ORIGINAL ARTICLE

Seroprevalence of COVID-19 Amongst Health Care Workers in a Tertiary Care Hospital of a Metropolitan City from India

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Abstract

Background: Seroprevalence studies for COVID-19 evaluate the extent of undetected transmission in a defined community, with special significance among health care workers (HCW) owing to their greater exposure and potential to transmit.

Methods: A total of 1122 HCW (approximately 25% of the employees) of a large tertiary care hospital in India were recruited for this cross-sectional study. COVID PCR-positive HCW were excluded. Based on their risk-assessment, participants were grouped into three categories. A questionnaire was administered and they were tested for SARS-CoV-2-IgG antibodies using the chemiluminescence.

Results: The overall seroprevalence among workers was 11.94%, which included 19.85% in COVID units, 11.09% in non-COVID units, and 8% in administrative workers ($p=0.007$). Antibody prevalence was highest in the department of gastroenterology (11.94%), followed by oncology (10.53%), pathology (10.26%), emergency medicine (7.84%) and critical care medicine (7%). Housekeeping staff, food and beverage staff, lab assistants and technicians had higher seroprevalence rate than doctors and nurses ($p < 0.0001$). HCW with a history of BCG vaccination in childhood and those who received an adequate prophylactic dose of hydroxychloroquine (HCQ) had a lower seroprevalence as compared to those who did not (7.31% vs. 16.8% and 1.30% vs. 11.25% respectively).

Conclusion: BCG vaccination, HCQ prophylaxis, and the job profile influence the seroprevalence rate in HCW. Seroprevalence rate and follow-up evaluation of its durability may help hospitals to triage their staff at risk, rationalize their placement, prioritize the use of PPE, thereby potentially reducing the risk.

Introduction

COVID-19 (Coronavirus disease 2019) was declared a pandemic illness on 11th March 2020 and is still evolving. Clinical presentation of the disease varies from mild upper respiratory tract symptoms to severe pneumonia and acute respiratory distress syndrome. It is difficult to predict the exact number of individuals being infected, since many of them may be asymptomatic carriers for several weeks.[1–4] The current data suggest that the pre-symptomatic and asymptomatic patients can be potential source of infection, though the extent of transmission of infection via asymptomatic individuals is unclear.[5,6]

Health care workers (HCW) are subjected to a greater risk of contracting the infection due to their direct contact with the infected patients. An infected HCW poses a risk to other patients under his or her care as well as to a fellow HCW.[7] It is, therefore, vital to understand the true prevalence rates of COVID-19 infection among the HCW. There is a sparse data on the seroprevalence of SARS-CoV-2 infection among HCW, with only a few published studies.[7–19] Since no data is available for the COVID-19 infection in HCW in India or the adjoining geographical areas, this research attempted to study the seroprevalence of anti- SARS-CoV2 antibodies in HCW from a tertiary care hospital in India.

Methods

A. Study design: It is a cross-sectional study extending over 6 weeks starting from 12th July 2020. A survey was conducted using an online questionnaire followed by antibody testing using chemiluminescence. The study was approved by the internal ethical committee of the institute.

B. Sample selection: A list of hospital employees was obtained from the human resource department**.** Subjects who were COVID RT-PCR positive either before or during the study were excluded. Subjects were divided into three groups based on the risk of exposure to the COVID positive patients:

1. Category A: High risk

• Working/ have worked in a COVID ward/Intensive Care Unit

• Regularly involved in the testing or investigating a COVID- 19 patients

2. Category B: Intermediate risk

• Those not belonging to category A or category C i.e., HCW who are managing patients or performing procedures on patients not diagnosed/ suspected to be having COVID. These included but were not limited to, staff working in emergency, aerosol-

