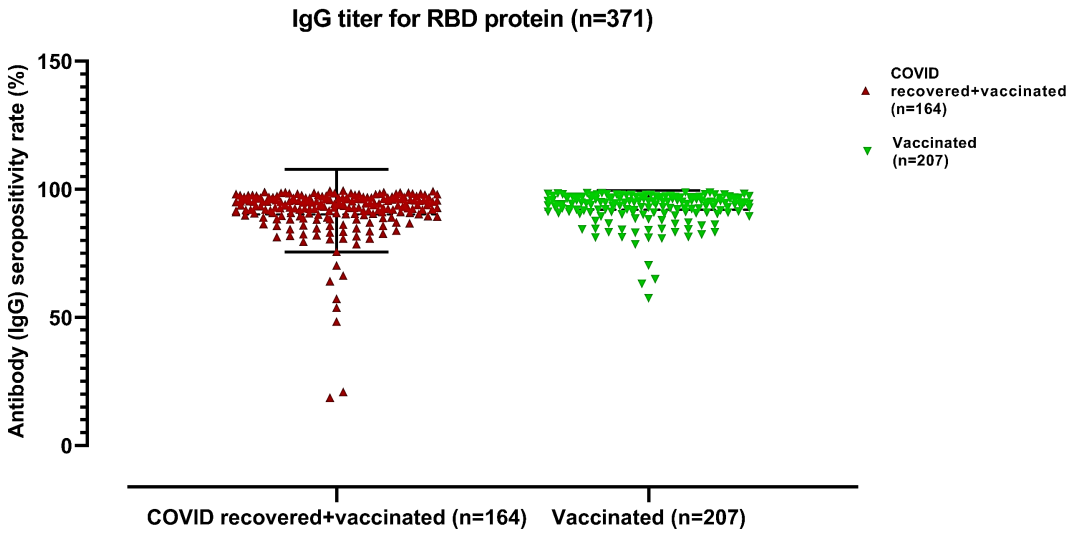

Figure 4. Neutralization efficacy after three doses vaccination among the HCW. Comparison of Nab titer between Covid recovered + vaccinated (red, n = 164) and vaccinated cases (green, n = 207). Administrated with three doses of vaccine (sera collected 12 months after the third (booster) dose of COVISHIELD™).

The participants were all of Indian nationality. None of the participants experienced any of the following symptoms prior to vaccination: cough, fever, sore throat, exhaustion, diarrhea, difficulty breathing, fatigue, muscle aches, or change or loss of sense of taste or smell. Our study group was further divided according to participants whose samples were used in previous and current studies. After 12 months of vaccination, their NAb titer had significantly ($p < 0.001$) increased compared to their NAb titer of the previous study (Figures 4-7). Flow chart of survey recruitment and serum sampling among healthcare workers (HCW) at Sri Jayadeva Institute of Cardiovascular Sciences and Research. First-line serological screening was performed using ELISA assay: detecting antiReceptor Binding Domain (RBD) antibodies and Novel coronavirus COVID-19 IgG ELISA detecting the anti-nucleocapsid protein (N) IgG.

3.2. COVID-19 vaccine cohort

Several studies have found that certain vaccinations, including mRNA-1273, NVX- CoV2373, BNT162b2, and ChAdOx1-nCoV19, are less effective at neutralizing B.1.1.7, B.1.351, and B.1.1.28 P1 variants. Indian citizens have received more than 2,137 million doses of the two approved vaccines Covishield (Astrazeneca-Oxford) and Covaxin™ (BBV152). Most of the immunization programs in India consist of Covishield. Therefore, understanding the capacity of the Covishield vaccine to counteract this newly discovered variant is crucial [9]. We evaluated the Covishield vaccination recipient sera 24 months after the second dose of COVISHIELD and 12 months after the third dose (booster) of COVISHIELD for their ability to neutralize the Covishield vaccine (n = 371).

