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Original Article

Public Health Section

Serosurveillance of SARS-CoV-2 among the Healthcare Workers of a Tertiary Care Teaching Institution during the Post Lockdown Phase in Central Kerala, India

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ABSTRACT

Introduction: The hallmark of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection is high infectivity, pre symptomatic transmission and asymptomatic prevalence which could result in high cumulative numbers of infections, hospitalisations, and deaths. Kerala was the first state to confirm community transmission in July 2020. Healthcare Workers (HCWs) being in the forefront in the war against Coronavirus Disease-2019 (COVID-19) are more prone to acquire the infection and could possibly be asymptomatic sources for cluster formation. Knowing the development of immunity as shown by the presence of anti COV-2 antibodies in the population contributes to the epidemiological understanding of the disease.

Aim: To determine the pattern of seropositivity of SARS-CoV-2 among the HCWs at Jubilee Mission Medical College and Research Institute, Thrissur, Kerala, India, six months after revoking the lockdown.

Materials and Methods: This cross-sectional study was carried out among 423 HCWs of the medical college from September 5th to December 15th, 2020. Multistage sampling was done with the hospital block as the first stage and departments as the second stage. Blood sample was collected and Anti SARS COV-2 IgG antibody testing which targets the Spike Protein 1 (SP1) was done using the vitros chemiluminescence platform (Orthoclinical diagnostics, USA). For the summary of demographic characteristics, continuous variables were summarised as mean values and Standard Deviation (SD) while categorical variables were summarised as proportions. The χ^2 test was used for comparing the epidemiological features between positive and negative cases. Chi-square test for trend analysis was done for exploring the relationship of the degree of severity with test positivity. All analyses were conducted using Statistical Package for the Social Sciences (SPSS) version 25.0.

Results: Jubilee Mission Medical College had 2785 working staff at the time of study. A total of 423 staff consented and their samples were tested. Thirty seven staff members tested positive for COVID-19 antibody, yielding an overall prevalence of 8.75% (95% CI, 6.23-11.86). A 86.5% (32/37) of them were having a history of COVID-19 Antigen/Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) Positivity. A statistically significant linear trend (p-value=0.00001) was observed, between seropositivity and the degree of severity of COVID-19. Among the various factors which increase the risk of seroconversion, history of undergoing quarantine (p-value <0.001), contact with a confirmed case (p-value=0.002), contact with a caregiver for COVID-19 (p-value=0.001) and history of upper respiratory symptoms (p-value=0.001), were found to be significantly associated with positive serology.

Conclusion: The pattern of seropositivity across the different category of HCWs observed in the present study showed a higher prevalence among nurses. Being an educational institution, it was obligatory to train all the elements of care delivery to the future generation of HCWs. Acquiring experience from a small but relevant sample was expected to facilitate larger community study envisaged in peripheral areas Jubilee Mission Hospital served.

Keywords: Coronavirus disease 2019, Pandemic, Seropositivity, Spike protein

INTRODUCTION

The hallmark of SARS-CoV-2 pandemic is high infectivity, pre-symptomatic transmission and asymptomatic prevalence which results in high cumulative numbers of infections, hospitalisations and deaths [1]. In India, Kerala was the first state affected by COVID-19, and Thrissur district had a good number of hospital admissions in the post lockdown phase [2]. By early March, the state had the highest number of active cases in India mainly due to huge number of cases imported from other countries and states. Using the five components of trace, quarantine, test, isolate and treat, by 10 June 2020, Kerala managed to keep the basic reproduction number (Ro) at 0.454 against the India and world averages from 1.225 and 3.4 [3].

The state of Kerala had witnessed a long lag phase after the first reported case of COVID-19 on Jan 30 [4] till the phase of community spread and had managed to delay the peak of the pandemic by successful implementation of various control measures. In each phase starting from the reporting of the first case in India, this hospital along with other hospitals in the state had taken appropriate control measures to fight against the pandemic [5]. It was particularly important to have knowledge about the effectiveness of the steps taken, in planning the future strategies for disease control. Silent infection was a matter of major concern. It was also important to know if HCWs could act as a source of infection to patients during the pandemic, especially when hospitals serve other patients too. Being a novel

virus, little was known about various aspects of the virus; the factors contributing to its spread, the progression of the disease, development of immunity etc. Knowing the development of immunity as shown by the presence of antibodies in the population contributes to the epidemiological understanding of the disease. Studies on seroconversion rates in a population helps to find out the exposure to the virus in that population, be it symptomatic or asymptomatic [6]. World Health Organisation (WHO) continues to review the evidence on antibody responses to SARS-CoV-2 infection [7]. Seroprevalence among various categories of tested individuals reported from different parts of India ranged from 0.73 to 19.8 [8-13]. So far, only little was known about seroprevalence in Kerala. Seroprevalence studies conducted by the Indian Council of Medical Research (ICMR) during the first two phases showed a gradual rise from 0.33% in May to 0.8% in August 2020 [14]. Hence, the study was planned to find out the seropositivity among HCW for COVID-19, during the rising graph of pandemic incidence in this part of Kerala and to compare with reports from elsewhere in the country.

