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Original Article

Seropositivity of severe acute respiratory syndrome coronavirus 2 infection among healthcare workers of the Armed Forces medical services, India: A multicentric study

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ABSTRACT

Background: Serosurveys provide the prevalence of infection and over time will reveal the trends. The present study was conducted to estimate the seroprevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) among healthcare workers (HCWs) and to analyse various characteristics (risk factors) associated with SARS CoV-2 infection.
Methods: Eight government designated Corona virus disease -19 (COVID-19) hospitals were selected based on the hospital admission of patients with COVID-19 and the local epidemiological situation in the region. Multistage population proportion to size sampling was performed for the selection of HCWs. Serosurvey was conducted using the enzyme-linked

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immunosorbent assay–based IgG antibody test (COVID KAVACH). Bivariate and multiple logistic regression was performed to find out the factor/factors associated with the positive antibody test.

Results: Out of 3255 HCWs that participated in the study, data of 3253 were analysed. The seroprevalence was 19.7% (95% confidence interval: 18.5–21.3%). Factors associated were location, category of HCWs, male sex, previously tested positive by the molecular test, training on infection prevention and control, personal protective measures, handwashing technique, close contact with a patient confirmed with COVID-19, use of personal protective equipment and symptoms in the last 30 days. However, in multiple logistic regression, only location, category, previously tested positive by the molecular test and symptoms in the last 30 days were statistically significant.

Conclusion: HCWs are vulnerable to SARS-CoV-2 infection. One in five HCWs had detectable antibodies. The presence of antibodies among HCWs may help in their placement and triage. HCWs may be advised to report early in case of any symptoms of COVID-19. Preventive measures may be targeted based on the location, with particular emphasis on ancillary workers and nurses.

Introduction

Exactly a year after the first case of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection was reported in India, there have been more than 100 million confirmed cases and 2 million deaths from COVID-19.[1] No continent has been spared from the effects of the ongoing pandemic.[2] Most of the cases of COVID-19 are mild and aymptomatic.[3] These asymptomatic and presymptomatic cases play a significant role in disease transmission in the community.[4] The proportion of asymptomatic to symptomatic patients in various studies varies from 20% to 75%.[5] Hence, the true prevalence of the disease is difficult to estimate from the caseload.

Serosurveillance serves as an excellent tool to estimate the true prevalence and trends of infection. Healthcare workers (HCWs) involved in managing COVID-19 cases are at higher risk of SARS-CoV-2 infection. The Indian Council of Medical Research (ICMR), vide its guidelines in the context, recommended serosurveillance studies in this high-risk group.[6]

The ICMR study conducted in the early period of the pandemic, March–April 2020, showed a total of 2.3% of asymptomatic HCWs as positive in laboratory-based antigen testing surveillance using Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR).[7] However, all HCWs were tested as per the prevailing testing policy at that time; hence, bias in the findings cannot be entirely ruled out. Serosurvey studies have been conducted among HCWs across the globe and have shown seropositivity from 1% to 40%.[8–15]

A large number of HCWs of the hospitals under the central government have been involved in various aspects of COVID-19 management since March 2020, including management of quarantine facilities, clinical management of admitted patients in healthcare establishments and contact tracing activities. The prevalence of seropositivity among HCWs would provide valuable insight into the risks associated with contracting the infection and provide a better understanding of the lacunae existing in the current practices about use of PPE, disinfection procedures and patient management protocols, which would in turn help in efficiently managing the valuable health workforce. Serosurvey studies from individual investigators have been published since then; however, they are limited to a single centre and in small sample sizes.[16–23] Moreover, the associated factors of seropositivity need to be studied in detail.

Hence, this study was conducted to estimate the seroprevalence of SARS-CoV-2 among the HCWs in Armed Forces healthcare establishments and to identify factors associated with seropositivity.