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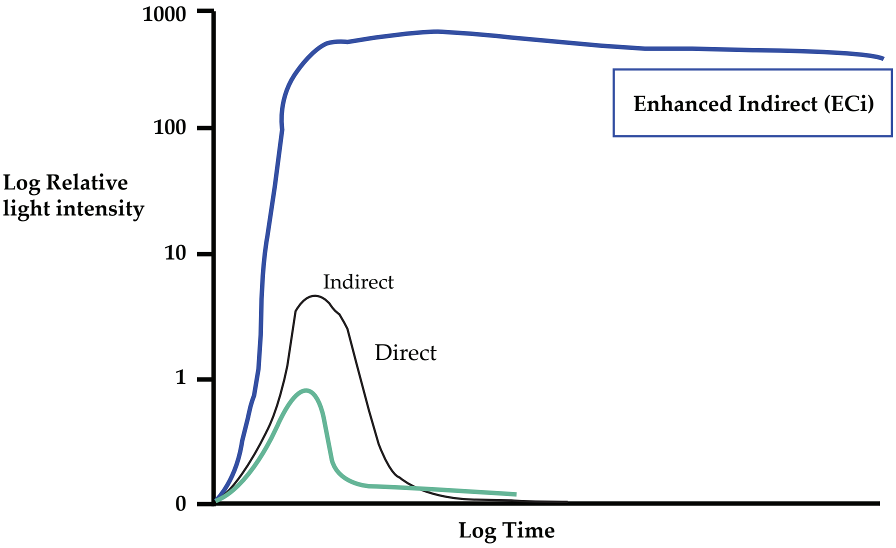


Fig. 1: Log relative light intensity generated by enhanced chemiluminescence method in comparison to direct and indirect methods

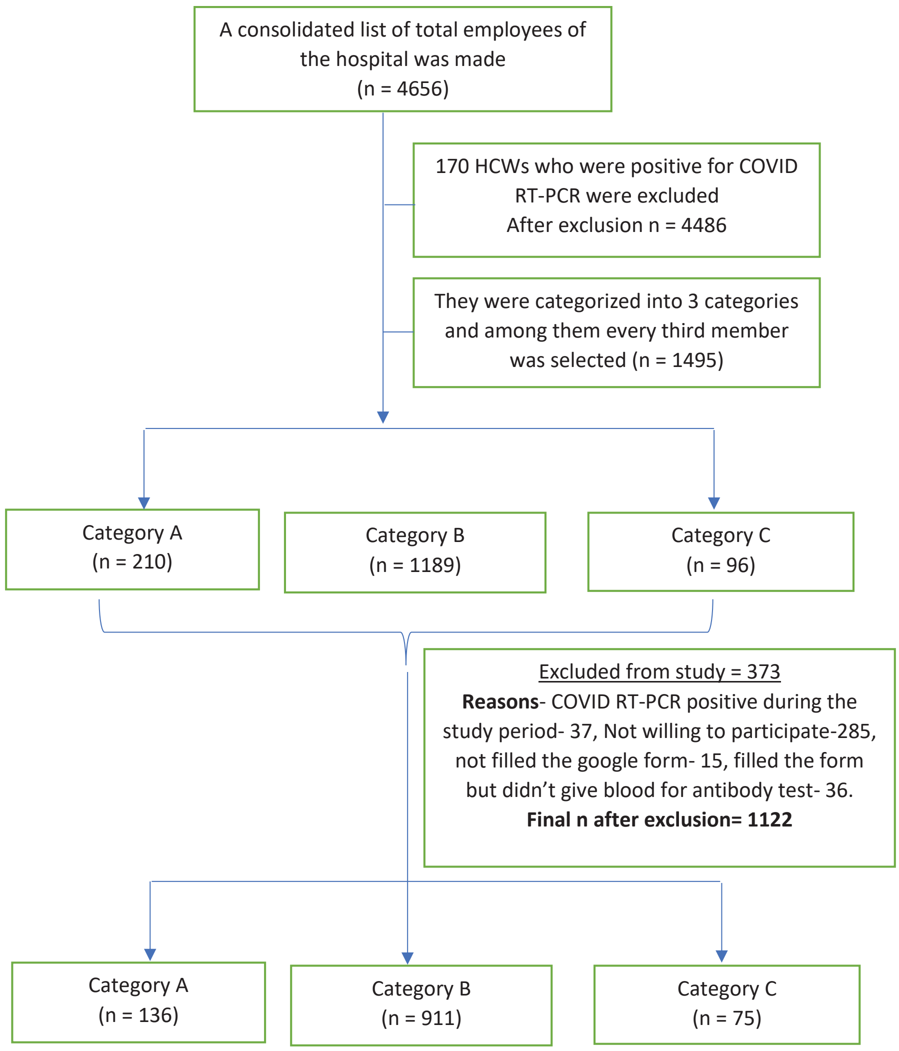


Fig. 2: Study flow chart showing the categorization of health care workers into three categories and the reasons for exclusion

generating facilities, and outpatient services.

