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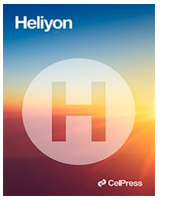

Research article

IgG responses against SARS-CoV-2 vaccines AZD1222 and BBV-152 and breakthrough infections among health care workers in southern India

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A B S T R A C T

IgG antibodies elicited in response to SARS-CoV-2 are critical in determining the protection achieved through vaccination. The present longitudinal study aims to assess the immune response generated through AZD1222 & BBV-152 vaccination among health care workers (HCWs) in a selected hospital. Serum IgG levels were measured approximately at 1.5 months and 6 months after the first and second vaccination. The final assessment was done 12 months after the first vaccination to analyse the sustained antibody levels. Results showed a progressive increase in antibody titres as a function of time. 26 HCWs in all had SARS-CoV-2 breakthrough infection, but their antibody titres were not significantly higher compared to COVID-19 naïve individuals. However, a comparative analysis showed considerably higher antibody titre in those who received the AZD1222 vaccine among this cohort. AZD1222 vaccination was significantly associated with seropositivity in the first and second assessments. Female HCWs showed significantly higher seropositivity, and participants above 60 years showed considerably reduced antibody titre in the first assessment. However, the final assessment showed no association with these variables, with 97.1 % of participants reporting to be seropositive. The results indicate good antibody response and potential protection against SARS CoV-2.

1. Introduction

Coronavirus disease 2019 (COVID-19) caused due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been the largest health burden globally, resulting in extensive mortality and morbidity across the world [1,2]. Two types of vaccines, namely BBV-152 (Covaxin™) and AZD1222(Covishield™), were launched in India on January 16, 2021, against SARS-CoV-2. AZD1222 is a recombinant Chimpanzee adenovirus vector-based vaccine manufactured by Serum Institute of India, Pune and BBV-152 was formulated by inactivated whole viral particles adjuvanted with imidazoquinoline (TLR 7/8) agonist manufactured by Bharat Biotech, Hyderabad in collaboration with Indian Council of Medical Research [3,4]. In western countries, mRNA-based vaccines have been used. These vaccines have been found to elicit a broad-spectrum neutralizing antibody, and other cellular responses and has extended protection against the variants. But the requirement for cold chain and high cost limit the application of these vaccines in resource limited countries like India [5].

The vaccination drive in India was initially implemented on health care workers (HCWs) followed by the elderly (more than 60

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years) and those with co-morbidities. From May 01, 2021, onwards adults (≥18 years of age) were vaccinated [6]. The administered dose of AZD1222 is 0.5 ml which contains 5 x 10^{10} adenovirus particles, carrying genes for SARS-CoV-2 spike protein, whereas 0.5 ml of BBV-152 has a 6 μg dose of the inactivated whole virion of strain NIV-2020-770. The vaccine effectiveness is generally determined by the factors like antibody production, induction of memory cells, and cell-mediated immunity [7,8]. The antibody-mediated immunity is an essential factor in controlling viral infections. Induction of sustained immune response is crucial for the overall success rate of the vaccine.

Various variants of SARS-CoV-2 have emerged worldwide and effectivity of available vaccines against these variants has been a great concern. The variant Delta (B.1.617.2) and Delta Plus (B.1.617.2.1) were the biggest concern for India [8]. With the emergence of new variants in China and many countries, it is important to assess the immune response of individuals, in particular healthcare workers. Long-term follow-up of the antibody kinetics will give a picture of total vaccine efficacy. HCWs have a high risk of exposure to SARS-CoV-2 due to their profession. Here we present a summary of follow-up data on antibody levels of HCW post-vaccination.