Material and methods

Study design

The study was conducted as a multicentric crossectional study. Eight government-designated COVID-19 hospitals had been chosen, based on hospital admission rates of patients with COVID-19 and the local epidemiological situation prevalent in the region. The study was carried out from August 21, 2020, to November 20, 2020. All HCWs in the selected hospital were included in the study.

A questionnaire duly pilot tested and checked for content validity by experts was adapted from the World Health Organization (WHO) questionnaire on serosurvey.[24] The questionnaire consisted of information on the interviewer, demographic details, information on the healthcare facility and the HCWs' basic knowledge of infection prevention and control (IPC) and personal protective equipment (PPE) use, activities carried out during interaction with patients with COVID-19, adherence to IPC measures and use of PPE. All

questions as marked essential by the WHO formed part of the questionnaire. Data were collected using an Open source tool without any personal identifiers using the freely available Kobo Toolbox application in the mobile with built-in logic checks and branching.[25]

A nodal officer was designated from each centre, and two training sessions of 2-h duration each were conducted. An operational manual was prepared and circulated to all. The form was transferred into the mobile of each data collector at centres. A central team addressed any difficulty in data collection via mobile and video conferencing. Data management was carried out centrally.

COVID KAVACH anti–SARS-CoV-2 human IgG enzyme-linked immunosorbent assay (ELISA) manufactured by Trivitron Healthcare for which technology is developed by Indian Council of Medical Research- National Institute of Virology (ICMR-NIV) 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.[26]

Trained phlebotomists collected venous blood samples (approximately 3 ml) under aseptic conditions. The blood was transported to the hospital laboratory for serum separation and testing for SARS-CoV-2–specific IgG antibodies as per the specified optical density cut-off value of 0.29.

Sampling technique

A multistage population proportion to size method was adopted. Population proportion to size was used for enrolment from a given site, wherein the number of personnel selected from the site depended on the posted strength of HCWs in the hospital. Within the hospital chosen, a list of all eligible participants was prepared as per various categories (doctors, nurses, nursing assistants, ambulance assistants, and so on). These categories were further compressed into three groups, namely, doctors, nurses, and ancillary workers, during the analysis. Study participants were selected by random sampling from each category, proportional to the population size in each category.

Sample size

Sample size was estimated for the entire population of HCWs with the assumptions regarding the desired level of confidence (1-α) as 95%, expected prevalence of seropositivity among HCWs (π) as 20%, absolute precision (d) as 2% and design effect (δ) as 2 (standard).

The formula (sample size $= Z^2_{1-\alpha/2}*\delta*\pi(1-\pi))/d^2$) yielded a sample of 3073 subjects. However, considering the possibility of dropouts, a sample size of 3255 was taken. The sample size calculation differ from what is given in protocol of the study published as we revised the expected prevalence of seropositivity and absolute precision for the article.[27]

Requisite ethical clearance was obtained from the institutional ethical committee at the institute level/centrally and from each participating centre. Patient confidentiality was maintained by censoring personal identifiers, and a final report was presented in aggregate numbers without any personal identifier.

Statistical analysis

All categorical variables were presented as numbers and percentages, and quantitative variables were presented as mean and standard deviation. Contingency tables were prepared for the association between seropositivity and other studied variables. Multiple logistic regression was carried out with seropositivity as a dependent variable and other variable as independent variables. Collinearity among the categorical variables was checked using contingency table. If the association between variable was significant (p value < 0.001) in the contingency table, then only one variable was selected. All variables having p values less than 0.05 were included in the model. A parsimonious model (a model with minimum variables) was made using forward techniques. The techniques were used until the time there was no difference between the parsimonious model and complete model. The Hosmer–Lemeshow goodness-of-fit test was used for model fit. Models were compared using log-likelihood ratios. Data analysis was carried out using StataCorp. 2019 (Stata Statistical Software: Release 16; StataCorp LLC, College Station, TX). A P value of 0.05 was taken as statistically significant.

