Original Article

Anti-SARS-CoV-2 antibodies among vaccinated healthcare workers: Repeated cross-sectional study

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Abstract

Background: Since the novel SARS-CoV-2 has been detected and the ensuing pandemic, the search for a cure or prevention has been the only target of the medical fraternity. As the second wave racked havoc, vaccines seemed to be the only viable option to stop this global surge. World Health Organization (WHO) and subsequently the Government of India have issued emergency use authorization to two vaccines. Our study aims to estimate the prevalence of the anti-SARS-CoV-2 antibodies and identify predictors of antibody titers in vaccinated healthcare workers in VIMSAR, Burla. **Methods:** This is a part of the ongoing, repeated cross-sectional study. Participants were enrolled well above the sample size (322) to increase precision. Two rounds of the survey were conducted and are being reported. Serum IgG antibodies against spike protein of SARS-CoV-2 were estimated using Elecsys® anti-SARS-CoV-2S is an immunoassay by ECLIA-based Cobas e411 analyzer. Univariate and multivariate regression were used in statistical analysis. **Results:** Our results show that 95.1% and 99.5% of the vaccinated individuals have developed antispike protein antibodies after the first and second doses, respectively. Previous COVID-19 infection was significantly correlated with antibody production, and age was negatively correlated. No difference was reported for sex, occupation, and diabetes. **Conclusion:** Our interim analysis report is coherent with the available literature and research regarding the high efficacy of the COVID-19 vaccine as far as seroconversion is concerned.

Keywords: ChAdOx1 vaccine, healthcare worker, regression, SARS-CoV-2, seroconversion

Introduction

Coronavirus disease (COVID-19) is a highly infectious disease caused by type 2 SARS coronavirus. The majority of infected people experience mild-to-moderate respiratory illness and do not require hospitalization.[1] People developing serious illnesses are either old or people with comorbidities.[2] SARS-CoV-2 is transmitted mainly through aerosol and droplets.[3] COVID-19 was declared as a Public Health Emergency of International Concern on January 30, 2020. Since then, it has spread to all the World Health Organization (WHO) member states.[4] There have been nearly 175 million confirmed cases of COVID-19, including 3.7 million deaths. As of June 9, 2021, a total of 2,156,550,767 vaccine doses have been administered.[5] Recognizing the need for vaccines, WHO published a road map for vaccine development as early as March 6 with SOP for clinical trials.[6]

Till June 8, 2021, there are 102 vaccines in the clinical development phase, and a further 185 vaccines are

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in the preclinical phases. Out of the 102 vaccines in clinical trials, 31 are protein subunits and 16 each of viral vector-borne (nonreplicating), RNA, and inactivated vaccines.[7] The majority of vaccines developed so far have been assessed by their ability to elicit antibody responses, and the generation of neutralizing antibodies has been the primary goal of vaccination.[8] Antigen-specific antibodies have been formally demonstrated as conferring vaccine-induced protection against many diseases.[9] Current vaccines predominantly mediate protection through the induction of highly specific IgG serum antibodies. In practice, to evaluate vaccine-mediated protection, estimation of antibody titer is the only feasible and available option as in rubella.[8]

In India, Covaxin (BBV152) and Covishield (ChAdOx1) were given emergency approval for restricted use in 2021.[10] Covishield (ChAdOx1) is a vector (adenovirus)-based recombinant nCoV-19 coronavirus vaccine with spike protein antigen.[11] It has been demonstrated in phase 1/2/3 clinical trials that there is a good antibody response after a single dose of the Covishield vaccine and that is neutralizing in nature, which further escalates after the second dose.[12]

India started its vaccination drive on January 16, 2021, with the healthcare workers being the initial beneficiaries. The phase 3 trial data for Covishield shows 70% efficacy 14 days after the second dose of the vaccine.[13]

The Spike protein (S glycoprotein) of coronaviruses (CoVs), which helps the virus fuse with the host cell membranes, is the primary immunogenic target for virus neutralization and vaccine design.[14,15] Also, most serological assays detect the neutralizing antibodies against the spike (S) protein.[16]

Serological testing provides direct evidence of population immunity, and quality serological surveys help in crucial insights into the community's immunological profile. They identify immunity gaps to prioritize additional vaccination interventions in these communities.[17,18] Also, these serological surveys tend to be more accurate than indirect estimations based on extrapolation of coverage and incidence data in this COVID-19 scenario with limited testing capabilities.[19]