2. Methods

2.1. Study design

The present report is a longitudinal study conducted on the 123 HCW of Madras Medical Mission, Chennai, a hospital in Southern India. The study participants were vaccinated either by AZD1222 or BBV-152 were surveyed and consented for the study. The antibody titres were measured three times post-vaccination using VIDAS SARS-CoV-2 IgG II (9COG) semi-quantitative assay kit that is FDA and CE marked [9]. The first sample was analysed 41 ± 22 days after the first dose, the second sample 186 ± 76.09 days after the second dose, third sample 368 ± 22.46 days after the first dose. Study participants with past infections, as well as breakthrough infections, were also included in the analysis. Data collection started from the first day of vaccination, i.e. 03 Feb 2021, until the third assessment, i.e. 16 Feb 2022. The serum or plasma samples were collected on the days mentioned earlier in EDTA vials and were analysed at the Department of Clinical Microbiology, Serology and Infectious Molecular biology at the Madras Medical Mission in Chennai.

The overall vaccination regimen is provided in Fig. 1.

2.2. Study participants

One twenty-three HCW were recruited for the study. The study received clearance from the Institutional Ethics Committee, The Madras Medical Mission (ECR/40/Inst/TN/2013/RR-20). Written informed consent was obtained from the participants for collection and analysis of the sample Relevant history of past COVID-19 infection, comorbidities such as diabetes and hypertension, age and sex were collected on each assessment to check their possible association with seropositivity or antibody titre. The participants' socio-demographic & clinical data is provided in S1.

2.3. Serologic assessment

VIDAS SARS-CoV-2 IgG assay kit was used to measure the IgG antibodies generated against SARS-CoV-2 spike protein using Enzyme Linked Fluorescent Assay (ELFA) method. The assay detects the antibodies attaching to the receptor-binding-domain (RBD) for SARS-CoV-2 "S" protein. The test was run on serial serum samples collected and run as per manufacturer's instructions. The sensitivity was 96.6 % and specificity of the kit was 99.9 % as per the manufacturer's protocol. Antibody levels of index value I ≥ 1 was considered

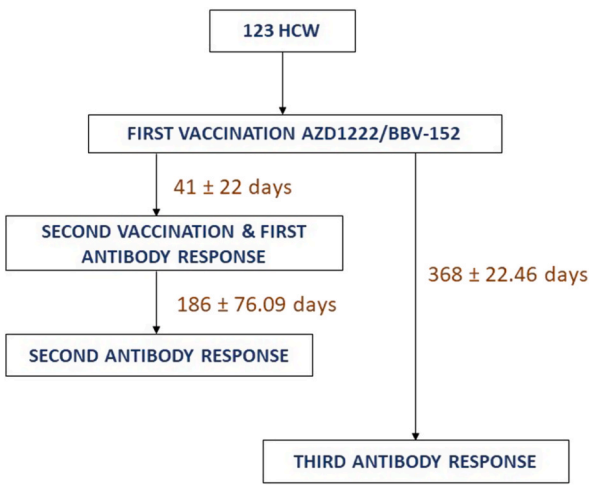

Fig. 1. CONSORT flow diagram showing the vaccination regimen.

seropositive, while antibody levels of index value I < 1 were considered seronegative. The limit of detection defined as the lowest concentration of the analyte that can be differentiated from an analyte free sample with a probability of 95 % was I = 0.27 and limit of quantitation defined as the lowest concentration of the analyte that could be detected and measured with acceptable precision (within lot precision set at 20 % CV) was I = 0.40 of the diagnostic kit. The test was considered semi-quantitative as a cut-off value (index = 1.00) was considered equivalent to 20.33 BAU/mL (Binding antibody unites/mL) of the WHO international standard for anti-SARS-CoV-2 antibodies (NIBSC, code 20/136).

2.4. Statistical analysis

Descriptive statistics was used to summarise and present the data. Continuous data is presented as mean ± Standard error, and categorical data is shown as percentages. To understand the effect of SARS-CoV-2 infection contributing to enhancing the antibody titre level apart from vaccination, the HCW were assigned into two cohorts for analysis purposes. Only 7.3 % of HCWs received booster dose; hence this cohort was excluded from the analysis. Chi-square test was used to check the association between any categorical variable. The *t*-test was done to find the significant difference between IgG levels among the grouping variables mentioned earlier. The Generalised linear mixed model with a binomial distribution (seropositivity as binomial variable) was fitted to link the magnitude of variance of each variable with the shift in the seropositivity to get an overall picture. The model worked well for the first and second assessment data. Since no evident variability was observed in the predictive/dependent variable in the final assessment (seropositivity was 97.1 %), it was excluded from the analysis. Repeated measures ANOVA was performed to check the variability in the antibody titre in different assessments in each cohort. P value less than 0.05 was considered a significant observation.