MATERIALS AND METHODS

This study was designed as a cross-sectional study. Testing of samples was done from 20th December to 30th December. Analysis was completed by 15th January 2021. The Institutional Review Board approval was obtained for this study (Ref. No: 33/20/IEC/JMMC&RI). The study was conducted among the HCWs at Jubilee Mission Medical College and Research institute, Thrissur, a 1600 bedded teaching hospital with around 3000 staff in the regular pay rolls, daily wagers, and workers of service contractors. All categories of staff working in the hospital and medical college comprised the study population. A rising graph of incidence was operationally defined as a period up to daily incidence in the state above 10000 and or daily COVID-19 in patient strength in our hospital as 100 or above.

Sample size calculation: Sample size was calculated by the formula $n=(Z\alpha)\ 2\times p\times q/d^2$. $Z\alpha$ is the Z value at α error of 0.05. i.e., 1.96 for a 95% confidence interval p, 23%, is the proportion of subjects with positive SARS-CoV-2 antibodies according to a study done by Percivalle E et al., during the peak of epidemic in Italy [6]. The q is 100-p; d is clinically allowable error which was taken as 20% of prevalence. The minimum sample size required was calculated to be 320.

Sampling Technique

Multistage sampling was done with the hospital block as the first stage and departments as the second stage. In the final stage of sampling the test individuals were selected on a first come first serve basis, after the antibody test availability was declared open and free for all staff. A consent form and a google form were given to all staff who volunteered to participate in the study. Research staff helped the volunteers to detail the consent form and in filling up of the proforma in google form. Each consented participant recruited into the investigation completed a questionnaire which covers demographic information, exposure history, residence details (containment zone or not), travel and details of family exposure.

Sample Collection

After obtaining informed consent, 3 mL of blood was collected in Ethylene Diamine Tetraacetic Acid (EDTA) vacutainers, centrifuged and the plasma was separated. The plasma was subjected to antibody testing by Chemiluminescence Immunoassay (CLIA). The kit used was manufactured by Ortho clinical diagnostics (USA). The kit was used in the vitros equipment. The blood samples were tested for the presence of Anti SARS-CoV-2 IgG antibody. Testing Time period: The process of recruitment and sampling started on 5th September 2020 and ended by 15th December 2020.

Testing Methodologies

Chemiluminescence technology was used for antibody testing. These 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. As compared to other coronaviruses, S1 protein is more specific and unique to COVID-19. The test kit used in the present study has a sensitivity of more than 90% and specificity of nearly 100%. Lin D et al., reported the superiority of CLIA over the Enzyme-Linked Immunosorbent Assay (ELISA) [15].

The details and results of the tests done were recorded. For those HCWs who tested positive in antibody testing, their details were shared with the institutional medical board and further necessary action if found was offered as per Kerala government guidelines and institutional policy [16]. Antibody positive status among tested samples were calculated and considered as seropositivity. It was calculated overall for all HCWs and separately for different categories of them.

STATISTICAL ANALYSIS

For the summary of demographic characteristics, continuous variables were summarised as mean values and SD while categorical variables were summarised as proportions. The χ^2 test was used for comparing the epidemiological features between positive and negative cases. Chi-square for trend analysis was done for exploring the relationship of the degree of severity with test positivity. All analyses were conducted using SPSS version 25.0.

RESULTS

Jubilee Mission Medical College and Research Institute had 2785 working staff at the time of study. A total of 423 staff [Table/Fig-1] consented and their samples were tested. This included 77 Doctors, 104 nursing staff, 85 technicians, 31 administrative/ministerial staff and 126 support staff. During the study period, 37 staff members tested positive for COVID-19 antibody, yielding an overall prevalence of 8.75% (95% CI, 6.23-11.86). Among them, 86.5% (32/37) of them were having a history of COVID-19 Antigen/RT PCR positivity.

