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Antibody Response Against Covid-19 Among Vaccinated Health Care Workers - A Cross-Sectional Serosurveillance Study

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Abstract

Background: The vaccination drive against COVID-19 in India began on January $16^{th,}$ 2021, with two approved vaccines - Covishield™ and Covaxin™. Despite hurdles in the production and supply of vaccines, the vaccination rollout envisaged the successful vaccination of the entire adult population of the country by the end of this year. The humoral response against COVID-19 induced by the administration of two doses of any one of the two available vaccines was estimated among health care workers (HCW). **Subjects and Methods:** Semiquantitative estimation of SARS-CoV-2 IgG antibody against S1 domain of spike protein was done in the serum obtained from 89 HCWs using ELISA. The antibody response was correlated with age, sex, body mass index (BMI), and co-morbid conditions. A comparison of antibody response was done among the infection naïve and naturally infected study population. The common adverse effects following vaccination were identified. **Results:** The seropositivity rate of this study was 86.5%. Covishield recipients reported higher seropositivity (89%). There was no significant correlation of antibody response with age, sex, BMI, and co-morbid conditions. The antibody response was significantly higher in participants who had previous COVID infection when compared with the infection naïve group. **Conclusion:** Vaccination is essential for containing the current pandemic. Significant antibody titer is present in vaccinated individuals. The immune response of participants who had been previously infected is significantly improved with vaccination.

Keywords: COVID-19, SARS-CoV-2, Vaccine, Antibody response, Enzyme linked immunosorbent assay.

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Introduction

Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2), a novel coronavirus emerged in December 2019 in China. The disease it causes was named COVID-19 and it spread rapidly throughout the world and soon became a pandemic. The spectrum of COVID-19 disease is wide, ranging from mild asymptomatic infection to severe respiratory illness. Till January 3, 2022, India had reported 3,49,22,882 confirmed COVID cases with 4,81,893 deaths according to the data of the Ministry of Health and Family Welfare. Vaccination has remained the mainstay of protection against the COVID crisis globally and in India, two vaccines were approved – Oxford-AstraZeneca vaccine manufactured by Serum Institute of India with the trade name Covishield and Covaxin developed by Bharat Biotech in association with the Indian Council of Medical Research (ICMR) and National Institute of Virology (NIV). The vaccination rollout was initiated on the 16^{th} of January 2021 and is successfully being carried out throughout the country. The vaccination program was introduced in phases and in the first phase, health care workers and frontline workers were targeted. Following this, was the second phase which targeted elderly adults, and currently, the third phase targeting adults aged > 18 years is in progress. Since the introduction of vaccination, studies evaluating antibody response and neutralization titers are being done.[1–6] Despite the US Food and Drug Administration (FDA) statement against post-vaccination antibody evaluation tests, studies have been done in different parts of the world to assess the efficacy of vaccination using standardized antibody assays among various study populations.[7–10]

Subjects and Methods

Study design and participants

This was a cross-sectional study. The study was approved by the Institutional Ethical Committee, of Dr. ALM PG Institute of Basic Medical Sciences, University of Madras (UM/IHEC/F.RM/2021/XIII). Health care workers aged 18 years and above, who were vaccinated with two doses of either Covishield or Covaxin were included in this study. The study group also included participants who had previously been exposed and recovered from COVID infection. Participants who were suffering from acute illness or who were diagnosed with COVID-19 infection six weeks before the study were excluded from the study. A written consent form was obtained from all the participants. A total of 89 participants were included in the study. Approximately one ml of venous blood was collected from each participant and processed after serum separation.

Anti-SARS-CoV-2 ELISA

Semiquantitative determination of human antibodies of the immunoglobulin class IgG against SARS-CoV-2 in serum samples of study participants was done using ELISA (EUROIMMUN Medizinische Labordiagnostika AG). The ELISA kit contained wells coated with the S1 domain of the spike protein of SARS-CoV-2. Briefly, 100μl of the calibrator, positive and negative controls, and diluted patient samples (1:100 dilution) were transferred to respective microplate wells and incubated at 37°C for 60 minutes. The plate was then washed three times using 300 μl of wash buffer for each well. Following this, 100 μl of enzyme conjugate was pipetted to each well and incubated for 30 minutes at 37°C. The washing step was repeated and 100 μl of substrate solution was added to each microplate well and incubated in dark for 30 minutes at room temperature. Finally, 100 μl of stop solution was added to each well and the color intensity was photometrically measured at a wavelength of 450 nm, 30 minutes after adding the stop solution. Semiquantitative evaluation of the results was done by calculating a ratio of the extinction of the control or patient sample over the extinction of the calibrator. A ratio <0.8 was interpreted as negative; ≥0.8 to ≤1.1 was interpreted as borderline and ≥1.1 was interpreted as positive.

Statistical analysis

Descriptive statistics and ANOVA were used in SPSS version 21.

