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Research Article

SEMI-QUANTITATIVE ANALYSIS OF SARS-COV-2 IGG ANTIBODIES FOLLOWING CHADOX1-NCOV (COVISHIELD™) VACCINATION

RAVISH KUMAR M*, PRAVEEN KUMAR DODDAMANI, NAGARKAR RAJHANS KISHANRAO, PARANDEKAR PRASHANT K, JISHA M, MARAPPA NARAYANA, PRATHIBA MAHADEVAIAH

Employees' State Insurance Corporation Medical College and Hospital, Gulbarga, Karnataka, India. Email: drravish@rocketmail.com

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ABSTRACT

Objectives: The objectives of this study were semi-quantitatively analyze SARS-COV-2 IgG antibodies following covishield vaccination in healthcare workers and to follow-up them for 6 months for persistence of antibodies and for getting infected with SARS-COV-2.

Methods: This was a prospective cohort study which was conducted at tertiary care hospital, South India. The blood samples were collected after second dose of vaccine at 28 days, 60 days, and 120 days. The serum was subjected for detection of IgG antibodies against S1 RBD (Receptor binding domain) of the spike protein antigen by Euroimmun kit (PerkinElmer company, Germany) using ELISA.

Results: Out of 30 healthcare workers, 28 (93.3%) were seropositive and 2 (6.7%) were seronegative. Out of two seronegative, one participant acquired SARS-COV-2 infection with severe symptoms. There was approximately 50% reduction in antibody levels in almost all seropositive individuals after 3 months of second dose. Even after 6 months, 25 (83.3%) were seropositive, 2 (6.7%) were seronegative, and 3 (10%) were borderline. When the IgG antibody ratio levels of 28 days following second dose of vaccination were compared with levels after 6 months, which showed, p value of 0.024 which is <0.05 implies statistically significant.

Conclusion: Covishield vaccine induced good immune response in majority of the participants, the levels were sustainably positive until 6 months but decreasing pattern. The vaccine-induced antibodies prevented the severe symptoms among vaccine breakthrough infections.

Keywords: SARS-CoV-2, India, Healthcare workers, IgG antibodies.

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INTRODUCTION

COVID-19 disease is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV-2) which is a novel virus emerged in late 2019 and believed to be zoonotic in nature [1]. Consequently, it has spread throughout the globe covering 188 countries and 25 territories due to highly contagious nature and it spread by inhalation of respiratory droplets [2]. SARS-COV-2 infection produces antibodies to spike(S) protein and nucleocapsid protein and based on animal studies it was noticed that anti-spike antibodies are protective and have neutralizing activity [3]. It is unclear about protective and long-lasting immunity following infection/vaccination. Immune responses specific to SARS-CoV-2 have now been detected up to 6 months following infection and same is anticipated following vaccination as suggested by provisional results [4]. With all possible measures taken for containing the burden of SARS-COV-2 infection, it is still difficult to stop or break the chain of transmission. Recently, the new strains have emerged with variation in the spread of infection. Now, the only possibility is to vaccinate everyone to develop efficient herd immunity for preventing the impact of COVID-19 on health-care setting, social, and global economy [5]. At present, various vaccines have been approved worldwide for emergency use. The following is the vaccines with their efficacies Pfizer/BioNTech, Gamaleya, Moderna, and AstraZeneca declared the vaccine efficiency as 95%, 92%, 94.5%, and 70%, respectively [6]. There is a dearth of knowledge about the quantity and duration of persistence of antibodies following vaccination. Healthcare workers are the group of people who are and will be constantly exposed to SARS-COV-2 infection at hospital. Hence, our objectives of the study were to semiquantitatively analyze SARS-COV-2 IgG antibodies following covishield vaccination in healthcare workers (Doctors, Nursing staff, Paramedical staff) who took two doses of covishield vaccine and to follow-up them for 6 months for persistence of antibodies and for getting infected with SARS-COV-2.

METHODS

This was a prospective cohort study which was conducted at tertiary care hospital, South India. The healthcare workers (Doctors, Paramedical staff and Nursing staff) between ages 18 years to 70 years who had taken two doses of covishield vaccine and voluntarily willing were enrolled in the study and informed consent was obtained. Ethical approval was obtained from the Institutional Ethical Committee for conducting the present study. Those who had past history of infection with SARS-COV-2 or any other respiratory illness were excluded from the study.

Sample size

For the study was 30.

Study duration

The study duration was 8 months.

Data were collected from the participants related to age, gender, profession, height, weight, blood group, habbits, and allergy, comorbidities, and any significant past history. Follow-up data were also collected related to vaccine breakthrough infection.

Blood sample (5ml) was collected from the subjects at three intervals: First sample was collected after 28 days of second dose vaccination, second sample after 3 months (90 days), and third sample after 6 months (120 days) after second dose vaccination. All the blood samples were allowed for clotting and serum was separated. The serum was subjected for detection of IgG antibodies against S1 RBD domain of the spike protein antigen by Euroimmun kit using ELISA. Diagnostic sensitivity and specificity of the kit was 93.8% and 99%, respectively.

