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Original Article

Findings of second multicentric follow-up serosurvey among Health Care Workers in government hospitals

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ABSTRACT

Background: The change in serological status of community may be used as input for guiding the public health policy. Hence, the present study was conducted to determine change in seroprevalence of COVID-19 among healthcare workers (HCWs).

Methods: From the baseline multicentric study sample, a subsample was followed up, and a seroepidemiological study was conducted among them between 6 and 22 weeks after the second dose of the vaccination. Multistage population proportion to size sampling was performed for the selection of subsample of HCWs. The serosurvey was conducted using the enzyme-linked immunosorbent assay–based IgG antibody test (COVID KAVACH).

Results: Follow-up serological testing was done in subsample of 1122 participants of original 3253 participants. The mean age of the participants was 34.6 (8.13) years. A total of 300

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(26.7%) participants were females. The seroprevalence was 78.52, (95%CI:76–80.1). Among those who were seronegative at initial test, 708 (77.04%) were seroconverted. Those who were not seroconverted (241 (21.5%)) have longer duration from the second dose of the vaccination (93 (31.4) vs. 56 (38.4); p value < 0.001). The COVID-19 infection was significantly associated with seropositive status and being a medical staff was associated with remaining seronegative on follow-up. The higher age (≥50 years) was found to be significantly associated with seroreversion.

Conclusion: Four in five HCWs had detectable antibodies. Seroepidemiological studies carry vital information to control the public health response in the course of the pandemic. The study can also further help as a platform to study the seroconversion and effect of vaccination among HCWs for newer variants of SARS-CoV-2.

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Introduction

The first round of multicentric serosurvey was conducted among Health Care Workers (HCWs) of Armed Forces Medical Services from August to November 2020.[1] The study estimated seroprevalence of 19.7% (95% Confidence Interval [CI]: 18.5–21.3%) among 3253 participants. Since then, there has been rapid developments in the course of the COVID-19 pandemic. At national level, COVID-19 cases decreased till Feb 2021 and start rising again from March 2021 onwards, which heralded the second wave of COVID-19 in India. The Government of India gave emergency use authorization to two vaccines, Covishield and Covaxin on 03 January 21, and the vaccination drive using these vaccine was started on 16 January 21.[2] The vaccination drive was started in a phased manner with HCWs and frontline workers and then expanding to high-risk population and finally to general population. In Armed Forces, the vaccination of HCWs also started from 16 January 21 onwards using Covishield vaccine. The complete dose of vaccination included two doses of Covishield 4–6 weeks apart.

As per the WHO global research map for population-level serosurveys for COVID-19 to generate data on the levels of infection, WHO has suggested protocol for conducting such population-based serosurveys.[3] The WHO has suggested three opportunities to conduct the serosurveys: First, cross-sectional survey, most suitable after the peak transmission is recognized; second, repetitive cross-sectional surveys in the same population or in the same geographic area to establish drifts in an evolving pandemic; and lastly longitudinal cohort study with sequential sampling of the similar individuals. Creating cohorts throughout a pandemic being resource intensive, however the last option is the most appropriate choice to guide public health response.[4] In Armed Forces, we undertook the longitudinal cohort approach using a subsample of initial cohort.

The seroprevalence study from India conducted at national level using repetitive cross-sectional approach after the second wave (fourth serosurvey conducted in June–July 2021) had observed that the seroprevalence of IgG antibodies (COVID KAVACH) among 7252 HCWs was 88.6%.[5] Ten percent of those HCWs were unvaccinated. Seropositivity was found to be higher among those who had previous history of natural infection. However, the immunity acquired through natural infection as well as vaccination is expected to wane over time.[6,7]

Keeping in view the need for data for the Armed Forces, the present study was undertaken to study change in seroconversion after vaccination of HCWs taking into account symptomatic infections during the course of the COVID-19 pandemic.

Material and methods

The initial serosurvey was conducted among the HCWs of the Armed Forces. The detailed protocol is given elsewhere.[8] The findings of the first phase of the study has been published.[1] The brief of the methodology is given here. The first phase of the study was conducted as a multicentric cross-sectional study among eight government hospital between August 2020 and November 2020. The second serosurvey was undertaken in the subsample selected through multistage sampling from the first-phase cohort irrespective of their seroconversion status in the first serosurvey between 6 and 22 weeks after the second dose of the Covishield vaccination. The duration of the present study period was from May 21 to Jul 21.