3. Results

A total of 123 healthcare workers vaccinated were assessed for antibody titre levels and seropositivity. Among the healthcare workers, 26 confirmed SARS-CoV-2 infections diagnosed by RT-PCR during the study period, most reporting after two vaccinations. Only one person had a history of COVID-19 before the first vaccination dose. Diabetes and hypertension were observed in less than 5 % of the HCW, whereas only 7.3 % of the study participants received booster dose. Table 1 summarises the overall data by grouping variables like gender, type of vaccine and age.

3.1. Overall trend in seropositivity in different assessments in COVID-19 naive cohort

The generalised linear mixed model was used to determine the association of age, gender, type of vaccine, and comorbidities with seropositivity in the different assessments of COVID-19 naïve HCWs (Fig. 2). Each variable was also independently assessed using the chi-square test for their significance. Seropositivity achieved in consecutive assessments revealed a progressive rise in percentage being; 86.5 %, 89-69 % & 97.67 %. The third assessment was excluded from the analysis as maximum seropositivity was observed with no variability associated with the factors considered. Consistently in the first and second assessments, recipients of AZD1222 showed good association with seropositivity as tested by chi-square ($p < 0.001$), and this observation was also well predicted by the model (coef = 2.5, p = 0.003-first assessment; coef = 2.3, p = 0.004- second assessment). Female HCWs were more associated with seropositivity compared to males. However, this was not the predictive variable per the generalised linear model since the model accounts for overall variability related to the predictive variable. Overall, no significant difference was observed in seropositivity concerning age. However, HCW above 60 years had lower antibody titre than HCW below 60yr with marginal significance and the mean difference ranging from 5 to 10 BAU/mL. Table 1 depicts the overall summary of seropositivity in different assessments.

3.2. Analysis of variables associated with antibody titres in different assessments among COVID-19 naive cohorts

Various variables such as type of vaccine, age and gender were analysed to check their association with the shift in antibody titre separately among the uninfected/COVID-19 naive and COVID-19 breakthrough infection cohorts. The breakthrough infection cohort and COVID-19 naive were separately analysed to rule out infective condition contributing to the serum antibody levels. The highest noted antibody titre value in the first assessment was 54.96 BAU/Ml. No significant association was observed with age, gender, type of vaccine and comorbidities. The generalised linear model was fitted, but no significant determinants were obtained. Among the uninfected cohort, antibody titres significantly varied in each assessment (21.78 ± 18.42, 18.90 ± 14.8 BAU/mL, and 28.84 ± 11.79

Table 1
Seropositivity pattern and the associated variables in the first and second assessment.

Variable	Seropositivity (%) First assessment	P value	Seropositivity (%) Second assessment	P value
Male	80.28	0.02*	87.32	0.37
Female	94.23		92.30	
AZD1222	91.81	<0.001*	92.7	0.001*
BBV-152	38.4		61.5	
≥60 yr	83.33	0.66	75	0.07
<60 yr	87.73		91.5	