Category	Working	Tested	Positive	Seropositivity
Doctors	486	77	5	6.5
Nurses	978	104	12	11.5
Technicians	154	85	6	7.1
Administrative/Ministerial	48	31	0	0.0
Support	1119	126	14	11.11
All	2785	423	37	8.75

[Table/Fig-1]: Test and result status of Anti SARS-COV2 antibody among HCWs.

The mean age of positive and negative employees was 35.35 and 34.46 years, respectively [Table/Fig-2]. Employees who were females comprised a greater proportion of study subjects and there was no statistically significant difference in the seroprevalence (p=0.327).

Attribute		SARS-CoV-2 antibody		p-value
		Positive (n=37)	Negative (n=386)	
Mean age (years)		35.35±9.69	34.46±10.59	0.624
Gender	Male	8 (21.6%)	120 (31.08%)	0.327
	Female	29 (78.4%)	266 (68.91%)	

[Table/Fig-2]: Anti SARS-COV2 antibody in relation to age and gender. Independent samples t test and chi-square test

The various risks for seroconversion were analysed. Among the various factors which increase the risk of seroconversion, history of undergoing quarantine (p-value <0.001), contact with a confirmed case (p-value=0.002), contact with a caregiver for COVID-19 (p-value=0.001) and history of upper respiratory symptoms of COVID-19 (p-value=0.001), were found to be significantly associated with positive serology [Table/Fig-3].

S. No.	Attribute		SARS-CoV-2 antibody		p-value
			Positive (n=37)	Negative (n=386)	
1	History of symptoms suggestive of COVID-19 in the past three months	Yes	26	91	0.001*
		No	11	295	
2	History of contact with a confirmed case/quarantined individual	Yes	24	147	0.002*
		No	13	239	
3	History of travel outside Kerala since COVID-19 outbreak	Yes	1	9	0.997
		No	36	377	
4	History of being in quarantine	Yes	27	85	<0.001*
		No	10	301	
5	History of taking COVID-19 testing (RT-PCR/Antigen)	Yes	30	160	<0.001*
		No	7	226	
6	Adherence to PPE protocols of HICC	Yes	35	370	0.715
		No	2	16	
7	History of contact with a caregiver for COVID-19 patients	Yes	24	142	0.001*
		No	13	244	

[Table/Fig-3]: Chance of exposure and SARS-CoV-2 antibody status.
PPE: Personal protective equipment; HICC: Hospital infection control committee; Chi-square test

The degree of severity of symptoms and the antibody responses were also analysed. A statistically significant linear trend was observed (p-value=0.00001), between seropositivity and the degree of severity of symptoms of COVID-19 [Table/Fig-4].

Severity of symptoms in past three months	SARS-CoV-2 antibody status		Odds ratio
	Positive (n=37)	Negative (n=386)	
Asymptomatic	9 (24.3%)	287 (74.4%)	1
Mild upper respiratory symptoms	8 (21.6%)	69 (17.9%)	3.22
Moderate symptoms	17 (45.9%)	29 (7.5%)	18.62
Severe	3 (8.1%)	1 (0.3%)	95.33

[Table/Fig-4]: Severity of symptoms and SARS-CoV-2 antibody status.
Chi-square for trend. p-value=0.00001

DISCUSSION

The initial "Kerala model" of response to the COVID-19 pandemic was to trace, contact and quarantine or test, isolate and treat all travelers from foreign countries as well as neighboring states, coupled with lockdown and break the chain measures. This practice had made the state succeed in preventing community spread in the initial months of the pandemic.

With unlocking according to national policy and easing of restrictions, the community transmission was inevitable. Kerala was the first state in the country to declare community transmission in Trivandrum district among the coastal region [17]. There is always a high risk of transmission to HCWs from presymptomatic and asymptomatic patients reporting with non COVID-19 illness, especially in non COVID-19 hospitals. Identifying infected HCWs, including asymptomatic ones, is important to reduce nosocomial spread [18].

Initially, a stratified random sampling was decided as the sampling technique. Risk groups were considered as the strata of the study. Hospital employees were stratified into four risk groups namely high risk, moderate risk, low risk and very low risk based on the likelihood of exposure to a suspected COVID-19 patient, frequency and duration of exposure and the nature of interventions being performed. WINPEPI was used to select the target HCWs. It was planned to study HCWs during the rising curve, plateau, falling, and post fall steady state of incidence graph. However, 'no direct benefit to the tested individual' was raised and honoring the voice of dissent, it was decided to withhold target individual selection and make it an open invitation to all HCWs for volunteering. The sample gathered were 423, one hundred more than initial target. This reflects the enthusiasm of the HCWs in volunteering for a study which can have only collective benefit.