Results

A total of 3255 HCWs participated in the study. Two of the tests were indeterminate. Hence, data of total of 3253 HCWs were analysed. Sociodemographic details, IPC, PPE practices and other variables regarding antibody results have been depicted in Table 1. The seroprevalence in the study was 19.7% (95% confidence interval [CI]: 18.5–21.3%). However, there was wide variation, with the lowest estimate among HCWs located at Jaipur hospitals (7.8%) and highest estimate among HCWs located Jammu hospital (42.8%). There was no difference in the age of seropositive and seronegative members (p value = 0.9). The prevalence among male and female HCWs was 20.8% and 17.1%, respectively (p = 0.02). The prevalence was lowest among medical officers (10.6%), followed by nursing officers (18.2%), and highest in ancillary workers (22.3%). The ELISA test was positive in 44.2% of individuals tested positive by the molecular test earlier. The seropositivity was 18.1% among those who attended any training in IPC as compared with 26.1% among those who did not attend (p < 0.001). Similarly, seropositivity was more among those without any training in PPE than among without training (p < 0.0001). Seropositivity was more among selective handwashers than those washing hands as prescribed by the WHO (p = 0.002). There was no difference in seropositivity among those who did or did not provide direct care and the availability of alcohol-based hand rub at the point of care to the COVID-19 case (p value = 0.7 for each). Seropositivity was more among HCWs with a history of close contact with the COVID-19 case and those who did not wear prescribed PPE during interaction within the past one month (p = 0.009 and 0.03, respectively). There was no difference in seropositivity

Table 1 – Characteristic of healthcare workers with reference to antibody testing.

S.No.	Characteristics	Number (%)	Antibody test result		P-value
			Negative	Positive	
1	Centre				
	Pune	321 (9.9)	243 (9.4)	78 (12.1)	<0.001
	Ahmedabad	131 (4.8)	124 (1.1)	7 (4.1)	
	Kolkata	623 (17.6)	458 (25.6)	165 (19.2)	
	Delhi	655 (20.2)	603 (23.2)	52 (8.1)	
	Jammu	229 (5.1)	131 (15.2)	98 (7.1)	
	Jaipur	243 (7.5)	224 (8.6)	19 (3)	
	Mumbai	625 (19.8)	516 (16.9)	109 (19.3)	
	Bangalore	426 (11.9)	308 (18.3)	118 (13.1)	
2	Sex				
	Female	829 (25.5)	687 (26.4)	142 (22)	0.022
	Male	2424 (74.6)	1920 (73.7)	504 (78.1)	
3	HCW category				
	Doctors	461 (14.2)	412 (15.8)	49 (7.6)	<0.001
	Nurses	610 (18.8)	499 (19.1)	111 (17.2)	
	Ancillary workers	2182 (67)	1696 (65.1)	486 (75.2)	
4	Previous tested positive by the molecular test				
	No	2873 (88.4)	2395 (91.9)	478 (74)	<0.001
	Yes	380 (11.7)	212 (8.2)	168 (26.1)	
5	Training on IPC				
	No	701 (21.6)	518 (19.9)	183 (28.4)	<0.001
	Yes	2552 (78.5)	2089 (80.2)	463 (71.7)	
6	Training on PPE				
	No	383 (11.8)	273 (10.5)	110 (17.1)	<0.001
	Yes	2870 (88.3)	2334 (89.6)	536 (83)	
7	Handwashing technique				
	Don't know	58 (1.8)	49 (1.9)	9 (1.4)	0.002
	Each time	2120 (65.2)	1737 (66.7)	383 (59.3)	
	Selective	1038 (32)	791 (30.4)	247 (38.3)	
	Don't have time	37 (1.2)	30 (1.2)	7 (1.1)	
8	Direct care to a COVID-19 case				
	No	1258 (38.7)	1004 (38.6)	254 (39.4)	0.706
	Yes	1995 (61.4)	1603 (61.5)	392 (60.7)	
9	Availability of alcohol-based hand rub				
	No	211 (6.5)	171 (6.6)	40 (6.2)	0.7
	Yes	3042 (93.6)	2436 (93.5)	606 (93.9)	
10	Close contact (within 1 m) with a patient confirmed with COVID-19				
	No	1331 (40.1)	1096 (42.1)	235 (36.4)	0.009
	Yes	1922 (59.1)	1511 (58)	411 (63.7)	
11	PPE used				
	No	288 (8.9)	217 (8.4)	71 (11)	0.033
	Yes	2965 (91.2)	2390 (91.7)	575 (89.1)	
12	Smoking				
	No	3114 (95.8)	2496 (95.8)	618 (95.7)	0.931
	Yes	139 (4.3)	111 (4.3)	28 (4.4)	
13	Symptoms in the last 30 days				
	No	3002 (92.3)	2439 (93.6)	563 (87.2)	<0.001
	Yes	251 (7.8)	168 (6.5)	83 (12.9)	
14	Risk factors				
	No	3076 (94.6)	2460 (94.4)	616 (95.4)	0.3
	Yes	177 (5.5)	147 (5.7)	30 (4.7)	
15	Duration of contact (if there are multiple contacts)				
	<15 min	626 (32.6)	478 (31.7)	148 (36.1)	0.093
	>15 min	1296 (67.5)	1033 (68.4)	263 (64)	