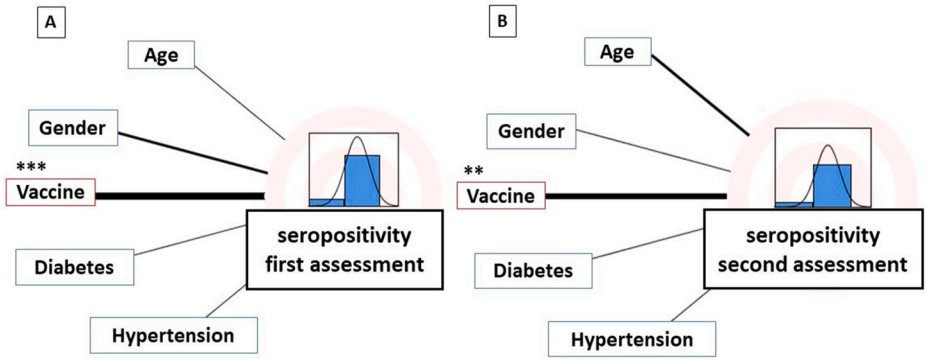

Fig. 2. Generalised linear model depicting the overall determinants of seropositivity in first assessment (A) and second assessment (B) with its significance (denoted by asterisk).

BAU/mL in the first, second and third assessments accordingly, $p < 0.001$) and the levels significantly increased by the end of third assessment compared to the initial evaluation ($p = 0.01$). Overall, the antibody titre considerably increased by the end of the third assessment. Antibody titre levels of HCW above 60 years showed reduced titre values in the first assessment with marginal significance. However, the second and third assessments did not report any significant difference in the antibody titre levels concerning age. Diabetes and hypertension were reported by less than 5 % HCW overall; hence antibody titre mean levels in these cohorts could not be compared. Gender and type of vaccine didn't show any association with the antibody titre value. Overall data is summarised and presented in Table 2.

3.3. *The pattern of COVID-19 breakthrough infection affecting seropositivity and antibody titre values*

Among the cases analysed, 26 HCWs had SARS-Cov-2 breakthrough infection at different study time points. Only one person had past COVID-19 infection, i.e. before the first vaccination. Most of the breakthrough infections occurred just before the second assessment. Hence the basis of considering it a cohort in the first and second assessments didn't seem relevant. Considering the time of infection, the comparison between the cohort was made only for the last assessment, which gave an overall picture of the long-term effect of past SARS-Cov-2 infections and vaccination. The mean antibody titre observed in HCW with a breakthrough infection was not significantly higher compared to the COVID-19 naive cohort (28.84 ± 11.79 BAU/mL -uninfected cohort, 30.50 ± 9.66 BAU/mL breakthrough infection cohort, $p = 0.52$). Apparently, a significantly higher antibody titre was observed in the recipients of AZD1222 who had a breakthrough infection (32.44 ± 7.73 BAU/mL – AZD1222, 19.82 ± 13.41 BAU/mL – BBV-152, $p = 0.01$) (Fig. 3).

Meanwhile, whether SARS Cov-2 infections were higher among the AZD1222 recipients was checked, but no association could be proved between the type of vaccine and occurrence SARS COV-2 infection. Hypertensive and diabetes cases were very negligible in this cohort. Hence comorbidity as a factor affecting the antibody titre or seropositivity was undetermined. At the end of the third assessment, 97.1 % seropositivity was achieved; hence any association with breakthrough infection gender age susceptibility type of vaccine could not be proved. Apart from this, no difference in SARS-Cov-2 infection occurrence was observed concerning gender and age above/below 60. Fig. 4 depicts the overview of antibody titre between the cohorts and the time point for the events like vaccine COVID-19 infection.

Table 2
Antibody titre levels of COVID-19 naive HCW in different assessments.

Variable	Group 1	%	Group 2	%	Mean ± SE Group 1 (3 assessment value in order)	Mean ± SE Group 2 (3 assessment value in order)	Significance
Age	≥60 yr	10.2	<60 yr	89.8	12.47 ± 15.35	23.73 ± 18.40	0.04*
					13.67 ± 14.41	20.06 ± 14.79	0.19
					27.41 ± 16.48	29.67 ± 10.74	0.77
Gender	Male	57.7	Female	42.3	20.62 ± 18.35	23.50 ± 18.64	0.45
					19.62 ± 15.5	17.82 ± 13.83	0.55
					29.56 ± 12.02	27.90 ± 11.74	0.64
Vaccine	AZD1222	89.4	BBV-152	10.6	22.5 ± 18.19	14.75 ± 16.31	0.3
					19.22 ± 14.56	15.72 ± 17.67	0.57
					29.38 ± 10.39	24.63 ± 21.05	0.19

Note: SE= Standard error. Independent sample *t*-test is employed to find the significant difference in the mean. $P < 0.05$ considered statistically significant. Significant p values are indicated by asterisks.

