Anti SARS-CoV 2 IgG antibody response in fully vaccinated Covishield (AZD1222) and Covaxin (BBV-152) recipients: a study done in southern part of West Bengal, India

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ABSTRACT

Background and Objectives: Vaccination being the only way to reduce mortality from the dreaded COVID-19 disease, the vaccine was introduced in India as per the advice of the National Expert Group on January 16, 2021. Duration of immune response elicited by the vaccines has always been a matter of content. With new variants emerging every other day, the study was done to look for the antibody response in vaccine recipients post second dose of vaccination.

Materials and Methods: A longitudinal observational study was conducted from August 2021 to February 2022 in fully vaccinated individuals who took either Covishield (AZD1222) or Covaxin (BBV-152). Blood was collected from the individuals at 12-16 weeks post-vaccination to look for IgG antibody response against S1 spike protein of SARS-CoV2 by ELISA. Follow-up was done at 32 weeks post the second dose in individuals who had received Covishield.

Results: Among 176 individuals, IgG antibody against S1 spike protein was found to be positive in 89.7% (158). Covishield recipients showed higher antibody response (99.1%) as compared to Covaxin recipients (71%). Antibody response was higher in males, individuals less than 50 years, and non-comorbid individuals. Of 38 Covishield recipients, IgG antibody response was positive in 28 (73.6%) individuals when followed up at 32 weeks post the second vaccination dose.

Conclusion: The study gives us input with regard to the long-term antibody kinetics of both vaccines. The study has a follow-up plan to co-relate the antibody response to the neutralization test.

Keywords: Immunoglobulin G; Vaccination; India; Spike glycoprotein

INTRODUCTION

The year 2020 took the world turtle as it faced the dreaded COVID-19 pandemic, with the first COVID-19 case being detected in December 2019 from Wuhan in Hubei province in China (1). Early in the pandemic, the WHO recognized the potential of a vaccine to end the pandemic and started rolling out protocols for trials for vaccine candidates (2). India rolled out its largest vaccination drive against COVID-19 from January 16th, 2021, with the Emergency Use approval of two vaccines. Covishield, an...
adenovirus vector-based vaccine (AZD1222 or ChAdOx1-nCOV) produced by Serum Institute of India and developed by Astra Zeneca in collaboration with Oxford University, followed by Covaxin (BBV-152), an inactivated vaccine developed by Bharat Biotech and Indian Council of Medical Research a few days later (3). Covaxin was initially recommended to be used under clinical trial mode as there was insufficient Phase 3 trial data. However, Bharat Biotech announced on March 3, 2021, the results of the third round of clinical trials, following which the subject expert committee gave emergency use authorization for this vaccine (4). COVID vaccination was rolled out in a phased manner with health care workers being vaccinated on a priority basis, followed by the front-line workers, citizens more than 60 years of age, citizens more than 45 years of age, and eventually citizens more than 18 years of age (5). As on 23rd October 2021, 30% of the Indian population had been fully vaccinated, and 707 million had the first dose (5). Studies done emphasize that the immune responses induced by COVID-19 vaccination exceed those induced by natural SARS-CoV-2 infection, and so people who have recovered from a COVID-19 infection still stand to benefit from vaccination (6). However, the duration of protection offered by these vaccines is still unknown, along with the need for booster doses (6). Both Covishield and Covaxin are given under the National immunization program in India.

With the emergence of many new variants of the SARS-CoV 2 virus globally, it becomes necessary to know the antibody dynamics associated with vaccination as well as the duration of protection provided by the vaccines currently under use. Though many studies have been done till date to know about the antibody kinetics of Pfizer-BioNTech and Moderna vaccine post two doses, few studies have been done in India. Studies done by Singh et al. (7) and Choudhury et al. (8) in India showed a significant difference in humoral immune response between Covishield and Covaxin recipients. Our study aims to know the long-term antibody kinetics of both the vaccines among recipients in Southern districts of West Bengal at 12-16 weeks post the second dose and also to look for the waning immune response over a section of those individuals over a period of time. The study also aims to know if there is any difference in antibody response with respect to both the vaccines in SARS-CoV 2 naive and SARS-CoV 2 infected individuals.

