Original Article

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Declining Effect in the Immunogenicity of Covishield Vaccine among Healthcare Workers Postvaccination- A Prospective Cohort Study

Microbiology Section

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ABSTRACT

Introduction: The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic had a severe impact on global healthcare systems and the economy. The introduction of Coronavirus Disease 2019 (COVID-19) vaccines has provided a solution for the disease. However, with mutations in the strains of COVID-19, there is a need to understand the immunogenicity and duration of protection provided by the vaccines.

Aim: To assess the immunogenicity of the covishield vaccine among Healthcare Workers (HCWs) postvaccination following the administration of the second dose of the covishield vaccine.

Materials and Methods: This was a single-centre prospective cohort study in the Department of Microbiology, Sapthagiri Institute of Medical Sciences and Research Centre, Bengaluru, Karnataka, India, from January 2022 to June 2022. Study included HCWs who had received the first and second doses of the Covishield vaccine and were willing to participate in the study. Only 30 HCWs who had received two doses of the COVID-19 vaccine (Covishield) at least two weeks before registration were enrolled in the study. The serum was tested to determine the qualitative detection of COVID-19 Immunoglobulin G (IgG) antibody in HCWs using the Enzyme-linked Immunosorbent Assay (ELISA) method using COVID Kawach IgG Microlisa kit. The P/N ratio is the Optical Density (OD) value of the sample tested/average OD value of the negative control. The P/N ratio was calculated and the participants were divided into different groups based on the months after receiving the second dose of the vaccine. The non parametric Kruskal-Wallis test was applied to test the difference between the P/N ratio based on the months of vaccination since the second dose.

Results: In the present study, the majority of HCWs, 20 (66.6%), were uninfected by the SARS-CoV-2 virus, while 10 (33.3%) became infected after receiving two doses of the covishield vaccination. Out of 30 samples, 29 (96.6%) samples had crossed the cut-off OD value of 0.3970, and the P/N ratio was greater than 1.5, suggestive of the presence of antibodies against SARS-CoV-2. Only 1 (3.3%) sample had an absorbance value lower than the cut-off, and the P/N ratio was <1.5, suggestive of the absence of antibodies against SARS-CoV-2. The P/N values of the participants were compared between different groups, which were divided based on the months since the second dose of the covishield vaccine. Participants who had completed five months since the last vaccine dose showed the highest P/N value (median 13.9), followed by median values of 7.1, 4.8 and 3.3 on completion of 6, 7 and 8 months and beyond, respectively. A steady decline in the median P/N ratio was observed from 5 to 8 months.

Conclusion: The immunogenicity of the covishield vaccine among HCWs Postvaccination after the second dose of the covishield vaccine showed a steady decline in the subsequent months.

Keywords: Antibody titer, Coronavirus disease 2019, Enzyme-linked immunosorbent assay

INTRODUCTION

Coronavirus disease 2019 is a highly contagious infectious disease caused by SARS-CoV-2, and it continues to threaten global socio-economic and healthcare systems [1]. As of January 23, 2022, over 346 million confirmed cases and more than 5.5 million deaths have been reported worldwide [2]. HCWs constitute a high-risk group for acquiring the disease. Studies conducted in various regions and countries have quantified HCW infections and case fatalities, showing a median HCW infection rate of 10.04% (range: 0-24.09%) among total cases [3]. From January 2020 to May 2021, 135 million HCWs were engaged in combating the pandemic, with 80,000-180,000 HCWs reported to have succumbed to COVID-19, according to the World Health Organisation (WHO) [4]. Furthermore, HCWs face a 7.4 times higher risk of developing severe COVID-19 compared to non essential workers, and an 11.6 times higher risk of testing positive for the COVID-19 nucleic acid test [5,6].