3. Category C: Low risk

• No direct contact with the patients or their belongings, for example, staff belonging to the administrative office, human resource department, and marketing.

A systematic random sampling method was applied to recruit participants. As categorized above, every third employee in each category was selected and was offered to participate in the study. A total of 1122 (out of a total of 4656) HCW participated in the study. Written informed consent was obtained from each participant who agreed.

C. Procedure: A questionnaire was formulated after discussion with scientific committee. The same in Google Forms was sent to the participants, either through a registered phone number or email address to collect demographic and clinical data. The form had twenty-six questions with multiple-choice option answers, requiring either a single or multiple replies. Survey questions were divided into the following three categories:

1. Demographic details of the study participants

2. Details of participant's job profile and working pattern

3. Relevant medical history.

D. COVID antibody testing: Antibodies to COVID-19 were tested using the enhanced chemiluminescence method (Vitros ECi, Ortho Clinical Diagnostics, New Jersey, US). It involves a 'signal generating' reaction using a luminol derivative in the presence of peroxide. Horseradish peroxidase (HRP) provides electrons from peroxide to luminol to produce light. The enhancer, 3-chloro 4-hydroxy acetanilide, acts as a catalyst for the luminal reaction. It accelerates electron transfer and increases the oxidation of luminol by HRP almost 1000 times maintaining the signal ~20min (Figure 1). The signal is read by a luminometer 16 times in 1.6 seconds in 'Glow' type chemiluminescence.

Statistical Analysis

All statistical tests were performed using SPSS version 20.0. Categorical variables were expressed as frequency and percentage of patients. These were analyzed using Pearson's Chi-Square

Table 1: Demography and medical history of study group and seropositivity rate

Parameter	Group	Total number	Positivity, n (%)
Gender	Male	734	101 (13·76%)†
	Female	388	33 (8·51%)
Age	<30 years	364	43 (11·81%)‡
	30-50 years	665	88 (13·23%)
	>50 years	93	3 (3·23%)
Diet	Vegetarian	60	4 (6·67%)†
	Non vegetarian	1062	130 (12·24%)
Blood group	A group	210	21 (10.00%)†
	Non A group	832	88 (10·58%)
Rh factor	Rh Positive	1005	105 (10·45%)
	Rh Negative	37	4 (10·81%)†
Job profile	Administration	75	6 (8·00%)**
	Doctor	255	10 (3·92%)
	Ward executives	71	5 (7·04%)
	Nurses	224	21 (9·38%)
	Housekeeping	226	59 (26·11%)
	Dietician/FB	49	9 (18·37%)
	Lab assistants/pharmacists	72	11 (15·28%)
	Technicians	99	12 (12·12%)
	Others	51	1 (1·96%)
Mode of transport	Walking	91	18 (19·78%)†
	Personal Vehicle	545	49 (8·99%)
	Public transport	451	56 (12·42%)
Residence in containment zone	Yes	293	31 (10·58%)†
	No	656	73 (11·13%)
Place of residence	Metropolitan	501	46 (9·18%)†
	Suburbs	621	88 (14·17%)
Number of persons in room	1 to 2	537	49 (9·12%)‡
	3 to 5	435	58 (13·33%)
	> 5	91	22 (24·18%)
Time spent in hospital in a week	< 48 hours	731	102 (13·95%)†
	48 hours or more	373	29 (7·77%)
BCG vaccine	Received	561	41 (7·31%)**
	Not Received	77	13 (16·88%)
MMR vaccine	Received	336	29 (8·63%)†
	Not received	303	25 (8·25%)
HCQ prophylaxis	Not received	885	115 (12·29%)**
	Inadequate dose*	160	18 (11·25%)
	Adequate dose$	77	1 (1·30%)
Comorbidities	Diabetes	65	6 (9·23%)†
	Hypertension / CAD	107	5 (4·67%)
	Lung disease	31	3 (9·68%)
	None	919	120 (13·06%)