Test evaluation
The optical density of the calibrator will be the ceiling limit of the reference range in non-infected individuals (cutoff) approved by EUROIMMUN. The values above the cutoff are to be considered as positive, those below as negative.

Results were evaluated semi-quantitatively by calculating a ratio of the optical density of the control or patient sample over the optical density of the calibrator

Optical density of the control or patient sample/optical density of the calibrator (Ratio) Euroimmun recommends interpreting results as follows:

- Ratio <0.8: Negative
- Ratio ≥0.8 to <1.1: Borderline
- Ratio ≥1.1: Positive [7].

Statistical analysis

Statistical methods

In the present study, descriptive and inferential statistical analysis has been carried. Results on continuous measurements were presented on Mean ± SD (Min-Max) and results on categorical measurements were presented in Number (%). Significance was assessed at 5% level of significance. The following assumptions on data were made, assumptions: (1). Dependent variables should be normally distributed and (2). samples drawn from the population should be random. Cases of the samples should be independent. The paired t-test was used to test the null hypothesis that the average of the differences between a series of paired observations is zero. Student t-test (two-tailed, dependent) was used to find the significance of the study parameters on continuous scale with in each group [8].

Significant figures

- +Suggestive significance (p value: 0.05<p<0.10)
- *Moderately significant (p value: 0.01<p ≤ 0.05)
- **Strongly significant (p value: p≤0.01).

Statistical software

The statistical software, namely, SPSS 22.0 and R environment ver.3.2.2 were used for the analysis of the data and Microsoft Word and Excel were used to generate graphs, tables, etc.

RESULTS

The data of 30 healthcare workers who received two doses of covishield vaccine and the anti-spike antibodies against S1 antigen of SARS-COV-2 were statistically analyzed. The mean age of the participants was 37.66±10.02 years (Table 1) with 50% (15/30) males and 50% females (Table 2). Out of 30 participants, 7 (23.3%) were aged <30 years, 21 (70%) were between 30 and 50 years, and 2 (6.7%) were above 50 years (Table 1). Gender-wise both males and females were 50% (Table 2). Out of 30 participants, two had diabetes mellitus, one had Hypertension, two had Bronchial asthma, and one was allergic to cold and dust.

Table1: Age in years-frequency distribution of participants studied

Age in years	No. of patients	%
<30	7	23.3
30–50	21	70.0
>50	2	6.7
Total	30	100.0

Mean±SD: 37.66±10.02

Table 2: Gender-frequency distribution of participants studied

Gender	Number of patients	%
Female	15	50.0
Male	15	50.0
Total	30	100.0

IgG SARS-COV-2 antibodies after 28 days of second dose covishield vaccine
Out of 30 healthcare workers, 28 (93.3%) were seropositive and 2 (6.7%) were seronegative (Table 3). Out of 2 seronegative, one participant acquired SARS-COV-2 infection with severe symptoms.

The IgG antibodies ranged from 0.75 to 5.7 with mean ratio of 3.11±1.32.

The mean antibody ratio of the participants <30 years (3.47±1.32), 31–50 years (3.26±1.34), and >50 years (2.48±1.05) showing strong immune response in younger individuals in <30 years.

IgG SARS-COV-2 antibodies after 3 months of second dose covishield vaccine
27 (90%) were seropositive, 2 (6.7%) were seronegative, and 1 (3.3%) was borderline (Table 3).

Two participants who were seropositive following vaccination acquired the infection with mild symptoms/asymptomatic.

There was approximately 50% reduction in antibody levels in almost all seropositive individuals.

IgG SARS-COV-2 antibodies after 6 months of second dose covishield vaccine
Even after 6 months, 25 (83.3%) were seropositive, 2 (6.7%) were seronegative, and 3 (10%) were borderline (Table 3).

When the IgG antibody ratio levels of 28 days following second dose of vaccination was compared with levels after 6 months, which showed, p value of 0.024 which was <0.05 implies statistically significant (Table 4) (Graph 1).

DISCUSSION

The present study was a prospective cohort study conducted on 30 healthcare workers. The aim of the study was to analyze the production of anti-spike (RBD) antibodies following ChAdOx1-nCOV vaccine and follow-up them for 6 months to know about breakthrough infection and duration of antibody persistence.

The blood samples were collected on 28 days, 60 days, and 180 days following second dose of covishield vaccination. Out of 30 healthcare workers, 28 (93.3%) were seropositive for anti-spike antibodies after 28 days in comparison with randomized controlled trial phase 2 study, which had 96% seroconversion rate [9]. The antibody levels peaked by 28 days (Median 2.945) and by 56 to 60 days that it reduced to (Median 2.15) levels in contrast to Phase 1/2 RCT which showed steady increase in antibody levels by 60 days [10]. There was decreasing pattern