HCW = healthcare worker; IPC = infection prevention and control; PPE = personal protective equipment.

Table 2 – Multiple logistic regression for antibody response as positive.

S.No.	Variable	Unadjusted odds ratio (95% confidence interval)	Adjusted odds ratio (95% confidence interval)
1	**Location**		
	Jaipur	Reference	Reference
	Pune	3.7 (2.2–6.5)	3.6 (2.1–6.1)
	Ahmedabad	0.7 (0.3–1.6)	0.7 (0.3–1.6)
	Kolkata	4.2 (2.6–7)	3.9 (2.3–6.5)
	Delhi	1 (0.6–1.8)	0.7 (0.4–1.3)
	Jammu	8.8 (5.2–15)	7 (4–12.1)
	Mumbai	2.5 (1.5–4.2)	2.7 (1.6–4.5)
	Bangalore	4.5 (2.7–7.5)	4.1 (2.4–7)
2	**Category**		
	Doctors	Reference	Reference
	Nurses	1.9 (1.3–2.7)	1.7 (1.1–2.5)
	Ancillary workers	2.4 (1.8–3.3)	2.9 (2.1–4.02)
3	**Any symptoms of COVID-19 within 30 days**		
	No	Reference	Reference
	Yes	2.1 (1.6–2.8)	2 (1.4–2.7)
4	**Previously tested positive by the test**		
	No	Reference	Reference
	Yes	4 (3.2–5)	4.1 (3.1–5.2)

among smokers and non-smokers (p = 0.9) and with or without comorbidities (p = 0.3); however, any of the symptoms of COVID-19 in the past one month was associated with higher seropositivity (p value <0.0001).

The results of multiple logistic regression analysis are shown in Table 2. In multiple logistic regression, location of the HCWs, category, any symptoms in the last 30 days and previously tested positive by the test were statistically significant. The Hosmer–Lemeshow goodness-of-fit test indicates that our model fits well the data (p value = 0.5).

Discussion

Table 3 shows that antibody positivity among various serosurveys conducted among HCWs in India varied from 0% to 70%. Various government agencies have also conducted serosurveys. However, they had been conducted in the general population. Among nationwide household surveys, the second survey conducted in August–September 2020 shows a seroprevalence of 6.6% (95% CI: 5.8–7.4) as compared with 0.73% (95% CI: 0.34–1.13) during the first nationwide serosurvey conducted in May–June 2020.[26]

In our study, we have also tried to look for the association of various factors with positive antibody results. Interestingly, training of healthcare personnel in IPC and PPE was associated with decreased prevalence in bivariate analysis. However, in multivariate analysis, it was statistically not significant. The reason for the same may be due to exposure to the COVID-19 case without knowing the status of the patient, which makes professionals vulnerable. This is further substantiated by the factors significant in multivariable analysis such as the place of the hospital, symptoms in the past 30 days and already tested positive by RT-PCR in the past.