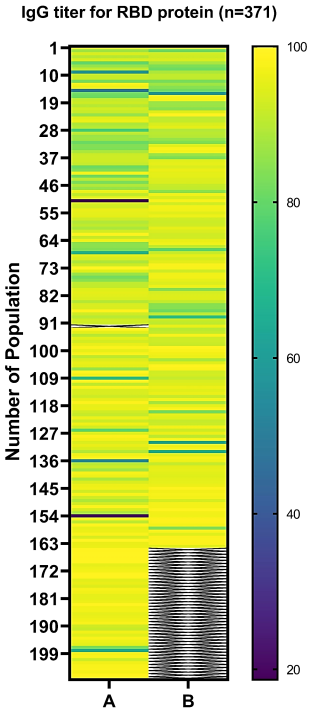

Figure 5. Anti-SARS-CoV-2 antibody responses to vaccines for 371 participants A (n = 207) and B (n = 164). Heat maps showing the kinetics of anti-SARS-CoV-2 neutralizing antibodies (anti-SARS-CoV-2 IgG antibodies).

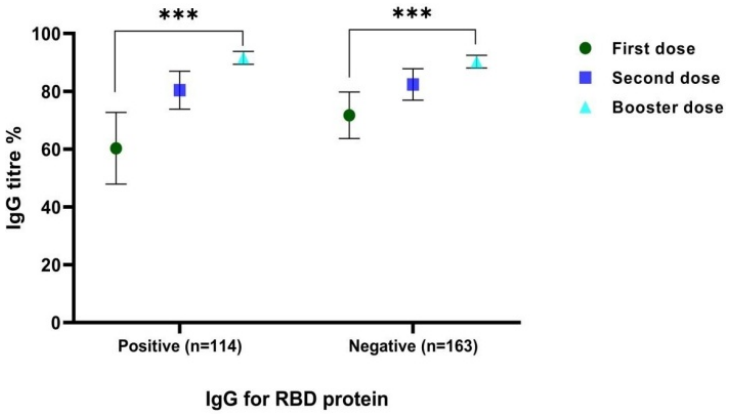

Figure 6. Binding IgG levels to spike RBD were measured by ELISA using serially diluted sera from naive and previously infected individuals collected from 3 months after dose 1, 6 months after dose 2, and 12 months after the 3rd dose of COVISHIELD vaccine. *** $p < 0.001$, calculated with Oneway Anova test using the Graphpad Prism version 9.0.0 software.

The COVID-19-positive recovered and vaccinated subjects were (n = 164) and vaccinated (n = 207). For both types of sera, the neutralizing-antibody (NAb) titer was measured. The results demonstrated that sera of COVID-19-positive recovered subjects (n = 164, red color) who received three doses of Covishield have a higher antibody response compared to the COVID-19 naive vaccine (n = 207, green color) with a significant difference ($p < 0.0001$) in NAb titer against SARS-CoV-2 infection (Figure 4 and 5). For the 371 participants, we successfully performed analyses of neutralizing IgG anti-SARS-CoV-2 antibodies after vaccination and then profiled the kinetics of the antibodies.

Figure 5 shows the trends in antibodies for each individual based on the time and dosage of vaccination, represented by heat maps. It's interesting to note that following the third treatment (12 months), the seropositivity rate increased significantly to 100% (371/371) and the IgG antibodies produced a strong response. After the third dosage of vaccination, compared to the first and second doses, the levels of these IgG antibodies increased once more and continued to rise for twelve months.

Furthermore, we measured the SARS-CoV-2 antibody, immunoglobulin (Ig) G s against full spike and RBD antigens from the previously infected and recovered participants, and only vaccinated individuals of those antibody neutralization titer studies were done in the previous and present investigation (Figure 6) to understand the antibody response following the doses 1 and 2 and 3 of COVISHIELD vaccines (Table 2).

The predominant response was found for the IgG antibodies in both naive and previously infected participants after doses 1, 2, and 3. Among naive individuals, IgG antibodies were higher after the booster dose (3rd) than after the first dose (Figure 6). However, IgG levels in the previously infected individuals were comparable with those in the naive individuals after dose 2, and were unchanged after dose 3.

Table 2. Participants who received the first, second, and third doses of COVISHIELD™ vaccine (n = 371).

Covid test result	Vaccine	N	Mean	SD	Median	Minimum	Maximum	p value
+ve	Dose 1	27	60.3	31.290	64.0	7.30	99.60	p <0.001
	Dose 2	39	80.4	20.240	86.4	35.30	99.50	
	Dose 3	48	91.6	7.554	94.1	57.45	97.91	
-ve	Dose 1	45	71.7	26.848	81.5	9.53	99.17	p <0.001
	Dose 2	52	82.4	19.529	91.8	32.00	99.70	
	Dose 3	66	90.3	8.904	92.6	48.37	98.69	

Table 3. Analysis of SARS-CoV-2 live virus neutralizing antibody titers (n = 53).