As antigen-specific antibodies are symbols of vaccine-induced immunity, estimation of serum antibodies will provide important information regarding vaccine efficacy in seroconversion and the overall community's immunization status. As there is limited study on antibody prevalence to SARS-CoV-2 post vaccination, our study aimed to estimate the prevalence of anti-SARS-CoV-2 antibodies and identify the predictors of antibody titers among vaccinated healthcare workers in VIMSAR, Burla. As vaccine hesitancy is a concern among the population and healthcare providers, the knowledge gained from the study will improve vaccine confidence among the primary care physicians to advocate for COVID vaccination, which will lead to increased coverage and herd immunity.

Methods

A repeated cross-sectional study was conducted in Veer Surendra Sai Institute of Medical Science and Research, Burla, Sambalpur, in the state of Odisha among healthcare workers, which include doctors, nurses, students, and other staff (laboratory staff, pharmacists, security, office staff) of hospital having a history of COVID vaccination. Two rounds of the survey were conducted—1 month after the first dose of vaccination and 1 month after the second. Participants were selected using a convenience sampling technique separately for each round of the survey. However, care was taken to include all types of healthcare providers from different age groups of both sexes. Convenience sampling that uses sera for the estimation of antibodies has adequate power to provide population immunity data, in addition to being significantly less expensive and time-consuming.[20]

Sample size

Phase 2/3 clinical trial for this vaccine had reported a seroconversion rate of more than 99%.[21] Taking the expected rate of seroconversion at 95%, acceptable margin of error to be 2.5, the estimated sample size was calculated to be 292 and considering 10% nonresponse rate, the final sample size was rounded to be 322. However, in the study, we overenrolled participants to increase the precision.

Study tools and techniques

The data were recorded in a predesigned Open Data Kit-based electronic data capture tool. Sociodemographic variables included age, gender, educational level, occupation, addictions, comorbidities like diabetes mellitus and hypertension, and history of previous COVID-19 infection diagnosed either by Rapid Antigen Test, RTPCR, or True NAT method. Neutralizing antibody titer of the participants was measured by ECLIA and reported in AU/mL.

Blood collection and transfer

Conforming to all aseptic precautions, a 3 mL blood sample was collected using a 5 mL syringe by trained laboratory technicians with venipuncture and transferred to vacutainers. Then the samples were centrifuged at 2,500 rpm for 5 min, and separated serum was transferred to the cryogenic vial (2 mL) and was stored at −20°C. The samples were transported to ICMR-Regional Medical Research Centre in Bhubaneswar for antibody testing and analysis, maintaining a cold chain (+2 to +8°C).

Laboratory procedures of antibody testing

Tests were carried out for quantitative detection of antibodies against spike protein of SARS-CoV-2 an electrochemiluminescence immunoassay (ECLIA)-based Cobas e411 machine, which followed the principle of double-antigen sandwich assay and provided the result within 18 min.

Anti-SARS-CoV-2S test principle-Elecsys® anti-SARS-CoV-2S is an immunoassay for the quantitative, *in vitro* determination

of antibodies to SARS-CoV-2 in human serum and plasma. The Elecsys antiSARSCoV2S assay uses a recombinant protein representing the receptor-binding domain of the S antigen in a double-antigen sandwich assay format. Quantification of the antibody response can help determine the specific antibody titer and aid in longitudinal monitoring of the dynamics of the antibody response in individuals. Through a blood sample, the test can measure the quantities of antibodies to the spike protein of the coronavirus. The cut-off of this test is 0.8 AU/mL. The highest detection limit is 250 AU/mL without diluents. This method has an overall specificity of 99.98%, clinical sensitivity of 100%, and 92.3% (95% CI 63.97–99.81%) positive agreement on correlation with VSV-based pseudoneutralization assay.[21]

Diagnostic Criteria: Samples with titer values more than equal to 0.8 U/mL were taken as reactive, and values less than 0.8 were taken as nonreactive or unfavorable.

Statistics

As the dataset was highly skewed, it was transformed with $\log_{10}$. Descriptive analysis and regression analysis were performed after log transformation of antibody titer levels. Descriptive analysis was done for both rounds of the survey, and linear regression was performed only for the second round of the survey to build a model after two doses of vaccination.

Multivariate linear regression analysis (stepwise method) was done, entering the factors with a *P* value less than 0.1 in univariate regression analysis. Cook's distance and leverage value were used for the identification of multivariate outliers.