Results

A total of 89 HCWs who had taken two doses of either of the vaccines were included in the study. The mean age of the study population was 28.71 years, with a standard deviation of 8.94 years. There were 30 males and 59 females. A questionnaire was used to obtain data on basic demographic details, presence of co-morbid conditions, history of previous exposure to COVID – 19, and vaccination details. Majority of the participants (93.25%) had been administered the Covishield vaccine. Out of 89 HCWs, 14 reported a history of natural infection with COVID-19 in the past. The semiquantitative ELISA assay identified the presence of antibodies in 77 samples (86.5%) which included 75 positives (ratio ≥1.1) and 2 borderline positives (ratio ≥0.8 to ≤1.1). The baseline characteristics of the antibody-positive and antibody-negative study population have been listed in [Table 1].

Differences in antibody status across the parameters such as age, sex, BMI did not show any significant association. However, significantly higher seropositivity was seen in participants who had natural infection with Covid earlier [Table 2].

Participants vaccinated with Covishield showed high seropositivity (89%) when compared to those vaccinated with Covaxin (50%) with a significant difference in the mean antibody ratio (F value = 7.915 ; p = 0.006) [Figure 1].

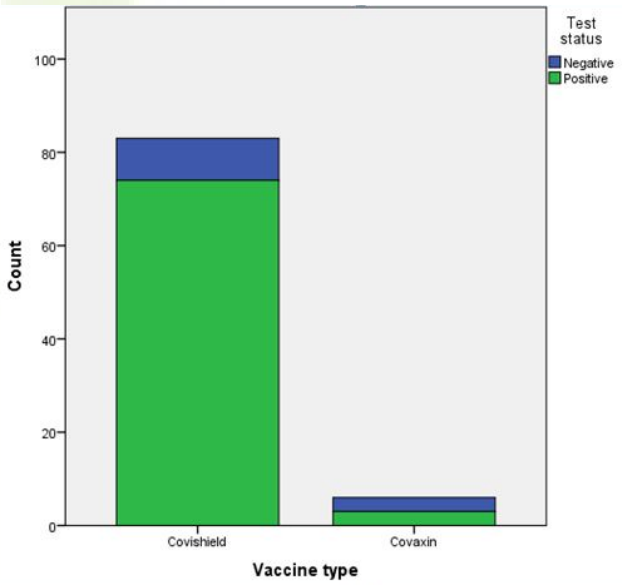

Figure 1: Bar chart showing the antibody status of participants administered with two different types of vaccines.

The duration between the second dose of vaccine and test for antibody detection ranged between 1 – 5months. Duration, when plotted against the mean ratio, displayed a steady decline in antibody with the increase in duration [Figure 2].

The antibody ratio levels were found highest at the first month after the second dose with a mean value at 5.03, thereafter reducing every month with a slight increase at the fifth month after the second dose. The low levels were found in the fourth month with a mean antibody ratio of 1.98.

These differences in mean antibody ratio at various months after the second dose of vaccine were found to be statistically

Table 1: Demographic characteristics, past exposure, and vaccination details of seropositive and seronegative cases.

Factors		Seropositive (*N= 77)	Seronegative (*N= 12)	Total
†Age Mean (SD)		28.130 (8.33)	32.41 (11.94)	28.71 (8.94)
Age Group	Less than 31	60	7	67
	31-40	7	3	10
	41-50	8	1	9
	51-65	2	1	3
Sex	Male	26	4	30
	Female	51	8	59
BMI	Underweight	4	0	4
	Normal	40	6	46
	Overweight	26	2	28
	Obese	7	4	11
Had Natural Covid	Yes	12	2	14
	No	65	10	75
Vaccine Type	Covishield	74	9	83
	Covaxin	3	3	6
Co-morbidity				
Diabetes Mellitus	Yes	2	0	2
	No	75	12	87
Hypertension	Yes	2	1	3
	No	75	11	86

*-N – frequency
†-Mean (SD) – Mean (Standard deviation)

Table 2: Comparison of the mean ratio of naturally infected with infection naïve participants

Previous COVID-19 infection status	Frequency	Mean ratio (SD)	F Value (significance)
Naturally infected seropositive	12	5.45 (1.19)	28.128 (p = 0.000)
Naturally infected seronegative	2	0.13 (0.05)	
Infective naïve seropositive	65	3.53 (1.55)	
Infection naïve seronegative	10	0.285 (0.224)	

Table 3: Frequency and mean antibody ratio up to five months after 2nd dose of vaccine

Duration between 2^{nd} dose of vaccine and antibody testing	Frequency	Mean (S.D)	F value (significance)
1 month	6	5.03 (1.34)	4.124 (0.004)
2 month	33	3.73 (1.91)	
3 month	27	3.49 (1.81)	
4 month	17	1.98 (1.95)	
5 month	6	2.77 (1.57)	