The similar enzyme-linked immunosorbent assay (ELISA) kit as used previously were used. COVID KAVACH anti-SARS-CoV-2 human IgG ELISA manufactured by Trivitron Healthcare was used in the study. It is a qualitative test and detects IgG antibody in serum/plasma samples. The reported sensitivity and specificity of the ELISA kit published by ICMR are 92.4% and 97.9%, respectively.[6]

The initial baseline data of all individual were already collected in the previous survey. Only the serotesting data were collected in MS Excel. The data were sent to the central place, where the data were merged using the subject unique identifier and telephone number. The continuous variables were described as mean and standard deviation. The categorical variables were described as number and percentage. COVID-19 infections were noted as persons having no COVID-

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19 infection, once COVID-19 infection, and twice COVID-19 infection. McNemars test was used for seroconversion status before and after vaccination. Multinomial logistic regression was done to find out the association with seropositives. Variables whose p value was less than 0.1 was taken into the model. The data were analysed using StataCorp. 2019. *Stata Statistical Software: Release 16*. College Station, TX: StataCorp LLC. The p value of less than 0.05 was taken as significant.

Results

A total of 1122 HCWs from the earlier 3253 underwent the second serotesting after mean days of 174 (95% CI: 170.4–177.6). The socio-demographic profile of 1122 HCWs are shown in Table 1. There was no difference in social-demographic characteristic of initial 3223 participants and 1122 HCWs. All participants were vaccinated with two doses of Covishield (ChAdox1). The mean age of the participants was 34.6 (standard deviation (SD) = 8.13) years. A total of 300 (26.7%) were females. A total of 881 (78.52; 95% CI: 76–80.1) participants were seropositive for COVID-19. Among various age groups of 19–29 years, 30–39 years, 40–49 years, and 50–59 years, the seropositivity was 78.5%, 79.5%, 78.6%, and 70.1%, respectively, with no significant difference in seropositivity among various age groups ($p = 0.45$). The mean duration of gap between the first dose of vaccine to the second dose was 4.4 weeks (SD = 1.3). During the follow-up period, a total of 167 persons had COVID-19 infection, the seropositivity among these individuals was 92.2%. On analysis as per their job profile in the hospital medical staff (doctor, nurses, paramedical staff) had significantly lower seropositivity (76.6%) than ancillary support staff working in the hospital (82.5%) ($p < 0.05$).

During the first serosurvey, 203 out of 1122 (18.1% 95% CI: 15.9–20.5) were seropositive. During the second serological testing, 881 out of 1122 (78.5% 95% CI: 76–80.8) were seropositive. The paired contingency table is highly significant (p value < 0.001) (Table 2). The four groups are made those who are positive during the first and second surveys (+/+), positive on the first and negative on the second (+/−), negative on the first and positive on the second test (−/+), and negative on the first and negative on the second (−/−). The distribution of variable within four categories is shown in Table 3. Because these four groups are nominal and may have different characteristic multinominal logistic regression was used to calculate relative risk in these categories. Only those variables were kept in the model (age, days, COVID-19 positive, and health occupation) for which p value was less than 0.1; other variables like sex, contact with patient, co-morbidity were not taken. The results of multinomial logistic regression are shown in Table 4.

Those who turn positive to negative (seroreversion) had statistically significantly higher age and longer duration (≥90 days) from the second dose of the vaccination compared with the baseline group (those converted seropositive after testing negative in the first test). The group that remained positive from the first test to the second test had statistically significant higher association with COVID-19 infections compared with the base group. In the group that remained seronegative in both the first and second test, statistically significant associations were observed with being a medical staff and longer duration from the second dose of the vaccination, whereas in the same group the association with COVID-19 infection was significantly lower compared with the baseline group.

Table 1 – Socio-demographic profile of the 1122 participants and original 3253 participants.

S. no	Variables	Participants in the current study (1122) N (%)	Participants at the time of initial serosurvey (N = 3253) N (%)
1	Sex		
	Female	300 (26.7%)	829 (25.5%)
	Male	822 (73.3%)	2424 (74.6%)
2	Healthcare group		
	Medical staff	751 (67%)	2329 (71.6%)
	Ancillary staff	371 (33%)	924 (28.4%)
3	Co-morbidities		
	Present	64 (5.7%)	177 (5.5%)
	Absent	1058 (94.3%)	3076 (94.6%)
		Mean (SD)	**Mean (SD)**
4	Age	34.6 (8.13)	34.3 (7.2)

Discussion

In the present study, the main aim was to estimate the seroprevalence among the HCWs in Armed Forces healthcare establishments. The infection in HCWs make the entire staff and patients vulnerable to the COVID-19. 78.5% seropositivity for SARS-CoV-2 antibodies among study participants were recorded, and maximum number of participants were ancillary workers. Understanding the seroprevalence and risk factors for SARS-CoV-2 among this high-risk group is paramount to ensuring their health and safety.