Vaccine	N	Mean	SD	Median	Minimum	Maximum	p value
Dose 1	53	68.0	27.466	69.5	7.30	99.17	p <0.001
Dose 2	53	82.9	18.074	91.6	35.30	99.50	
Dose 3	53	90.0	9.271	92.4	48.37	98.69	

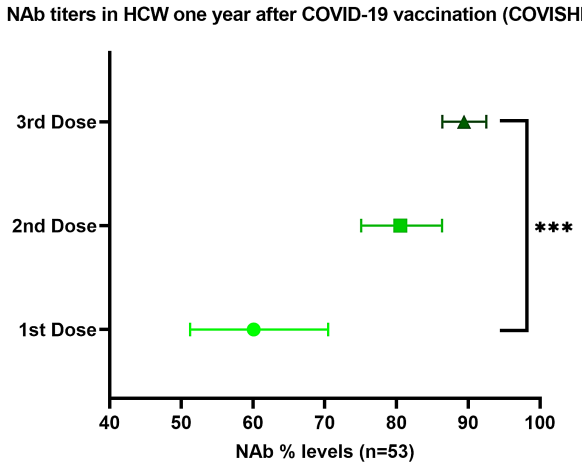

Figure 7. Geometric Mean (GM) with 95% Confidence Interval (CI) for the Nab titers in HCW 3 months after the 1st dose COVISHIELD, 6 months after the 2nd dose of COVISHIELD™ and 12 months after the 3rd dose (booster) of COVISHIELD (*** $p < 0.001$).

3.3. Analysis of neutralization titers of the same individual's sera variables associated with anti-spike anti-body titers after three doses of vaccine ChAdOx1-nCOV (COVISHIELD™)

114 participants never achieved positive conversion after vaccination during the three-dose regimen. Their IgG response was maximum after the third dose. In particular, participants who had achieved positive conversion after the second dose did not again exhibit positive conversion after the third dose (Figure 7). The IgG (anti-RBD/S) titer was significantly higher in vaccinated individuals after one or two vaccine doses, compared with the baseline values of individuals who had developed an immunity response after a previous SARS-CoV-2 infection. The GM of IgG (anti-RBD/S), after one vaccine dose from vaccinated individuals was significantly higher ($p < 0.001$) than GM found at the point of recruitment for individuals that had a previous infection.

The main objective of the current study was to compare the spike neutralization activity (n = 53) after determining the serostatus of SARS-CoV-2 among COVISHIELD vaccination recipients (Table 3). The IgG antibodies remained detectable in all participants for the study duration.

3.4. Present study findings and further implications for practice and research

Our prospective longitudinal study assesses over more than one year: the anti-SARS-CoV-2 antibody, persistence against anti-RBD after primary infection, the neutralizing capacity of these antibodies against live virus variants of concern the influence of host factors on antibody kinetics, the impact of vaccination on humoral responses against SARS-CoV-2 variants after COVID-19, and long-term risk of reinfection during SARS-CoV-2 variants spread. We found that vaccination with COVISHIELD™ increases anti-RBD IgG to a level that strongly neutralizes SARS-CoV-2 variants regardless of prevaccine IgG levels and dose-dependant manner. Furthermore, this study offered a unique opportunity to evaluate the risk of reinfection following previous COVID-19 with a longitudinal follow-up of convalescent and recovered individuals during the same period, which includes the three COVID-19 waves experienced in India. Analysis of clinical and virological data revealed that the risk of reinfection was reduced by 96.7% over one year.

4. Discussions

The severe acute respiratory syndrome coronavirus-2 is the cause behind the highly contagious respiratory ailment known as Coronavirus Disease of 2019 (COVID-19) (SARS-CoV-2) [16]. Through the angiotensin-converting enzyme 2 (ACE2) receptor, SARS-CoV-2 infects alveolar epithelial cells and primarily alveolar epithelial type 2 cells [14]. The innate immune system is also activated by SARS-CoV-2; macrophage stimulation results in an overproduction of pro-inflammatory cytokines, such as IL-6, and the "cytokine storm", which causes multiple organ failure and the systemic inflammatory response syndrome [17]. Understanding the COVID-19 pandemic critically depends on the duration that adaptive immunity against SARS-CoV-2 lasts as well as how effectively it works after the primary infection [18]. The ability to determine the occurrence of COVID-19, quickly identify asymptomatic patients, and assess COVID-19 patients after therapy is made possible by antibody testing against SARS-CoV-2 [19]. Monitoring the existence of antibodies against SARS-CoV-2 following vaccination is another function of antibody testing in the COVID-19 vaccine era [20]. The two main categories of the