Abbreviations: FB- food and beverage, BCG-Bacille Calmette-Guerin, MMR-Measles Mumps Rubella, HCQ- Hydroxychloroquine, CAD- coronary artery disease. *Inadequate dose was defined as 400 mg once a week for <6 weeks. $Adequate dose was defined as 400 mg once a week for >6 weeks; Note: some of the participants did not respond to certain parameters; †p=not significant; ‡p<0.05; **p<0.005

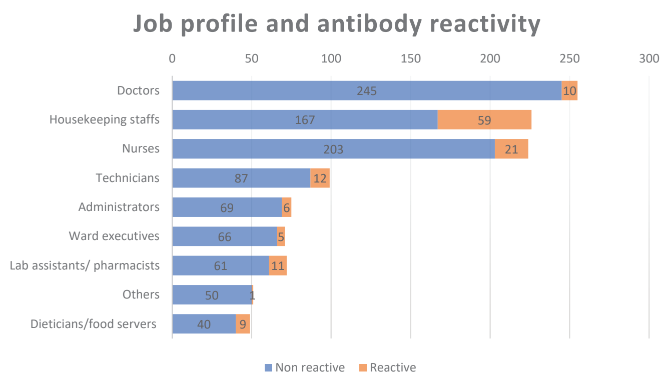
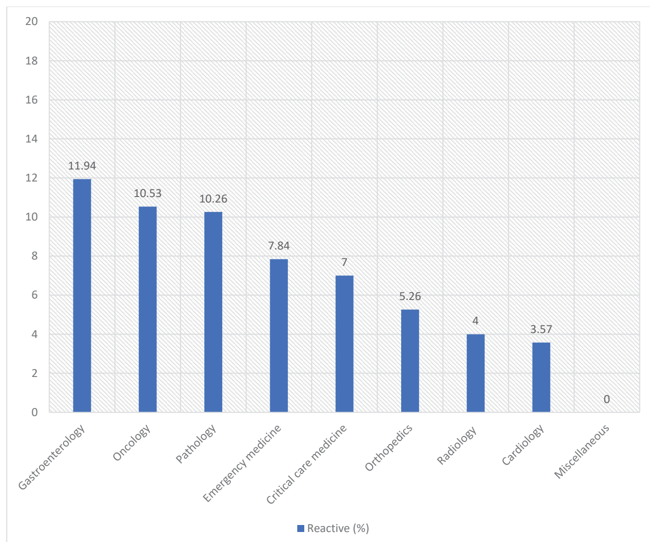


Fig. 3: Distribution of antibody reactivity among the health care workers based on their job profile. The seroprevalence rate was highest among housekeeping staffs (26.11%) followed by dieticians/food and beverage staff (18.37%), lab assistants/pharmacists (15.28%), nurses (9.38%), administrators (8%), ward executives (7.04%) and doctors (3.92%)



Miscellaneous departments that had zero prevalence include ambulance and transport, anaesthesia, medicine, nephrology, neurosciences, obstetrics and gynaecology, paediatrics, physiotherapy, pulmonology and surgery

Fig. 4: Distribution of antibody prevalence among various clinical departments. The antibody prevalence was highest in the gastroenterology department (11.94%), followed by oncology (10.53%), pathology (10.26%), emergency medicine (7.84%), critical care medicine (7%), orthopaedics (5.26%), radiology (4%) and cardiology (3.57%)

Test for Independence of Attributes/ Fisher's Exact Test as appropriate. We also used Univariate and Multivariate logistic regression analysis. In all cases, statistical significance was set at a p-value of less than 0.05.

Results

Clinical profile

A total of 1122 HCW (approximately 25% of the employees), categorized into three categories based on their risk assessment were recruited for this study. Figure 2 shows the study flow chart with distribution in the three categories and reasons for exclusion. A majority of participants belonged to 30-50 year age group (n = 665, 59.26%), with a male preponderance (n = 734, 65.42%). The clinical profile of these HCW is shown in Table 1. Figure 3 shows the distribution of participants in terms of their job profile. Doctors (n = 255, 22.72%) formed the most common group, followed by housekeeping (n = 226, 20.14%), nurses (n = 224, 19.96%), technicians (n = 99, 8.82%), administrators (n = 75, 6.68%) lab assistants/pharmacists (n = 72, 6.41%), ward executives (n = 71, 6.33%) and dieticians/food and beverage staff (n = 49, 4.37%).