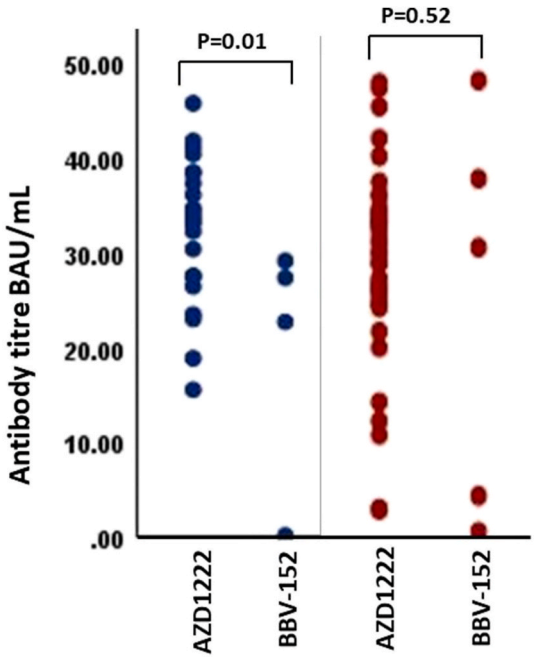

Fig. 3. A dot plot showing relation between type of vaccine, breakthrough infections & antibody titre.

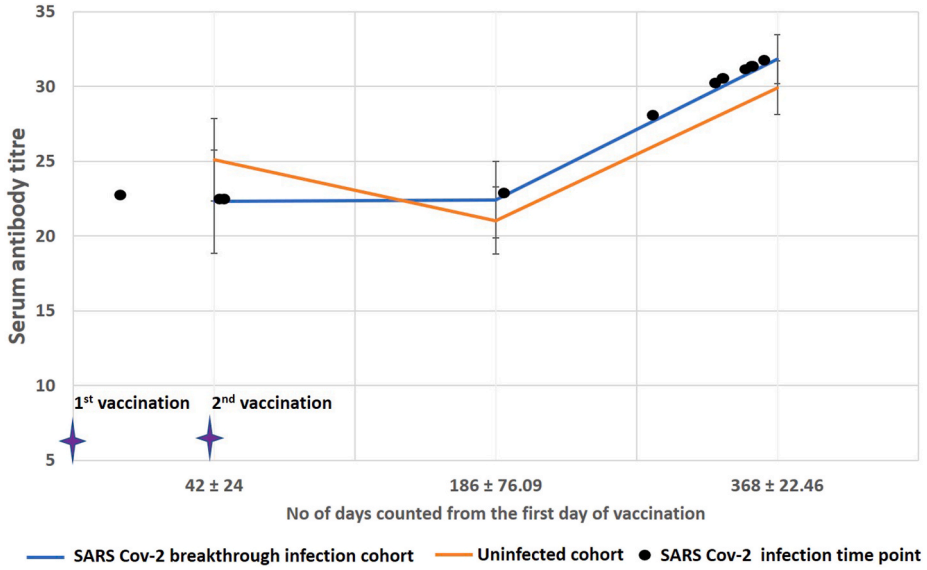

Fig. 4. Summary of antibody titre between the cohort (participant with SARS Cov-2 breakthrough infection and COVID-19 naïve participant) in each assessment.