Model assumptions were tested after obtaining the most predictive model by histogram, P-P plot, and scatter plot. The interaction was identified using the regression plot. Model validation was done using 50% of the samples randomly.

Ethical considerations

The study was conducted according to the Declaration of Helsinki and WHO Good Clinical Practice (GCP); it was approved by the state health research and ethics committee. Written consent was obtained before the enrollment of the participants.

Result

The number of participants included in the first-round survey and second-round survey were 391 and 423, respectively. Overall seroconversion was 95.1% after 1st dose (first-round survey) and 99.5% after 2nd dose (second-round survey). – [Table 1 and Figure 1].

In the first-round survey, 225 (57.5) were males, and 166 (42.5) were females, with females having higher log-transformed titer levels. As far as the occupation was concerned, nurses had the highest titer, and doctors had the least IgG level. Participants with hypertension ($n = 27$) had lower serum IgG levels in comparison

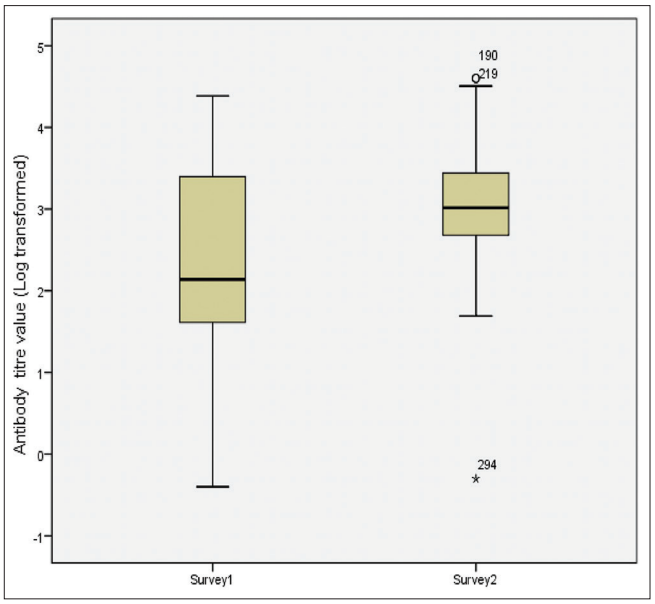

Figure 1: Box plot showing antibody titer level (log10 transformed) for survey round 1 and 2

with normotensives. Those with a history of previous COVID-19 infection ($n = 51$) had higher titer values than those without such a history.

In the second-round survey males ($n = 205$) and females ($n = 218$) had comparable IgG titer values. Doctors had the lowest titers. Hypertensives had a lower titer than normotensives. Previously infected persons had higher titer values compared with participants without COVID-19 history.

Regression analysis

On univariate regression analysis for the second-round survey, the following factors were identified ($P < 0.1$) for multivariate regression analysis, i.e., previous COVID-19 infection ($P < 0.0001$), age ($P = 0.025$), and hypertension ($P = 0.059$). Then a multivariate regression analysis was done, entering the above three variables in a stepwise manner. On running the regression method, two models were built.

By comparing the adjusted *R* square of Model 1 with the adjusted *R* square of Model 2, it was clear that adding age improves the model fit because the adjusted *R* square increased from 0.066 to 0.079, indicating that 7.9% of the variation can be explained. The *R* square change and the change statistics indicate that in Model 1 with the previous infection only, R^2 changed from 0 to 0.068, and in Model 2, with age included as a predictor, R^2 increased by 0.016. The corresponding *P* values were less than 0.05 and were significant; indicating the amount of variation accounted for by the model has significantly increased.

In the ANOVA, the regression mean square decreased from 9.785 in Model 1 to 6.016 in Model 2 when age was added because more of the unexplained variation was now explained. With high *F* values, both models were significant.

The standard error around the beta coefficient for the previous infection remained at 0.71 in both model 1 and model 2, indicating that the model was stable. The coefficients table showed that previous infection with a standardized coefficient of 0.268 was more significant predictor of antibody titer than age with a standardized coefficient of −0.125 [Table 2].

The excluded variables in the model were age and hypertension. The collinearity statistic tolerance was close to 1, meaning that the predictor variables were not closely related to one another (collinear). The predicted values range from 2.7719 to 3.4851, and the unstandardized residuals range from 3.07 below the regression line to 1.55 above the regression line [Figure 2].