The collaborative efforts of the pharmaceutical industry, scientific community, and government agencies have resulted in the development of several vaccines against COVID-19. Vaccination is a crucial measure for preventing the disease. The National COVID-19 vaccination strategy, which commenced on January 16, 2021, focused on safeguarding HCWs and Frontline Workers (FLWs) during Phase I. Phase II, which ran from March 1, 2021, to April 1, 2021, aimed to protect the most vulnerable, specifically the population over 45 years of age, accounting for over 80% of COVID-19 mortality in the country [7]. Two vaccines, covishield (a recombinant, replication-deficient chimpanzee adenovirus vector encoding the SARS-CoV-2 spike glycoprotein) and covaxin (inactivated whole virions grown in vero cells), were authorised by the Indian drug regulator for emergency use [8]. Covishield has accounted for nearly 88% of all doses administered in the country to date and has been the sole vaccine used in certain regions [9]. Currently, limited data are available on the persistence of protective antibodies in vaccine recipients, particularly HCWs. The present study was conducted to assess the immunogenicity of the covishield vaccine among HCWs postvaccination after receiving the second dose of the covishield vaccine.

MATERIALS AND METHODS

The present single-centre prospective cohort study was conducted in the Department of Microbiology, Sapthagiri Institute of Medical Sciences and Research Centre (Tertiary Care Hospital), Bengaluru,

Karnataka, India, from January 2022 to June 2022. The study protocols were approved by the Institutional Ethics Committee (IEC Ref No: SIMS &RC/IEC/05/2021), and written informed consent was obtained from all participants.

Inclusion criteria: HCWs who had received both doses of the covishield vaccine at least two weeks before registration and were willing to participate in the study were included.

Exclusion criteria: Non vaccinated individuals and those who had only received one dose of the covishield vaccine and individuals who had not completed two weeks after the second vaccine dose were excluded from the study.

Sample size calculation: Non probability technique was used for sampling. All the subjects available during the study duration were included in the study. One hundred and forty-three HCWs were registered for the study. Total of 143 subjects included, were registered for the study. Of these, 62 had not taken the second vaccine dose, and 28 had not completed two weeks after the second vaccine dose. Among them 23 did not give consent for participation and hence did not meet the eligibility criteria. Therefore, only 30 HCWs who received two doses of the COVID-19 vaccine (Covishield) at least two weeks before registration were enrolled in the study.

Study Procedure

Blood samples were drawn from the subjects and subjected to an ELISA test for the qualitative detection of IgG antibody titers against SARS-CoV-2. By practicing all aseptic precautions, 4 mL of blood sample was collected from each participant. All 30 samples were kept aside for 45 minutes and then centrifuged. The serum samples were then tested for the qualitative detection of COVID IgG antibodies in HCWs by the ELISA method using the COVID Kawach IgG Microlisa kit (Microwell ELISA test for the qualitative detection of COVID-19 IgG antibodies in serum). This kit is approved by the Indian Council of Medical Research (ICMR) and the National Institute of Virology (NIV) with LOT number EKGO 10422, date of manufacture 04/2022, and an expiry date of 03/2023.

At the time of sample collection, demographic details of the HCWs were collected. The infection status of only those with a positive quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) report was considered. The test was performed according to the instructions in the COVID KAWACH IgG MICROLISA kit, which is a Microwell ELISA test for the qualitative detection of COVID-19 IgG antibodies in serum.

Optical Density (OD) value of the sample: P/N ratio=Optical Density (OD) value of the sample/ Average OD of Negative control. The cut-off value was determined as the average of the negative control +0.2, according to the kit literature [10]. In the current study, the average OD of the negative control was 0.1970, resulting in a cut-off value of 0.3970 (0.19705+0.2). The cut-off value was calculated based on the values obtained after performing ELISA using the formula [10].

The criteria for interpretation were as follows:

- A sample with OD greater than the cut-off and P/N ratio greater than 1.5 was considered positive.
- A sample with OD less than the cut-off and a positive to negative ratio (P/N) less than 1.5 was considered negative [10].

STATISTICAL ANALYSIS

The data collected was entered into Microsoft Excel, and the analysis was conducted using Statistical Package for the Social Sciences (SPSS) software version 20.0. The non parametric Kruskal-Wallis test was applied to test the difference between P/N ratio by months of vaccination since the second dose. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The study revealed that out of 30 HCWs, 20 (66.6%) remained uninfected by the SARS-CoV-2 virus, while 10 (33.3%) became infected after receiving two doses of the covishield vaccination. Only HCWs with a positive qRT-PCR testing report confirming their infection were considered, as depicted in [Table/Fig-1].