MATERIALS AND METHODS

Study setting. The study was carried out in VRDL, Department of Microbiology, Midnapore Medical College, which is an ICMR-certified laboratory. It was carried out for a period of 6 months, from August 2021 to February 2022. Any individual who was >18 years of age and had completed at least 12 weeks post two doses of vaccination were eligible to participate in the study. Post-vaccinated individuals with a history of SARS-CoV2 infection, as well as those who were SARS-CoV 2 naive, were included in the study. Individuals who had not completed at least 4 weeks post SARS-CoV2 infection were excluded from the study. A total of 176 individuals from different strata of the population including doctors, nurses, teachers, reporters, security staff, pensioners, laboratory personnel, etc. participated in the study. Random sampling strategy was followed. Informed written consent was taken along with a set of questionnaires which included their age, occupation, duration post-vaccination, and previous history of COVID-19 if any. Data regarding breakthrough infections were also recorded.

Study design. It was a longitudinal observational study where a percentage of vaccinees who showed positive antibody response against S1 glycoprotein of SARS CoV2 at 12-16 weeks post the second vaccination dose will again be tested for antibody response on completion of 32 weeks.

Sample collection and storage. 6-10 ml of blood was collected from vaccinated individuals by trained laboratory personnel. Blood was centrifuged at 3000 rpm for 5 minutes to get sera which was stored at -20°C till the test was performed.

Detecting serum IgG against SARS-CoV2. Anti-SARS CoV-2 IgG antibody was detected by Enzyme-Linked Immunosorbent Assay (ELISA) method using ELIsafe® TM COVID-19 IgG kit from Hi-media Laboratories Pvt Ltd. The kit is intended for the qualitative detection of IgG antibodies against the S1 fragment of SARS-CoV2 by the standard ELISA method from human serum/plasma samples. The kit has a sensitivity of 99% and specificity of 100%. The tests were performed as per the manufacturer's
instructions, and the final OD at 450 nm was measured in a Biorad microplate reader. Samples whose OD value was more than the cut-off were considered positive. A cut-off index of >0.2 was considered positive, and < 0.2 was considered negative.

**Ethical considerations.** The study was reviewed and approved by the Institutional Ethics Committee of Midnapore Medical College.

**Statistical analysis.** Data collected were uploaded in a Microsoft Excel sheet, and the data was verified for its correctness. A P-value less than 0.05 was considered significant. A Chi-square test for a 2 x 2 contingency table was calculated.

**RESULTS**

The study was carried out in Midnapore Medical College, and it included 176 individuals who volunteered to participate. Out of 176 individuals, 117 were males, and 59 were females. Both SARS-CoV2 infected individuals prior to vaccination (16) and SARS-CoV2 naïve individuals (160) participated in the study. Among 176 participants, 116 were Covishield recipients, and 60 were Covaxin recipients. Among the total 176 participants, IgG antibody against S1 spike protein was positive in 158 (89.7%) participants. Overall seropositivity was higher in males, in individuals less than 50 years and in non-comorbid participants (Table 1). Higher humoral immune response was observed in Covishield recipients (99.1%) as compared to the Covaxin recipients (71%) across all age groups (Fig. 1).

Out of 116 Covishield recipients, 115 (99.1%) showed positive antibody response against the S1 protein. One individual whose antibody was negative was found to be a chronic smoker and alcoholic for the past ten years. He was SARS COV 2 naïve and did not have comorbidity. There was no difference in positivity regarding age, gender, previous history of infection, and comorbidity in Covishield recipients. All 25 comorbid Covishield recipients were found to be positive for the anti-spike antibody.

Among 60 Covaxin recipients, 43 (71%) were reactive for IgG antibody against S1 protein, and the rest, 17 (4.2%), were negative. Covaxin participants below 50 years showed higher antibody response (88.4%) as compared to those above 50 years of age.