4. Discussion

The present study demonstrated a good antibody response to vaccination among the HCWs. Antibody titres were assessed after the first and second vaccination, followed by a final assessment a year later from the first vaccine shot. Overall antibody titres progressively increased in each assessment from a mean titre value of 21.16 ± 18 to 29.45 ± 11 at the end of the assessment. The antibody titre among the AZD1222 and BBV-152 recipients didn't show a significant difference as per the analysis. There are many studies revealing the comparative benefit of AZD1222 as well as BBV-152. Few studies reported a dip in antibody titre over the period in AZD1222 recipients [10–14]. Few other studies revealed a decline in antibody titre with AZD1222 and BBV-152 [12]. The present study aligned with the studies demonstrating a sustained antibody titre which was a good response from the immunological perspective [3].

Considering the type of vaccine, generally, a good response was obtained from the recipients of the mRNA-1273 (Moderna) vaccine, 26 CE.COV2.S (Johnson & Johnson), BNT162b2 (Pfizer-Biotech) vaccine [15–17]. Sustained antibody levels were also connected to the fact of having past COVID-19 infection as well as breakthrough infection. In such patients, the antibody titre sustained in the body for almost eight months and remained significantly higher than SARS Cov-2 naïve individuals, as indicated by several reports [7,17,18]. Overall higher vaccine effectiveness was observed among the vaccinated people with prior infection [6]. The study conducted by Pavan et al., 2021 in the same region showed a similar pattern antibody response between those who took BB-152 in single dose and Covid-19 naive people who received two doses. In the present study, the antibody levels were not significantly higher among the individuals who experienced COVID -19 infection, compared to COVID-19 naïve cohort but incidentally, AZD1222 recipients with breakthrough infection reported substantially higher antibody response. To add to this point, AZD1222 recipients showed significantly higher seropositivity compared to the recipients of BBV-152 in the first and second assessments. This observation of significantly higher antibody titre/seropositivity among AZD1222 recipients compared to BBV-152 was also reported by others [3,8,19]. In contrast to this there is also a report demonstrating significantly lower viral load in those who received BBV-152 compared to AZD1222 [20]. While connecting all the variables associated with seropositivity and antibody titres, it was found that only first and second assessment values seemed to be affected by variable such as age and type of vaccine gender. For instance, female HCWs showed significantly higher seropositivity, and participants above 60 years showed considerably reduced antibody titre in the first assessment. A decreased level of antibody titre in the HCW above 60 years was also reported by others [8,21–23]. The final assessment revealed 97.1 % seropositivity irrespective of the type of vaccine, gender and age. In the long run, the vaccine seems to have a protective role, as indicated by the results. We do acknowledge some limitations of the study like difference in the duration of assessment after first and second vaccination. There was longer time interval (approximately 6–8 months) between the assessment and second vaccination shot. The antibody titre in this duration could have affected by the contract of infection as Covid diagnosis trend largely reduced. Due to the different visiting points, there was bigger range in the duration adding to its variability. The study involved only health care workers thus may not accurately represent a general population.

5. Conclusions

Overall antibody titre showed a gradual rise by the end of the third assessment, and it was not dependent on gender and type of vaccine. The breakthrough infection didn't result in a significant rise in the antibody titre; however, individuals who received AZD1222, among this cohort reported a significantly higher antibody titre. A higher proportion of people opted for AZD1222, which was associated with higher seropositivity in the first and second assessment compared to BBV-152. Overall, it suggests that initial response of antibody titre and seropositivity varied with grouping variables such as age, type of vaccine and gender; however, the final assessment showed no association with the variables mentioned, with 97.1 % of participants reporting to be seropositive.

Data availability

The entire data is included in the supplementary file.

CRediT authorship contribution statement

Anusha Rohit: Writing – review & editing, Conceptualization. **Caroline DSouza:** Writing – original draft, Formal analysis, Data curation. **Suresh Kumar:** Methodology, Investigation. **Meenachi Ct:** Methodology, Formal analysis. **Vinothini V:** Methodology, Formal analysis. **Siva Perumal:** Methodology, Investigation, Formal analysis. **M. Philip:** Supervision, Resources, Project administration. **Raju George:** Supervision, Resources, Project administration. **Iddya Karunasagar:** Writing – review & editing, Validation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25528.

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