The standardized predicted values and standardized residuals have a mean of zero and a standard deviation of approximately or equal to 1, indicating normal distribution.

The histogram and normal *P–P* plot indicate that the distribution of the residuals deviates only slightly from a classically bell-shaped distribution. The variance around the residuals showed homoscedasticity, i.e., equal variance throughout the regression model.

The residuals statistics showed that the largest Cook's distance is 0.247, below the critical value of 1. The largest leverage value is 0.021, which was below the critical value of 0.05, meaning there were no influential outliers in this model. The maximum Mahalanobis distance was 8.917 (degrees of freedom 2). There was only one case with a standardized residual of more than three standard deviations from the regression. It had a log-transformed titer value of 0.3 compared with a predicted value of 2.70 and a standardized residual of −5.41.

When we conducted the linear regression including age *previous COVID-19 infection as interaction variable, the model fit improved with an adjusted R^2 of 0.098.

With two explanatory variables in the model, the regression line will be of the form of

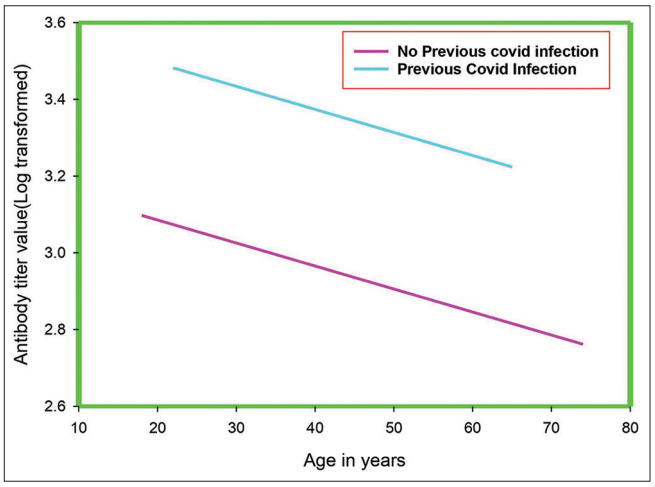

Figure 2: Regression lines by previous Covid infection status for predicting antibody titer

Table 1: Characteristics of the participants included in the study

	Survey round 1	Mean of Log transformed antibody titer (± SD)	Survey round 2	Mean of Log transformed antibody titer (± SD)
n	391	2.21 (1.01)	423	3.05 (0.58)
Age (mean, SD)	38.2 (11.9)		37.74 (12.37)	
Sex	M=225	2.12 (1.07)	M=205	3.06 (0.65)
	F=166	2.34 (0.89)	F=218	3.05 (0.51)
Occupation	Doctor=112	1.92 (1.07)	Doctor=149	2.98 (0.58)
	Nurse=74	2.40 (0.88)	Nurse=88	3.04 (0.52)
	Student=82	2.29 (0.74)	Student=53	3.15 (0.47)
	Other=123	2.32 (1.10)	Others=133	3.12 (0.66)
Diabetes	Yes=24	2.33 (1.21)	Yes=27	3.06 (0.58)
	No=267	2.21 (0.99)	No=396	3.03 (0.67)
Hypertension	Yes=27	1.64 (1.24)	Yes=32	2.87 (0.53)
	No=264	2.25 (0.97)	No=391	3.07 (0.58)
Previous COVID-19	Yes=51	2.94 (0.59)	Yes=76	3.38 (0.59)
Infection	No=340	2.10 (1.01)	No=347	2.98 (0.56)
Seroconversion	Yes=372 (95.1%)		Yes=421 (99.5%)	
	No=19 (4.9%)		No=2 (0.05%)	

*SD, standard deviation

Table 2: Multivariate linear regression model with explanatory variables

Model	Unstandardized Coefficients-B (CI)	SE	Standardized Coefficients-β	*P*
Intercept	2.98 (2.92,3.04)	0.03		<.0001
Previous Covid infection	0.39 (0.25,0.53)	0.072	0.261	<.0001
Intercept	3.206 (3.03,3.37)	0.087		<.0001
Previous COVID-19 infection	0.408 (0.26,0.54)	0.71	0.268-0.125	<.0001
Age	−0.006 (−0.01, −0.002)	0.002		<.0001

CI, confidence interval; S.E, standard error

$y = a + b_1x_1 + b_2x_2$,

Where x_1 is previous COVID-19 infection and x_2 is age.