![Fig. 1. Antibody positivity with respect to Covishield and Covaxin at 12-16 weeks post second dose of vaccination (Chi square: 32.505, p <0.00001)](image)

**Table 1. Anti-SARS CoV2 IgG positivity against spike protein among vaccine recipients at 12-16 weeks post second dose of vaccination.**

| Characteristics       | Overall positivity (%) (n=176) | P value | Covishield (n=116) Positivity (%) | Covaxin (n=60) Positivity (%) |
|------------------------|-------------------------------|---------|----------------------------------|-------------------------------|
| GENDER                 |                               |         |                                  |                               |
| Male, n (%)            | 110/117 (94.01)               | P=0.008 | 86/87 (98.85)                    | 24/30 (80)                    |
| Female, n (%)          | 48/59 (81.3)                  |         | 29/29 (100)                      | 19/30 (63.33)                 |
| AGE                    |                               |         |                                  |                               |
| <50 years, n (%)       | 113/116 (97.4)                | P<0.00001 | 90/90 (100)                      | 23/26 (88.46)                |
| ≥50 years, n (%)       | 45/60 (75)                    |         | 25/26 (96.15)                    | 20/34 (58.82)                 |
| COMORBIDITY            |                               |         |                                  |                               |
| Present, n (%)         | 36/45 (80)                    | P=0.012 | 25/25 (100)                      | 11/20 (55)                    |
| Absent, n (%)          | 122/131 (93.1)                |         | 90/91 (98.9%)                    | 32/40 (80)                    |
| PREVIOUS H/O INFECTION |                               |         |                                  |                               |
| Present, n (%)         | 16/16 (100)                   |         | 15/15 (100)                      | 4/5 (80)                      |
| Absent, n (%)          | 142/160(88.75)                |         | 99/100 (99)                      | 39/55 (70.90)                 |
age (58.8%) (p=0.011). Antibody response was also found to be significantly lower in comorbid individuals (p=0.042). Among 20 comorbid participants, the antibody response was absent in 9 (45%). Of these 9 individuals with negative antibody response, 5 (55%) were diabetic. There was no association of antibody response to gender in the case of vaccinated covaxin participants.

Various side effects were seen in the participants. Covishield recipients had higher rates of side effects as compared to Covaxin recipients (34.4% vs 8.3%, p=0.0001). The most common side effect observed in Covishield recipients was only fever (65%), followed by fever with body ache (12.5%). Covaxin recipients mostly complained of weakness and malaise.

Till 16 weeks post the second dose of vaccination, breakthrough infections were recorded in 4 (3.4%) participants, and all of them were Covishield recipients. All of them recovered at home, and the disease course was very mild. It was not recorded in any of the Covaxin recipients included in our study.

Antibody response was re-evaluated at 32 weeks post the second dose of Covishield vaccination to find out the waning response over time. Among 116 Covishield recipients, 38 participants who showed positive antibody response at 12-16 weeks post-vaccination were followed up at the 8th month (32 weeks). 28 participants (73.6%) were positive for antibody; rest 10 (26.3%) showed negative antibody response. These 10 participants were SARS CoV 2 naïve. Of 28 participants, 14 (50%) had a history of SARS CoV 2 infection. History of SARS-CoV 2 infection was found to be significantly associated (p<0.05) with positive antibody response (Fig. 2). Follow-up could not be done for Covaxin recipients.

**DISCUSSION**

In this study, we tried to find out the antibody response in the vaccine recipients at 12-16 weeks post two doses of vaccination. Out of 176 recipients (117 male, 59 females), 89.7% showed positive antibody response, thus showing the good rate of persistence of antibodies till 12-16 weeks. The similar higher overall positivity rate of 95% was reported by Singh et al. post two doses of vaccination (7).

The study demonstrated a significantly higher humoral immune response in Covishield recipients (99.1%) as compared to Covaxin recipients (71%). The difference in immune response elicited by two vaccines at the same period post-vaccination may be due to immune response elicited by different individuals to the vaccines in its own way, due to differences in vaccine makeup (vector-borne vaccine vs. inactivated vaccine), or due to elicitation of delayed immune response in some individuals and needs further in-depth study with added parameters and large cohort size. A study done by Choudhury et al. (8) also reported a higher median anti-S IgG titre among Covishield recipients.

Our study also showed a significant association of overall seropositivity to age and sex. Comorbid participants showed less seropositivity rate compared to non-comorbid participants. This may be due to suppressed immune response in comorbid individuals due to associated medical conditions. Study done by Singh et al. (7) also showed that people over 60 years or those with comorbidities had significantly lower seropositivity rate.