antibody test are binding and neutralizing antibody assays. Antibodies (IgG, IgM, or total) against the spike protein receptor-binding domain (RBD), partial spike protein (S1 subunit, S2 subunit), or nucleocapsid protein can be found using binding antibody assays (N). The presence of functional antibodies to stop SARS-CoV-2 infection is identified by the neutralizing antibody assay [21]. Our results demonstrated that robust seropositive rates were observed after the first and second vaccinations of Covishield. Corresponding well with these findings, our previous study reported that seropositive rates after the first and second vaccinations of Covishield vaccine were significantly high (92-98.5%) [9].

The humoral immune response after vaccination could differ according to age, sex, and ethnicity. This study demonstrated that age and gender did not statistically affect antibody response after the second and third (booster) vaccinations, and our previous studies showed conflicting results for vaccine response by age and gender. The causes driving this "antigenic shift" are likely to become stronger when most of the population develops resistance to the virus through infection, vaccination, or both. The SARS CoV-2 neutralizing antibody developed after immunization and is retained in the blood for several months depending on several physiological factors.

According to Floriane *et al*., a study that examined antibody responses up to 13 months after SARS-CoV-2 infection and the risk of reinfection, the slight antibody decline seen in convalescent individuals does not actually reflect a waning of humoral immunity but rather a contraction of the immune response, while antibody affinity maturation and anti-S memory B cells persist [22]. According to a recent study by Wang et al., memory B cell clones that express strong and broad anti-S antibodies are selectively preserved in the repertoire for at least a year after infection and persist after vaccination [23]. These findings provide highly encouraging evidence for the longevity of humoral responses induced by COVID-19 and imply that long-term protection against SARS-CoV-2 infection may be possible [24,25].

In conclusion, our study provides crucial information on the persistence of circulating antibodies against SARS-CoV-2 more than one year after COVID-19 vaccination, and on the long-term risk of re-infection. The SARS-CoV-2 vaccine may improve protection by raising the levels of cross-neutralizing antibodies, particularly against versions with antibody escape mutations like B1.351. The interpretation of serological results and the future calculation of a protective anti-RBD IgG level may benefit greatly from the high association between antibody levels and their neutralizing capacity against variations [26]. Furthermore, our findings demonstrate that even repeated booster shots can significantly boost a strong humoral immune response to protect against SARS-CoV-2 infection. A twelve-month study of polyfunctional CD4+ and CD8+ T-cells following the COVISHIELD™ booster is also being investigated.

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Disclosure statement

Conflict of interest: The authors declare that they have no conflict of interest. Ethical approval: The study was approved by the Institutional Ethics Committee of the Sri Jayadeva Institute of Cardiovascular Sciences and Research Centre (SJICR-EC), Bengaluru. (IHEC Number-SJICR/EC/2023-24/003). Written and informed consent was obtained from all participants enrolled in the study before the collection of specimens.

CRediT authorship contribution statement

Conceptualization: Naveena Jagadeesan, Kavitha Karur, Nandini Puttamasthi Gowda; Methodology: Naveena Jagadeesan, Kavitha Karur, Nandini Puttamasthi Gowda; Software: Praveen Kumar, Harsha Tumkur Kumar; Validation: Naveena Jagadeesan, Kavitha Karur, Nandini Puttamasthi Gowda; Formal Analysis: Naveena Jagadeesan, Kavitha Karur, Nandini Puttamasthi Gowda, Prapulla Kumari, Manjunath Cholenalli Nanjappa; Investigation: Naveena Jagadeesan; Resources: Naveena Jagadeesan, Kavitha Karur, Nandini Puttamasthi Gowda, Prapulla Kumari; Data Curation: Naveena Jagadeesan, Praveen Kumar, Harsha Tumkur Kumar; Writing - Original Draft: Naveena Jagadeesan, Praveen Kumar; Writing - Review and Editing: Naveena Jagadeesan; Visualization: Naveena Jagadeesan, Praveen Kumar, Kavitha Karur, Harsha Tumkur Kumar; Supervision: Naveena Jagadeesan, Manjunath Cholenalli Nanjappa; Project Administration: Naveena Jagadeesan.

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