Substituting the variables and the unstandardized coefficients from the coefficients table, the equation for the model is as follows:

Antibody titer (log transformed) = 3.206+ (0.408 × previous COVID-19 infection) − (0.006 × age)

Discussion

As vaccines protect by inducing effectors mechanism (antibodies), its level following vaccination will guide us in future strategy and policymaking. In our study, we found that majority of the ChAdOx1 nCoV-19 coronavirus vaccine recipients have developed antibodies against the viral spike protein that is 95.1% and 99.5% after the first and second dose, respectively, which is similar to the phase 1/2 and phase 2/3 clinical trial of the vaccine.[22,23] Our results show a significantly higher seroconversion rate after the 1st dose than the study by Singh *et al.*[24] (95.1% vs. 86.8%); however, the rate was comparable after the 2nd dose (99.5% vs. 98%). The observed difference may well be due to the difference in IgG estimation method (LIASON® S1/S2 quantitative antibody detection kit using indirect chemiluminescence immunoassay).[25] The same study reported seroconversion rate following the first dose of BVB-152 (Covaxin) at 43.8% and after the 2nd dose at 80%.[26,27] Clinical trial data reported seroconversion of 96.2%–98% after two doses of the BVB-152 vaccine.[28]

For Sputnik V (adenoviral vector vaccine), seroconversion levels were 94% and 100% after the 1st and 2nd doses.[29] Similar results were also obtained for BNT162b2 mRNA (Pfizer–BioNTech) COVID-19 vaccine with 99.9% becoming seropositive after two doses of vaccine.[30] Seroconversion rates after inoculation of mRNA-1273 (Moderna) COVID-19 vaccine have been shown to be between 73.8% and 89.9% and 97.7% and 100% after the 1st and 2nd dose, respectively.[31] Adenovirus type-5-vectored COVID-19 vaccine developed by the Beijing Institute of Biotechnology has also been shown to have similar rates of seroconversion of more than 96%.[32] Phase 1/2a clinical for Ad26.COV2.S COVID-19 vaccine (Johnson & Johnson) reported 90% seroconversion after the 1st dose and 100% after the 2nd dose.[33]

Seroconversion rates were better than (Influenza; 41.4%–88.8%,[34,35] Varicella: 91.5%–95%,[36,37] Rotavirus Vaccine; 80%[38]) or comparable with (Measles: 87%–98%,[39,40] Mumps: 87%–100%[41]) commonly used vaccines for vaccine preventable diseases.

Our sample size allowed us to establish a multivariate regression model to identify background factors that allow the prediction of antibody response. The strongest and significant factor was previous COVID-19 infection with $P < 0.001$. Similar associations were reported by multiple other studies where exponential antibody response was seen after the administration of the vaccine in previously infected individuals.[27,30,42,43] This association has been hypothesized to boost the effect of even a single dose of COVID-19 vaccine among previously infected cases and the need for serological assay before vaccination to prioritize recipients for COVID-19 vaccine.[44,45,47]

Another vital predictor in our study was age ($P < 0.001$) that correlated negatively with antibody production. Similar results were reported by Muller *et al.* and Tepros *et al.*[42,48,49] However, no significant difference was observed in many other studies.[30,31,46]

Our regression analysis showed no significant predictive ability for gender, occupation, or co morbidity that other studies have also reported.[25,42] Some studies have, however, reported better antibody response in females.[30]

The prevalence of anti-SARS-CoV-2 antibodies among the study participants was found to be 95.1% and 99.5% after the 1st and 2nd dose. On multiple linear regression analysis, we found previous SARS-CoV-2 infections and age as significant predictors of antibody titer.

Conclusion

Overall, our analysis supports most of the available literature regarding the high seroconversion rate after COVID-19 vaccine administration. Clinical trials and early research have also shown a good efficacy of the vaccine with sufficient neutralizing antibody production. Real-world effectiveness data will be known after significant coverage is achieved on long-term studies. However, many studies have reported waning of antibody titer with time, and its significance on seroprotection needs further evaluation. The ongoing long-term study will hopefully address these gray areas also.

Limitation

One of our limitations is that our study group includes participants from the healthcare community, which may be different from the general community in terms of sociobehavioral and other risk factors. Repeated cross-sectional design and convenient sampling are also the limitations; however, it was selected keeping in mind the long-term feasibility of the project.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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