Over the mean duration of 25 weeks of the study, seroprevalence increased from 19.7% (95% confidence interval: 18.5–21.3%) to 78.52%; (95% confidence interval 76–80.1). The increase in the seroprevalence was similar to findings from the other studies.[9,10] This was expected considering the vaccination of all HCWs and high infection rates observed during the second wave from April to May 2021. This type of trend also reflects the impact of government policies such as vaccination strategy, evolution of virus transmission, and appropriate behaviour by the public.[11]

For any seroprevalence study, it is critical to use an optimum test, in terms of the nature of the antibody (IgG, IgM, or both) and the procedure of the test.[12] In the study done by Chaudhari et al. to compare the performance of three immunoassays, the sensitivity of the assays was 84.7 (95 % CI 80.6–88.1), 82.6 (95 %CI 78.3–86.2), and 75.7 (95 %CI 71.0–79.9), respectively, for RBD, LIAISON, and Kavach. In addition, Kavach and the RBD ELISA showed a specificity of 99.5% and 100%, respectively.[13]

Even though seroepidemiological studies are substantial in assessing the burden of SARS-CoV-2 infection among the general population, their conclusions specifically the

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Table 2 – Contingency table for the first and second serosurvey (N = 1122).

		Second serosurvey		
		Positive (n/N*100)	Negative (n/N*100)	Total (n/N*100)
First serosurvey	Positive (n/N*100)	173(15.4%)	30(2.7%)	203 (18.1%)
	Negative (n/N*100)	708(63.1%)	211(18.8%)	919 (81.9%)
	Total (n/N*100)	881 (78.5%)	241 (21.5%)	1122 (100%)

Table 3 – Distribution of variables with different serological group.

S. No.	Variable	+/+ Gp n = 173 (%)	+/− Gp n = 30 (%)	−/+ Gp n = 708 (%)	−/− Gp n = 211 (%)
1.	Age				
	‘<50	162 (93.6)	25 (83.3)	679 (95.9)	199 (94.3)
	≥50	11 (6.4)	5 (16.7)	29 (4.1)	12 (5.7)
2.	Sex				
	Female	46 (26.6)	6 (20)	188 (26.6)	60 (28.4)
	Male	127 (73.4)	24 (80)	520 (73.4)	151 (71.6)
3.	Co-morbidity				
	No	162 (93.6)	27 (90)	667 (94.2)	202 (95.7)
	Yes	11 (6.4)	3 (10)	41 (5.8)	9 (4.3)
4.	Days (second dose of vaccination)				
	<90	94 (56.6)	13 (52)	539 (76.7)	57 (27.5)
	≥90	72 (43.4)	12 (48)	164 (23.3)	150 (72.5)
5.	COVID-19 infection				
	0 time	110 (63.6)	24 (80)	578 (81.6)	196 (92.9)
	1 time	39 (22.5)	3 (10)	86 (12.2)	12 (5.7)
	2 times	24 (13.9)	3 (10)	44 (6.2)	3 (1.4)
6.	Direct care to patient				
	No	64 (37)	8 (26.7)	299 (42.2)	90 (42.7)
	Yes	109 (63)	22 (73.3)	409 (57.8)	121 (51.4)
7.	Job profile				
	Ancillary worker	55 (31.8)	10 (33.3)	251 (35.5)	55 (26.1)
	Medical staff	118 (68.2)	20 (66.7)	457 (64.5)	156 (73.9)

+/+ positive during the first and second surveys, +/− positive on the first and negative on the second, −/+ negative on the first and positive on the second test, −/− negative on the first and negative on the second.

inference about antibody-mediated immunity need to be considered with due caution. Immunoglobulin G antibodies against SARS-CoV-2 decline over time, with faster waning of anti-nucleocapsid antibodies than anti-spike antibodies.[14] Therefore, there are higher chances that seroprevalence data could underestimate the true number of people who have been infected. Furthermore, the seroprevalence data do not represent the long-term immunity, as the durability of SARS-CoV-2 adaptive immunity is still uncertain.

Table 4 – Multinomial logistic regression.