Another interesting finding in the study was lowest risk among doctors and highest risk among ancillary workers. The reason for the same may be higher compliance of preventive measures by doctors and more contact of ancillary workers with patients and exposure during procedures. Similar findings have been found by Goenka et al.[21]

Table 3 – Antibody positivity among serosurveys conducted among HCWs in India.

S.No.	Author	Place of the study	Period of the study	Sample size	Seroprevalence
1	Singhal et al.[23]	Mumbai	June 2020		Asymptomatic HCWs: 4.3% Previously symptomatic untested HCWs: 70%
2	Hawaldar et al.[22]	Indore	March to June	307	7.82%
3	Goenka et al.[17]	Kolkata	July to August	1122	11.94%
4	Kumar et al.[19]	Kerala	July 11 to 24	635	0%
5	Khan et al.[18]	Srinagar	June 15 to 30	2905	2.5%
6	Dave et al. [29]	Udaipur	April to May	100	16%
7	Kumar et al.[16]	Mumbai	Not mentioned	801	11.1%
8	Baveja et al.[20]	Mumbai	May 2020	1552	6.9%
9	Goenka et al.[21]	Kolkata	August	117	32.5%
10	Singhal et al.[23]	Mumbai	June	244	4.3%

All these government hospitals were designated COVID-19 hospital. The study conducted elsewhere shows that HCWs working in the COVID-19 hospital have higher seroprevalence than those not working in the designated COVID-19 hospital.[28] The infection in HCWs can make the entire health system vulnerable; hence, there is a need to improve early detection and not to miss any COVID-19 cases among them. One of the important findings of our study is that those with any symptoms of COVID-19 must get themselves tested at the earliest. Second, only 44% of those previously tested positive by the molecular test have antibodies, and similar responses have been found in other studies.[16,18] It signifies the limitation of IgG antibodies by ELISA in determining the immune responses after COVID-19 infection.[30]

A recent news report of the third ICMR serosurvey conducted between December 17, 2020, and January 8, 2021, has estimated the seroprevalence among 7171 HCWs as 25.7%. However, the details of the same were not available.[31] We estimated lower prevalence than the national survey despite all hospitals being dedicated COVID-19 hospital, probably owing to better implementation and adherence to all preventive measures. The universal precautions, handwashing, correct and adequate use of PPE and refresher training on PPE and IPC may make significant difference in contracting infection. These were important variables in bivariate analysis; however, in multivariable analysis, they were not associated. Their implementation needs to be studied in detail and corrected. They continue to be an important public health measure for prevention of infection.

The data regarding duties of HCWs in different wards such as acute wards or chronic wards were not collected. The seropositivity of HCWs may be affected with the course of the pandemic as well as behaviour outside the hospital.

The study's strength is that it was conducted pan-India with a large sample size. All ELISA kits were of the similar make, and all the hospitals had quality control mechanisms in place for conducting the test. The training, standardization of the data collection form and procedure and collection of data on mobiles in real time made it possible for the central team to monitor and provide real-time inputs. The study can further help as a platform for studying the seroconversion and effect of vaccination among seropositive and seronegative HCWs.

Conclusion

HCWs are vulnerable to SARS-CoV-2 infection, with one in five having detectable antibodies, which is higher than the national survey conducted around the same time. The presence of antibodies among HCWs may help in prioritizing and placement of HCWs. HCWs may be advised to report any symptoms of COVID-19. Preventive measures may be targeted based on the location, with particular emphasis on ancillary workers and nurses.

Disclosure of competing interest

The authors have none to declare.

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