Antibody prevalence

1. Overall and as per risk assessment:

Table 2: HCW category according to the risk assessment and their seropositivity rate

Category	Total, n (%)	Reactive
A	136 (12·12%)	27 (19·85%)
B	911 (81·19%)	101 (11·09%)
C	75 (6·68%)	6 (8·00%)
Total	**1122 (100%)**	**134 (11·94%)**

Category A: High risk; Category B: Intermediate risk; Category C: Low risk (details in text)

Of the 1122 HCW evaluated in our study, 134 tested positive for IgG antibodies, giving a seroprevalence rate of 11.94% (Table 2). While most (n = 803 , 71.6%) of these individuals were asymptomatic in the past three months, 28.4% (n = 319) had mild or non-specific symptoms including headache, runny nose, and generalized body ache.

The seropositivity rate was significantly higher in category A (n = 27, 19.85%) in comparison to category B (n = 101, 11.09%) and C (n = 56, 8.00%) with a *P* value of 0.007 (Table 2). On comparing the individual categories with one another, the positivity rate was significantly lower among category C compared to category A (p = 0.023), while the rate of B vs C (p = 0.491) and A vs B (p = 0.106) were not statistically significant.

2. Prevalence according to the department: As shown in figure 4, among the various medical departments of the hospital, the antibody prevalence was highest in the gastroenterology department (11.94%), followed by oncology (10.53%), pathology (10.26%), emergency medicine (7.84%), critical care medicine (7%), orthopaedics (5.26%), radiology (4%) and cardiology (3.57%).

3. Prevalence as per demography and medical history: Table 1 shows the clinical profile, demography, and medical history of the study population and the related seroprevalence rate. Male HCW had a significantly higher prevalence rate as compared to females (13.76% vs. 8.51) and the younger population had a higher prevalence compared to those above 50 years. There was no statistically significant difference in relation to diet (vegetarian vs non-vegetarian) or blood group (A vs non-A or Rh factor positive vs negative). Regarding the job profile, the seroprevalence rate was highest among housekeeping staffs (26.11%) followed by dieticians/food and beverage staff (18.37%), lab assistants/pharmacists (15.28%), nurses (9.38%), administrators (8%), ward executives (7.04%) and doctors (3.92%). Mode of transport to the hospital or time spent in the hospital did not influence seropositivity (p = 0.094 and 0.201 respectively). HCW staying in the metropolis had a lower prevalence in comparison to those staying in suburbs i.e. traveling from down-town (9.18% vs. 14.17%). Those staying in a crowded residence (>5 inhabitants/room) had a higher prevalence rate (24.18%).

HCW with a history of BCG (Bacille Calmette-Guerin) vaccination in childhood had a lower seroprevalence rate than those without (7.31% vs 16.8%, p = 0.004). The positivity rate was significantly lower with adequate (>6 weeks) hydroxychloroquine (HCQ) prophylaxis (1.30%,) in comparison to inadequate or no prophylaxis (11.25% and 12.99% respectively, p = 0.009). Diabetes mellitus did not influence IgG antibody positivity, while patients with cardiac ailments (hypertension, ischemic heart disease, and others) had a lower seropositivity rate (4.67%) as compared to those without any co-morbidity (13.06%).

Table 3 shows the univariate and multivariate analysis of demography and relavent medical history. As shown, male gender, younger age, job profile (housekeeping, food and beverage staff, lab assistant/pharmacist, technician, and nurses), staying in downtown, crowded inhabitation, administration of BCG vaccine were significant influencing parameters during univariate analysis. On multivariate analysis, however, job profile (housekeeping, food and beverage staff), crowded inhabitation, history of receiving BCG vaccination, and adequate HCQ prophylaxis were significant.

Discussion

The seroprevalence in HCW in the concerned tertiary-care hospital was at 11.94%. Similar studies from other countries show a rate of as low as 0% in Malaysia,[9] 4% in Denmark,[14] 1.06% - 13.7% in the United States of America,[15,16] 6.4% in Belgium,[17] 9.3% in Spain,[10] 10.6% in the United Kingdom (UK)[8,12] to as high as 17.14% in China.[18,19] This difference may be related to the period of study, the prevalence in the local community, and hospital policy in terms of triage, social distancing, hand sanitization, use of PPE. It is worthwhile to mention that the tertiary care hospital in this study is a Joint Commission Accreditation approved facility with proper workforce education, patient triage, and strict PPE usage policy.