Infection status	n (%)
Infected after vaccination	10 (33.3)
Uninfected after vaccination	20 (66.6)
Total	30 (100)

[Table/Fig-1]: Infection status of the HCWs after vaccination.

[Table/Fig-2] shows that 29 (96.6%) samples crossed the cut-off value of 0.3970, indicating a positive result for COVID-19 IgG antibodies. Only one (3.3%) sample had an absorbance value lower than the cut-off. As per the kit instructions, the criteria for a qualitative positive result for COVID-19 IgG antibodies are that the absorbance OD value should exceed the test's cut-off value and the P/N ratio should be over 1.5. Out of 30 samples, 29 had a P/N ratio above 1.5, while only one sample had a P/N ratio below 1.5 [Table/Fig-2].

Status of COVID-19 IgG antibodies and P/N ratio	n (%)
Positive	29 (96.6)
Negative	1 (3.3)
Total	30 (100)

[Table/Fig-2]: Status of COVID-19 IgG antibodies in the tested serum samples.

Among the 29 participants included for analysis who tested positive for COVID-19 IgG antibodies, 7 (24.1%), 6 (20.6%), 10 (34.4%) and 6 (20.6%) had completed 5, 6, 7 and 8 months, respectively, since receiving the second dose of vaccination [Table/Fig-3]. The P/N values of the participants were compared between the different groups. The participants who had completed five months since the last vaccine dose showed the highest P/N value with a median of 13.9, followed by median values of 7.1, 4.8 and 3.3 at 6, 7 and 8 months and beyond, respectively [Table/Fig-3]. The mean P/N ratio was 13.8, 7.3, 4.4, and 3.1 at 5, 6, 7, and 8 months, respectively, after receiving the covishield vaccine [Table/Fig-3]. A steady decline was observed in the median P/N ratio from 5 to 8 months. The month-wise difference in the P/N ratio was statistically significant, with p-value=0.0000093 [Table/Fig-3].

Month	n (%)	P/N ratio				
		Mean	Median	Minimum	Maximum	p-value
5 months	7 (24.1)	13.8	13.9	10.5	16.6	**0.0000093**
6 months	6 (20.6)	7.3	7.1	6.4	9.3	
7 months	10 (34.4)	4.4	4.8	4.2	5.9	
8 months	6 (20.6)	3.1	3.3	2.1	3.8	

[Table/Fig-3]: Month-wise distribution of P/N ratio.
Kruskal-wallis test; The p-value in bold font indicates statistically significant value

DISCUSSION

The COVID-19 vaccination program in India commenced on January 16, 2021, for HCWs and other FLWs, which included police, paramilitary forces, sanitation workers and disaster management volunteers. It was later extended to elderly citizens in March 2021 [7]. India, being the second most populous country globally, aimed to vaccinate 300 million people in the initial phase, including 10 million HCWs and 20 million FLWs in the first phase and 270 million people aged over 50 years and/or with co-morbidities in the second phase by August 2021. The Ministry of Health and Family Welfare introduced the CoWIN website to simplify the process of registration and vaccination [11]. Additionally, from March 2022, vaccine eligibility was extended to children aged 12-14 years, exclusively to receive corbevax, a vaccine developed by Biological E [12,13].

Booster doses became available from January 10, 2022, starting with FLWs and residents over the age of 50 years with co-morbidities. Subsequently, the Ministry of Health and Family Welfare announced that all adults aged 18 years and over would become eligible for booster doses beginning April 10, 2022, available via private vaccination centres [14]. The extensive coverage of the Indian population with COVID-19 vaccination was seen as an essential step in controlling the global spread of the SARS-CoV-2 virus. India also supplied tens of thousands of COVID-19 vaccine doses free of cost to several countries [11].

Despite the vigorous expansion of COVID-19 vaccination, the omicron strain has spread significantly since the beginning of 2022. Omicron was first reported to the WHO on November 24, 2021, by South Africa and was declared a Variant Of Concern (VOC) on November 26, 2021, by the WHO. The Omicron variant features multiple unique mutations in the spike protein targeted by most COVID-19 immunisations at the time of its discovery [15].