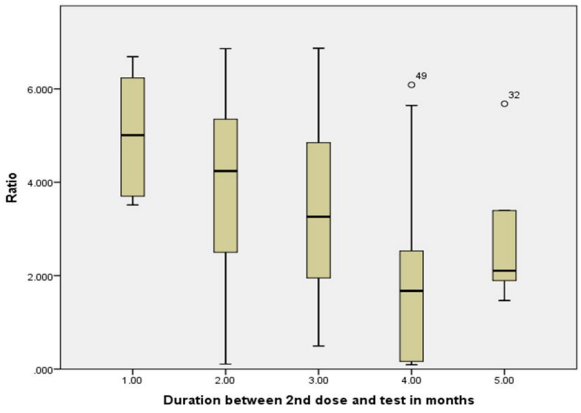

Figure 2: Box and whisker plot showing the relation between duration after the second dose of vaccine with the mean ratio.

significant [Table 3].

Adverse effects following vaccination were more pronounced following the first dose of vaccine and pain at the injection site was reported as the commonest side effect following both first and second dose of vaccine. The common side effects after vaccination are listed in [Table 4].

Discussion

Vaccination against COVID-19 has evolved as a key factor in controlling the current pandemic and has become indispensable in cutting the chain of transmission. Serological assays to detect antibodies against SARS-CoV-2 serve as an adjunct diagnostic test in addition to molecular assays and standard guidelines proposed by the International Federation for Clinical Chemistry and Laboratory Medicine (IFCC) Taskforce recommended the use of these assays in identifying immunity and antibody response to vaccines.[11] The immune response developed following vaccination is heterogeneous and poor responders are observed commonly in aged and immunosuppressed populations.[12,13] Hence, determination of post-vaccine immune response is essential to assess the protection against disease.

The present study detected the presence of anti-spike IgG antibodies against SARS-CoV-2 in a cohort of HCWs who were administered with two doses of either Covishield or Covaxin. Out of the 89 study participants, 83 (93%) were immunized with Covishield. This is in line with the recent studies done in India which reported 82.5% Covishield vaccinees.[5,6] Positive antibody response was observed in 77 out of 89 study participants (86.5%) in the current study.

The age of participants ranged between 19-65 years with a majority of them in the age group less than 31 years (75.3%). There was no significant difference in the antibody response rate among the study population with respect to age. Previous studies evaluating antibody response after the first dose of vaccination have reported an increase in non-responder rates with an increase in the age of the study population.[5,12,14] However, antibody response evaluated by studies after the second dose of the vaccine showed no change in seropositivity rate with increasing age.[6,14] There was no significant correlation of sex and BMI with antibody response in the present study which is similar to the previous study reports.[4,6] No significant association of co-morbidities with the immune response could be established in this study, unlike previous studies which observed a significant reduction in seropositivity rates among participants with type-2 diabetes mellitus and hypertension.[5,6]

Past history of COVID-19 infection either before or after the first dose of vaccination was present in 15.7% of participants. A significant difference was detected when the mean antibody ratio of the naturally infected was compared with the infection naïve population. Similar results were reported by Singh AK et al. who observed a significant rise in antibody titers in people who were naturally infected following both first and second doses of vaccine.[5,6] Salvagno et al. compared the post-vaccination antibody levels of baseline seropositive subjects with that of baseline seronegative subjects and found that the levels were 11 times higher.[1]

The seropositivity rates were higher in participants administered with the Covishield vaccine when compared to Covaxin in the present study. This may be due to the very small sample size (n=6) of Covaxin recipients. A pan-Indian study done recently also reported a similar finding. The number of Covaxin recipients was low in this study also.[6] The other reasons for this difference in efficacy between two vaccines may be differences in characteristics of vaccine components and recipients.

The frequency of adverse effects following vaccination was more after the first dose of vaccination. The common side effects noted in this study were pain at the injection site, tiredness, and fever. There was no significant difference in post-vaccination side effects between previously infected and infection naïve groups. A study by Ebinger et al. reported more pronounced side effects in previously infected people after the first dose of vaccination. However, following the second dose, no significant difference was observed among the two groups.[15]

The study has some limitations particularly the small sample size, and the small number of Covaxin vaccinees which made the comparison between the two vaccines difficult. Baseline serology before vaccination was not done and T-cell immune responses were not measured in this study.

Table 4: Post-vaccination adverse effects

Symptoms	After the first dose (Frequency)	After the second dose (Frequency)
Fever	46	15
Headache	31	11
Pain at Injection site	56	19
Chills	3	2
Myalgia	33	8
Tiredness	56	15
Allergy	1	0

Conclusion

The mean antibody ratio values were found to decrease with time from the first to the fourth month after the second dose. More studies on antibody titers at various point of time with larger sample for a longer duration is required to understand the duration of benefit, the vaccination offers. Since there is a definite and significant seropositivity level amongst the vaccinated, it may be inferred that vaccination holds the key to the control of the pandemic and has to be aggressively carried out to break the chain of transmission. Vaccination of a large proportion of the population, together with COVID appropriate behaviour, appears to be the way to transition to normalcy.

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