It is critical for any seroprevalence study to use an optimal test, both in terms of the nature of the antibody (IgG, IgM, or both) as well as the test technique. Iversen et. al and Pallett et. al, both have used a point of care testing for antibody, which has a lower sensitivity of 82.5%.[8,15] IgM antibody tests have lower sensitivity and specificity, shorter duration, and heterogeneity in results.[9] We have therefore used the IgG antibody for our seroprevalence study. The antibody tests can target the Spike-protein S1 antigen, Spike-protein S2 antigen, nucleocapsid antigen, or a combination. The assay which we used in this study was Vitros anti-SARS-COV-2 IgG, which targets the S1 spike protein.[20,21] As compared to other coronaviruses, S1 protein is more specific and unique to COVID-19.[22,23] In SARS-CoV-2 infection S1 is, therefore, more specific than S2 or nucleocapsid (N) protein.[24] Woon et al have used the test to detect the IgG antibody against nucleocapsid.[9] The test kit used in the present study has a sensitivity of more than 90% and specificity of nearly 100%. Lin et al reported the superiority of chemiluminescence-immunoassay over the ELISA method.[25]

During our study, 207 out of 4656 (4.44%) HCW from the hospital were diagnosed to have COVID by a PCR positive test just before or during our study period. This would mean an estimated 16.38% of HCW in the hospital had evidence of an active or recent COVID-19 infection. These figures are similar to study from Spain (11.2%) and the UK (18%).[8,10]

In univariate analysis of our study, males and younger HCW had a higher seroprevalence rate. Brant-Zawadzki et al also noted a lower mean age in seropositive HCW compared to antibody-negative HCW.[14] Iversen et al noted seropositivity to be higher in male HCW compared to females.[14] In contrast, Basteiro et al found no difference between the two genders.[10] Our multivariate analysis showed no significant difference in age and gender.

Table 3: Univariate and multivariate analysis of demography and medical history of study group

Variable		Univariate analysis				Multivariate analysis			
		OR	95% Confidence interval		p value	OR	95% Confidence interval		p value
			Lower bound	Upper bound			Lower bound	Upper bound	
Category	A	2·85	1·12	7.25	·03	·93	·13	6·79	·94
	B	1·43	·61	3.39	·41	·75	·11	5·15	·77
	C								
Gender	Female	·58	·39	.88	·01	·62	·34	1·11	·11
	Male								
Age	<30 years	4·02	1·22	13.26	·02	1·84	·51	6·62	·35
	30-50 years	4·58	1·42	14·77	·01	2·55	·75	8·67	·14
	>50 years								
Rh factor	Rh negative	1·04	·36	2·99	·94	·97	·32	2·95	·96
	Rh positive								
Job profile	Administrator	2·13	·75	6·07	·16	1·66	·55	5·01	·37
	Dieticians/ FB	5·51	2·11	14·41	<·001	3·05	1·01	9·24	·04
	Housekeeping staff	8·66	4·31	17·41	<·001	4·90	2·04	11·74	<·001
	Lab assistant/ pharmacist	4·49	1·79	10·88	·01	1·83	·61	5·51	·28
	Nurse	2·53	1·17	5·50	·02	1·61	·61	4·25	·33
	Technician	3·38	1·41	8·09	·01	2·27	·84	6·11	·11
	Ward executives	1·86	·61	5·62	·27	1·45	·44	4·74	·54
	Others	·49	·06	3·91	·50	·35	·04	3·01	·34
	Doctor								
Mode of transport	Walking	1·74	·97	3·13	·07	1·27	·66	2·45	·48
	Personal vehicle	·70	·47	1·05	·08	·81	·51	1·30	·39
	Public transport								
Number of persons in room	1-2	·32	·18	·55	<·001	·40	·20	·81	·01
	3-5	·48	·28	·84	·01	·45	·23	·88	·02
	>5								
Time spent in hospital in a week	> 48 hours	·52	·34	·80	<·001	·97	·54	1·73	·91
	≤ 48 hours								
Place of residence	Metropolitan	·61	·42	·89	·01	·77	·51	1·16	·21
	Suburbs								
BCG vaccine	Received	·39	·20	·76	·01	·37	·18	·74	·01
	Not Received								
MMR vaccine	Received	1·05	·60	1·84	·86	1·13	·64	2·01	·67
	Not received								
HCQ prophylaxis	Not received	11·35	1·56	82·42	·02	8·85	1·21	64·87	·032
	Inadequate*	9·63	1·26	73·56	·03	9·50	1·23	73·07	·03
	Adequate[$]								
Comorbidities	Diabetes	·68	·29	1·60	·38	·67	·28	1·61	·37
	Hypertension / CAD	·33	·13	·82	·02	·38	·15	·96	·04
	Lung disease	·71	·21	2·38	·58	·97	·27	3·15	·90
	None								