The elderly population (adults over 65 years of age) is at higher risk for severe COVID-19, accounting for approximately 80% of hospitalizations and a higher fatality rate, and require prioritization in vaccination campaigns (9, 10). In the Covishield group, antibody response was not related to age and gender but in Covaxin group, antibody response decreased with an increase in age.

Antibody dynamics differed substantially with a history of infection. IgG antibody was positive in 100% (16/16) participants with prior infective status as compared to SARS-CoV2 naïve participants (89.3%). Previous studies were done in BNT162b2 vaccinated healthcare workers also demonstrated higher antibody levels and slower waning with history of prior infection (11-13).

A vaccine breakthrough infection is defined as the
detection of SARS-CoV2 RNA or antigen in a respiratory specimen collected from a person ≥14 days after receipt of all recommended doses of an approved COVID-19 vaccine (14). Breakthrough infections were not reported in Covaxin recipients until the data lock date, similar to the one written by Singh et al. (7). This might be due to the fact that Covaxin had better protective coverage against the emerging variants. Breakthrough infection was recorded in 3.4% of Covishield recipients in our study. A study was done by Dash et al. (15) from an eastern state of India also reported a higher rate of breakthrough infections in Covishield recipients as compared to Covaxin recipients.

The durability of protection offered by two doses of vaccination is still under study, and controversies surround the perfect time or the role of a booster dose. When 38 Covishield participants were followed up at eight months (32 weeks) post-second dose, 14 of 28 participants who showed persistence of antibody level had a history of SARS CoV 2 infection. Though the whole cohort of Covishield participants could not be followed up, we can infer that the antibody level had started falling in the eighth month, and higher immune response in the form of persistence of antibody levels could be seen in participants with a history of infection. Several studies concerning Pfizer-BioNTech vaccine also show that long-term humoral response and vaccine effectiveness in previously infected persons were superior to that in recipients of two doses of vaccine (16). Whether this persistence could protect against reinfection can be understood only when the anti-spike antibody is correlated with neutralizing antibody. A study done by Levin et al. (17), found that six months after receipt of the second dose of the BNT162b2 vaccine (Pfizer–BioNTech), humoral response was substantially decreased. Since limited data exists for long-term antibody kinetics of Covishield and Covaxin, this study attempted to look at the waning immune response at the 8th month, though the cohort size was very small.

Anti-spike antibody titer is reported to correlate highly with the in-vitro virus neutralization test measured by PRNT (18). This study gives us a presumptive picture of vaccine efficacy and duration of protection though detection of neutralizing antibody titer would have given a better view. The immune system consists of antibodies, B cells, and T cells. Researchers found that vaccination resulted in durable cellular immunity. Memory B cells continued to grow in numbers for at least six months, and T-cell counts remained relatively stable, with a slight decrease in number (19).

Though the study was the first of its kind in this region, as per our knowledge, there were several limitations. First, though random sampling was done, most of them were healthcare workers. A larger community-based study would have given a better picture. Second, since ELISA is a qualitative test, antibody titer could not be compared between SARS CoV2 naive and SARS CoV2 infected individuals. Third, as the PRNT test could not be performed, we could not comment on the relation and level of protection offered by the anti-spike antibody against reinfection from COVID19. Fourth, follow-up in the eighth month could not be done for Covaxin recipients as the participants failed to turn up. So, the level of persistence of antibodies in Covaxin recipients could not be detected. Fifth, there was a lack of data on cellular immunity, which also has a significant role in protecting individuals.

CONCLUSION

Not many studies have been done before this to know about the long-term antibody kinetics of vaccines given in India under Emergency Use Authorization, and to the best of our knowledge, our study is an addition to these few numbers. In fact, to the best of our knowledge, the present study is the first of its kind to report the antibody kinetics of Covishield in the eighth month in India though the follow-up cohort was very small. Due to the ever-evolving nature of the SARS-CoV2 virus, strategies to modify vaccines to give better coverage to the variants should be thought of to protect humanity from the dreaded virus. The study has a follow-up plan to compare the positive antibody response to its neutralization activity by the PRNT test.

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