Characteristic	Group (−/+)	Group (+/+) Relative risk ratio (95% CI)	Group (+/−) Relative risk ratio (95% CI)	Group(−/−) Relative risk ratio (95% CI)
Age	**Base group**			
<50		1 (Ref)	1 (Ref)	1 (Ref)
≥50		1.1 (0.5–2.5)	4.6 (1.4–14.7)	1.6 (0.7–3.5)
Job profile				
Ancillary worker		1 (Ref)	1 (Ref)	1 (Ref)
Medical staff		1.1 (0.8–1.6)	0.8 (0.4–2)	1.5 (1.03–2.2)
COVID-19 infection				
0 time		1 (Ref)	1 (Ref)	1 (Ref)
1 time		2 (1.3–3.2)	0.8 (0.2–2.8)	0.3 (0.1–0.5)
2 times		3.3 (1.9–5.7)	1.3 (0.3–5.8)	0.3 (0.1–0.9)
Days				
<90		1 (Ref)	1 (Ref)	1 (Ref)
≥90		2.4 (1.7–3.5)	3.1 (1.4–7.1)	9.2 (6.4–13.2)

+/+ positive during the first and second surveys, +/− positive on the first and negative on the second, −/+ negative on the first and positive on the second test, −/− negative on the first and negative on the second.

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In the present study among the 203 seropositive participants from the first round of serosurvey, 30 (14.8%) participants had seroreversion despite two doses of vaccine, while among 919 seronegative participants from the first round, 211 (23%) remained seronegative on repeat test during the second serosurvey despite two doses of the vaccine. A study with 122 participants had shown no seroconversion in 0.8% participants after two doses of ChAdox1 vaccination, seroreversion in 6% cases, and declining antibody titre in 70% of participants after 6 months of the second dose of the vaccination.[15] The above study and present study differed in type of kits, type of assessment (quantitative vs qualitative) and period of follow-up, which could the reason for the observed difference. However, the phenomenon of seroreversion within 6 months of vaccination was observed in both the studies.

Some of the longitudinal studies had observed that rate of seroreversion was not associated with age, sex, BMI, or comorbidities.[15,16] However, in our study we found significant association of age (≥50 years) with seroreversion. In the present study, COVID-19 infection are significantly associated with seropositive status in the follow-up test, the study by Misra et al. also observed that participants with a history of COVID-19 infection have significantly higher mean titer values at 1 and 6 months after the second dose.[15] In the present study, medical staff had higher chances of remaining seronegative than ancillary support staff of the hospital. A large sample study done at AIIMS, New Delhi, found significantly higher seroprevalence in administrative staff than in medical staff (nurses and physicians), the study mentioned that it could have been due to better awareness and infection control practices followed by medical staff.[17-20]

Studies have reported fivefold drop in ChAdOx1 antibodies after 70 days of complete vaccination, which was consistent when results were stratified by sex, age, and clinical vulnerability. Studies from India have also shown significant decrease in antibody levels within 4–6 months of Covishield double-dose. An Indian studies have shown that the IgG antibodies levels in 6% of vaccinated drop to undetectable level and 30% had antibodies below protection levels. In the present study also, it was observed that longer duration, i.e. more than 90 days from the second dose of the vaccination was significantly associated with seronegative status.

Large-scale, population-based, seroepidemiological surveys are resource-intensive, and allotting scarce public health resources could be challenging for several developing nations. The subset of the cohort was studied for change in seroconversion. Post priori, the power calculated for the studied sample size was more than 99% for 5% change of discordant pair with 0.05 alpha error. The strong point of this study is that it was conducted in the different parts of the country with a large sample size. The consecutive nature of this study, along with an inter-survey period permissible for a sequential evaluation of changes in seroprevalence is its strength. All the ELISA kits were of the similar type, and all the hospitals had quality assurance devices in place for performing the test. The training, standardization of the data collection form and process and collection of data on smartphones/tablet in real time made it possible for the core team to monitor and deliver real-time inputs. The findings of the study had been used in policy-making. The study can also further help as a platform to study the seroconversion and effect of vaccination among HCWs.

The study has certain limitations. The present study kits could only provide qualitative assessment of IgG antibodies so drop in antibody titers with time could not be assessed, the study also did not collect data on clinical profile of the individuals who may had suffered natural infection both prior to the first round and during the follow-up, which could have further helped to assess the impact of mild, moderate, or severe disease on antibody response. Seroepidemiological studies do not measure T-cell-mediated immunity, and immune escape viral variants can cause resurgence of the disease despite high seroprevalence.[16] Despite above seroepidemiological studies do carry vital information to control the public health response in the course of the pandemic.

Conclusion

The study brought out the complexity associated with COVID-19 seroconversion, which seems to be affected by multiple parameters with possible interactions. Although majority of the seronegative individual have seroconverted, a small percentage remain seronegative. In seropositive participants at beginning, seroreconversion was also observed. Important sociodemographic variables were delineated for findings. However, molecular studies may be required to have an understanding of seroconversion and seroreversion in COVID-19.

Disclosure of competing interest

The authors have none to declare.

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