Abbreviations: FB- food and beverage, BCG-Bacille Calmette-Guerin, MMR-Measles Mumps Rubella, HCQ-Hydroxychloroquin , CAD- coronary artery disease. *Inadequate dose was defined as 400 mg once a week for <6 weeks. [$]Adequate dose was defined as 400 mg once a week for >6 weeks.

Our lack of significant difference in seroprevalence rate for HCW performing duty in the COVID unit and non-COVID unit could be related to the strict use of personal protective equipment by individuals entering the COVID units. Moreover, among non-administrative staff, the housekeeping, food and beverage staff, lab assistants/ pharmacists and technicians, had a higher rate of seroprevalence, while doctors (3.92%) and nurses (9.38%) had a lower rate. One explanation could be that those with higher rates were moving in and out of different hospital areas, whereas nurses and doctors were working in the well-defined designated location. Higher awareness and better implementation of hospital protocols could also be responsible for a lower rate among doctors and nurses. Among various medical departments gastroenterology (11.94%), oncology (10.53%), pathology (10.26%) and emergency services (7.84%) had relatively higher seroprevalence. This could be attributed to exposure to aerosol-generating procedures (gastroenterology), handling immunosuppressed patients who carry silent infections (oncology), lab sample handling (pathology), or exposure to a mixed patient population during triaging (emergency).[26] However, due to the lack of available data on such a correlation, contrasting findings, like a Spanish study which did not find any relation between working in COVID unit or professional category with seropositivity, become difficult to explain.[10] Iversen et al, however, did notice higher rate among medical students and lower rate in laboratory personnel.[14] We noted significantly higher antibody positivity in those staying in suburbs (compared to those in the metropolis) and with crowded housing. This may be due to the lower socioeconomic status of these individuals.

An interesting finding in the present study was a significantly lower prevalence in HCW who had received BCG vaccination in childhood and also in those receiving adequate HCQ prophylaxis in the recent past. While the protective role of BCG vaccination and HCQ in the occurrence of COVID-19 is still debatable, there is literature supporting the role of both these interventions in either disease prevention or progression.[27-30] Sharma et al have reported that the rate of confirmed cases and mortality is lower and recovery rate is higher in those countries who have BCG vaccination in their universal health program, in comparison to countries where BCG vaccination is not implemented.[28]

Strengths and Limitations

The strength of the present study includes a well-defined cohort and the inclusion of only asymptomatic or mildly symptomatic individuals. The hospital HCW were classified into three categories with an intent to have a realistic and representative sample for analysis. Also, a standardized sensitive, and specific immunoassay was used. The authors also plan to follow up and retest the cohort with a positive antibody to understand the dynamics and durability of these antibodies. Limitations of the study include a moderate sample size for any seroprevalence study. However, 33% of the total HCW were enrolled and ultimately 25% of the total eligible hospital staff could be included, which is fairly representative. The study period was 6 weeks which is too long for evaluating a dynamically changing pandemic. However, this could not be avoided due to frequent lockdown in the geographical area during the study. Also, a clear seroprevalence data from the general community to compare with was not available. Such comparison would have helped us to study the difference, if any, between HCW and the general population.[15]

Conclusions

This study gives a fair idea about the existing seroprevalence among HCW of similar large hospitals in the country. Obtaining data about seroprevalence and subsequent follow up evaluation of durability and protective nature of this antibody may help hospitals to triage the staff at risk, rationalize their placement, prioritize the use of PPE and potentially reduce the risk of transmission.

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