Antibody titers in SARS-CoV-2 and the duration of their elevated levels are important indicators for confirming the efficacy of COVID-19 vaccines. According to Zhuang C et al., although the results of Vaccine Effectiveness (VE) might begin to decline over time, around three months after full vaccination [16]. Antibodies against SARS-CoV-2 decrease over time after the second dose of the vaccine, if there is no spontaneous COVID-19 infection [17].

A longitudinal cohort study was conducted to determine the dynamics of vaccine-induced IgG antibodies against SARS-CoV-2 among 614 vaccinated HCWs from three different districts of Odisha, India. This 6-month follow-up study documented that the antibody levels in the covaxin and covishield recipients dropped significantly after two months and four months, respectively [18].

A cross-sectional study conducted at Believers Church Medical College (a tertiary care centre), Thiruvalla, Kerala, India, reported that the covishield vaccine after two doses showed an excellent immune response. However, greater immunogenicity after the first dose was seen among those with previous COVID-19 infection, even surpassing the titre achieved by the second dose of the vaccine in SARS-CoV-2 naive recipients [19].

Another cross-sectional study was conducted among 199 senior citizens aged 60 years and above residing in Belagavi city, Karnataka, India, from November 2021 to January 2022. They received two doses of the COVID-19 vaccine (Covishield) at least six months before recruitment into the study, and it showed waning of immunity levels and a decline in response after eight months, especially in those in the higher age groups [20].

There was a high prevalence of COVID-19 infection despite rigorous screening and the implementation of lockdowns, due to asymptomatic cases that drove COVID-19 transmission in the community [21]. The global escalation of the disease was a testament to the essentiality of the project. This was a step towards assessing the vaccine's immunogenicity and efficacy. It is of utmost importance to understand more about the perplexing disease that has shaped the world through drastic measures. The present study has helped shed more light on the matter. Putting an end to the global COVID-19 pandemic is of primal concern at the moment. Achieving these goals necessitates safe and effective vaccines. The study has aided in assessing the role of the vaccine under these pressing circumstances.

There is little consensus concerning the nature, stability and durability of antibody responses over time in COVID-19 vaccines. The postvaccination seroconversion data was minimal, and the study fills the gap in the related field. The current study centres on the principle of assessing the immunogenicity of the vaccine administered among healthcare professionals. With the recent advent of the administration of the booster dose, conclusion on the necessity of booster vaccine administration at this juncture need to be assessed. The IgG titers will aid in understanding the level of effectiveness of the vaccine after two doses but before availing the booster dose. Since the mechanism of action of vaccines developed along different technical routes is different, the VE is undoubtedly closely related to the type and characteristics of the vaccine.

The results of the kinetics of live virus-neutralising antibody responses showed that the Pfizer BioNTech (BNT162b2) COVID-19 vaccine and Moderna COVID-19 vaccine {Messenger Ribonucleic Acid (mRNA)-1273} were characterised by high peak neutralising antibody responses that declined sharply by six months, and the responses declined further by eight months [22-24]. The VE of the covishield vaccine also shows a steady decline after two months onwards, as measured by the antibody titers [18-20]. In the present study, waning of antibody response against COVID-19 in HCWs showed a steady decline from the 5th month to the 8th month of receipt of the vaccine. It was confirmed that the SARS-CoV-2 antibody titer decreases with time. Based on the current results, the safety of vaccines was reassuring; the VE and the immunogenicity can be greatly improved after booster vaccinations with the same or different vaccines.

Limitation(s)

Asymptomatic breakthrough infections result in higher responses. Miscategorisation of quantitation occurs when the P/N ratio of optical densities is used as a surrogate marker for immune response quantitation. Another limitation of the study was the unavailability of seroconversion data.

CONCLUSION(S)

The immunogenicity of the covishield vaccine among healthcare workers postvaccination after the second dose showed a steady decline in consecutive months. The waning of the vaccine's immunogenicity confirms that the SARS-CoV-2 antibody titer decreases over time and suggests the need for a third/ booster dose of the vaccine.

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