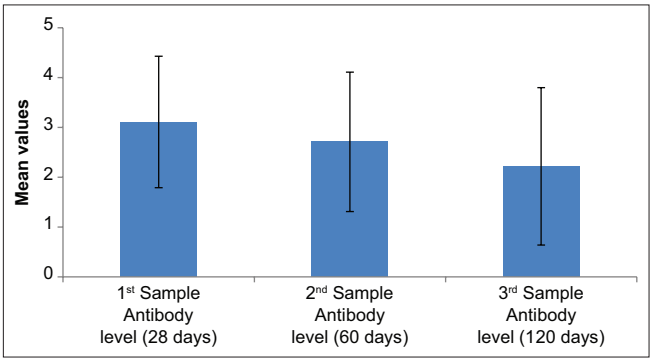
Table 3: Anti-SARS-COV-2(RBD) IgG antibodies

Variables	Number of Patients	%
Seropositivity after 28days		
Borderline	0	0.0
Negative	2	6.7
Positive	28	93.3
Seropositivity after 3 months		
Borderline	1	3.3
Negative	2	6.7
Positive	27	90.0
Seropositivity after 6 months		
Borderline	3	10.0
Negative	2	6.7
Positive	25	83.3
Total	30	100.0

Table 4: Descriptive statistics

Variables	Minimum	Maximum	Mean	Standard deviation	p-value
1st Sample Antibody level(Ratio)	0.74	5.70	3.11	1.32	-
second Sample Antibody level(Ratio)	0.75	5.70	2.71	1.40	0.153
3rd Sample Antibody level(Ratio)	0.55	7.76	2.22	1.58	0.024*

p value comparison employed with 1st sample



Graph 1: Anti-spike IgG antibody levels at different intervals

of antibody levels in first 3 months, but most were seropositive even after 6 months. Decrease level of antibodies may warrant for booster dose after 6 months [11]. Two (6.7%) were seronegative for anti-spike antibodies, among them one healthcare worker got severely infected with 90% SPo2, 14 days fever, high values of inflammatory markers suggesting that antibodies play an important role in preventing severe symptoms, and his antibody levels were increased sustainably to such a level that, his was the highest among all participants after 6 months. Severity is proportional to antibody levels [12]. Question is why he was seronegative following vaccination but became seropositive following infection. Probably, his antibody levels were not in the detectable range. The other healthcare worker who was seronegative at 28 days remained seronegative even at 6 months and didn't get infected, may warrant further evaluation for non-responding. Among two healthcare workers who were seropositive got infected, one had mild symptoms of cold, and vomiting who is a known asthmatic and allergy to dust (antibody was proportionately increased to double the value before infection), whereas the other one was asymptomatic who has past history of allergy to dust suggesting that antibody levels might prevent development of severe symptoms in contrast to seronegative person who developed symptoms. Our study had three vaccine breakthrough infection following two doses of vaccination after 6 months in comparison with Lumley *et al*. and Borgonovo *et al*. who showed that incidence of reinfection is significantly lower in healthcare workers with SARS-COV-2 anti-spike antibody [13,14]. Majority of the participants had sustained response of anti-spike (RBD) antibodies after 6 months of vaccination in comparison with a study by Ketas *et al*. who monitored 45 healthy recipients of either Pfizer BNT162b2 or Moderna mRNA-1273 mRNA vaccines showed sustained antibody response to all anti-SARS-COV-2 RBD antibodies after 3 months of complete vaccination [15]. One healthcare worker probably got exposed to his SARS-COV-2 infected wife; he had no symptoms and was RTPCR negative. His antibody levels sustained/marginally raised during 5 month following two doses of vaccination.

One healthcare worker was seronegative after 3rd month developed cold symptoms and was RTPCR negative, but antibody ratio level was in borderline at 6th month.

The association of variables such as age, gender, weight, height, and comorbidities with the level of antibodies was found to be not significant in our study.

Limitations in our study

Our sample size is small, so it is difficult to generalize the results on community.

We could not be able to study on neutralizing antibodies and cell-mediated response which could have given clear picture about predominant immunity, but some of the study has shown that anti-spike antibodies behave more likely as neutralizing antibodies [16].

We could not take the baseline antibody levels before vaccination and also after 1st dose.

CONCLUSION

Our present study highlighted the fact that ChAdOx1-nCOV vaccine being used is highly immunogenic giving 93.3% seropositivity among vaccinated healthcare workers; however, the antibody levels were found to be sustainably above positive range but decreasing pattern which necessitates the booster dose. The study also provides the information that although vaccine could not prevent infection, but it prevented the severity of infection in majority of the healthcare workers.

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AUTHORS CONTRIBUTIONS

Dr. Ravish Kumar M, Dr. Parandekar Prashant K, and Dr. Nagarkar Rajhans Kishanrao planned the research, contributed to data analysis and interpretation, writing, and critical review. Dr. Ravish Kumar M and Dr. Praveen Kumar Doddamani conducted the research study design, data collection, data analysis, interpretation, writing, and critical review. Mrs, Jisha M, Mr. Marappa N, Dr. Prathiba conducted the experiment contributed to data collection, writing, and critical review.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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ETHICS STATEMENT

Approval taken

Institution Name: ESIC Medical College.

Approval no: ESICMC/GLB/IEC(2)/05/2021.

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