The overall seropositivity in the current study was found to be 8.75%. This estimate was comparable to that of seroprevalence studies conducted among general population in the United States and Wuhan in China which have reported a seroprevalence of 6.9% and 3.8% respectively [19,20]. The prevalence of antibodies largely depends on the stage of the epidemic in the area at the time of the study. The findings of the present study contrast with the results of the population based studies done in India. The nationwide study done in India in the early phase of the pandemic by the Indian Council of Medical Research (ICMR) revealed a seroprevalence of 0.73% while the seroprevalence study conducted across Delhi showed the prevalence of IgG antibodies to be 23.48% [21,22]. Studies done in similar settings among HCWs in Italy and India reported a seroprevalence of 14.4% and 11.94%, respectively [21,22]. The variability in prevalence in a large country with multiple local communities having varying health status, demographic profile and ecology is understandable. The containment strategies adopted by the local governments had to follow the national guidelines, but the efficiency of it varied. This would also explain the variation.

The pattern of seropositivity across the different category of HCWs observed in the present study showed a higher prevalence among nurses. This result agrees with a recently published report from United States [22]. These findings imply a higher occupational risk for SARS-CoV-2 infection among nurses. The seropositivity was lowest among those working in non clinical environments without patient contact. History of upper respiratory symptoms in the past three months and a history of undergoing quarantine were significantly associated with a higher probability of SARS-CoV-2 specific antibody positivity. This result was found to be consistent with a similar report from a hospital in North India [23].

A significant linear association was found with COVID-19 antibody positivity and severity of disease with the likelihood of being seropositive being highest for those with severe respiratory tract infections. During the initial phase of the pandemic, strategies that aimed to increase herd immunity by exposing young low risk individuals to the virus were under consideration [24,25]. But early evidence suggested that acquired immunity may be short-lived in individuals with mild or asymptomatic infections [25]. But newer evidence confirmed that a stronger antibody response was associated with disease severity [26].

Seropositivity among the staff of the present institution showed a comparatively low figure. Even after a period of 10 months after the first case detection in the district and six months after first case detection in this hospital, the development of immunity against COVID-19 remaining low is a matter of concern. Only when herd immunity develops or enough people are vaccinated, the pandemic of viral disease will get controlled and eradicated.

Right from the beginning of this pandemic and since reporting of the first COVID-19 case from India our hospital had adopted strict infection control measures. Various measures were adopted by our institute to contain the spread. Classroom teaching for MBBS

and nursing students were suspended and shifted to online mode, though with certain concerns [27,28]. A multi disciplinary institutional medical board was constituted to take necessary actions regarding COVID-19 positive patients and infection control among staff. The hospital management under the guidance of the Hospital Infection Control Committee (HICC) provided necessary Personal Protective Equipment (PPE) for the staff as per the exposure risk and working environment and organised training through online mode on infection control measures [29]. COVID-19 testing for all in patients and their bystanders were also made mandatory.

Staff surveillance team was assigned to assess exposure and quarantine of staff and their contacts. In-house testing facility for COVID-19 PCR, antigen and antibody testing were started. Authors assessed and facilitated willingness and emotional preparedness of staff in managing the cases well before the first admission [30]. The audit conducted after all preparations showed a fairly good level of practice. All these have reduced the chance of exposure to virus during duty or outside and hence, the antibody development in them was low. By knowing the immunity status of HCWs, the institution would be able to contribute authentically to the development of intervention strategies and guidelines from time to time [31-33].

Limitation(s)

This study has several limitations. It included only a relatively small proportion of HCWs from a single teaching institution and cannot be considered as representative of the general population. The randomness was not ensured in the last stage of sampling, and it was not possible to accurately elicit the history of mild symptoms which may have resulted in overestimation of asymptomatic cases.

CONCLUSION(S)

Keeping track of the pattern of development of immunity in the community is part of understanding the illness and forecasting the spread. For the tested HCWs, it would boost their morale by ending uncertainty. For the hospital administration it would help in decision making about relative focusing of interventions on patients in general and HCWs. By knowing the immunity status of HCWs, the institution would be able to contribute authentically to the development of intervention strategies and guidelines from time to time, besides following the available guidelines. This is a responsibility of a leading academic institution with serving experts, whose expertise will remain dormant otherwise.

Being an educational institution, it is obligatory to train all the elements of care delivery to the future generation of HCWs. It includes how to observe, confirm observations, identify the mechanisms behind it and develop intervention strategies with rationale. Understanding the development of personal and herd immunity to a virus infection is hence inevitable. Getting experienced from a small but relevant sample was expected to facilitate larger community study envisaged in peripheral areas Jubilee served.

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