



“SEROPREVALENCE OF SARS-CoV-2 ANTIBODIES AMONG HEALTHCARE WORKERS: A SINGLE CENTRE STUDY IN A TERTIARY CARE HOSPITAL”

Microbiology

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ABSTRACT

Introduction: Study of antibody production against SARS-CoV-2 in Health care workers (HCWs) either after infection or vaccination has been a subject of debate and holds an important correspondence with the severity of the disease. This study was done to help provide a clearer perspective on the impact of this virus on HCWs, who are the front line workers against this infection. **Methods:** A total of 934 samples were collected and processed for detection of IgG to SARS-CoV-2 by Enzyme Linked Immuno-Sorbent Assay (ELISA) using ICMR (Indian Council of Medical Research) approved COVID Kawach IgG Microlisa kit (J. Mitra & Co. Pvt. Ltd). **Results:** This study detected 595 positive cases of antibody production with a prevalence rate of 63.7% irrespective of their history of previous laboratory confirmed infection. **Conclusion:** This study concludes the need of vaccination and booster dose. It also provides better understanding of the pandemic and paves the way for a larger population based serosurveillance study.

KEYWORDS

SARS-COV-2, antibodies, healthcare workers, seroprevalence

INTRODUCTION

COVID-19, first identified in December 2019 in Wuhan, China was declared an emergency of international concern in January 2020 and a pandemic in March 2020. Having effectively managed the first two waves of the pandemic, India sought to achieve utmost preparedness to combat the next wave. In order to achieve this, the country began its vaccine drive against COVID-19 in January 16, 2021 and today, it is considered the largest vaccinator in the world with the highest number of people having been completed the vaccination schedule.[1]

Health-care workers (HCWs) stand as the front line workers against COVID-19 pandemic exposing themselves to high risk of infection. The prevalence of SARS-CoV-2 infection among HCWs is about 11% in spite of using personal protective equipment (PPE) and 14.6% of HCWs are affected by breakthrough infection even after receiving two doses of COVID-19 vaccination.[2,3] This puts healthcare system under a tough situation augmenting the already existing low manpower and risk of easy transmission to their families.

Antibody production to infection or vaccination begins after 7-14 days and persists for several months.[4] Antibodies are directed against the Critical neutralizing domain (CND) present in the Receptor Binding Domain (RBD) of the Spike protein and nucleocapsid protein of the SARS-CoV-2 virus. Antibody detection helps in evaluating the epidemiology and burden of SARS-CoV-2 infection and also helps in gauging the effectiveness of infection control measures including vaccination.[5]

This study aims at measuring the seroprevalence of SARS-CoV-2 antibodies in various categories of HCWs and allied staffs working in a teaching tertiary care hospital in Imphal, Manipur.

MATERIALS AND METHODS

It is a single centre, cross sectional study which included a total of 934 HCWs working in various departments of Regional Institute of Medical Sciences, Imphal, Manipur, India for a duration of 6 months (July to December 2021). Whole blood samples were collected by a phlebotomist by venepuncture under strict standard precautions and samples were processed in the department of Microbiology. Sera were separated from the samples and stored at -20°C until processing.

Detection of IgG to SARS-CoV-2 was done by Enzyme Linked Immuno-Sorbent Assay (ELISA) using ICMR (Indian Council of Medical Research) approved COVID Kawach IgG Microlisa kit (J. Mitra & Co. Pvt. Ltd). IgG antibodies from serum binds to the SARS CoV-2 virus whole cell antigen coated onto the microtitre plate (ELISA wells). In the next step, anti human IgG horseradish peroxidase, HRP binds to captured human IgG antibodies. Following which, chromogenic substrate (TMB/H2O2) was added and the reaction was stopped by 1N H₂SO₄. The intensity of colour/optical

density was measured at 450nm. Samples were tested according to manufacturer's guidelines using positive and negative quality controls provided in the test kit. All testing procedures were done using appropriate personal protective equipments following standard precautions.

Interpretation of the test: A cut off value of the test run and a P/N ratio of positive control were first calculated. P/N ratio of positive control is defined as the ratio of O.D (Optical Density) value of positive control divided by O.D value of average O.D of the two negative controls. Similarly, P/N ratio of each test sample was calculated. The test results were given as positive or negative if:

Positive: If O.D value > cut-off value and P/N ratio is more than 1.5

Negative: If O.D value < cut-off value and P/N ratio is less than 1.5.

All testing and interpretation procedures were done under steady lighting and strict monitoring. The sensitivity and specificity of this kit as validated by ICMR, NIV, Pune are 96.33% and 100% respectively obtained after analysis of the performances of three batches of this kit.

Statistical Analysis: Statistical analysis of the variables were done using SPSS version 21. Association between the variables were done using Chi-square test and Fisher's exact test. P value of <0.05 was considered significant.

RESULTS

This study included 934 HCWs from various departments of the hospital of which 595 (63.7%) were detected to be seropositive for production of Ig G antibodies against SARS-CoV-2. Maximum number of samples were received from HCWs belonging to the age group (21-30) years while highest seropositivity was seen in the age group 41-50 years with a seropositivity rate of 67.4% as shown in table 1.

Table 1: Seropositivity Rates Among Different Age Groups

Age group (in years)	Total number of cases	Seropositive cases (prevalence rate)
21-30	297	192(64.6%)
31-40	279	177(63.4%)
41-50	239	161(67.4%)
51-60	110	62(56.4%)
61-70	9	3(33.3%)
Grand Total	934	595

Of the 934 HCWs, female HCWs contributed to a larger number of samples (Females=625, 66.9%, Males=309, 33.1%) and females were also found to have higher seropositivity rate (404/625, 64.6%) as compared to males (191/309, 61.8%) at a male:female positivity ratio of 1:2.

This study also analysed the seropositivity rates of the HCWs by the

different areas of their work space as shown in table 2. Highest number of the study population were doctors with a total of 347 samples, but highest seropositivity rate was detected among the allied HCWs (N=143 out of 193, 74.1%) which included technicians, multi task service providers, pharmacists and other such HCWs. Out of the 143 positive cases amongst this group of HCWs, 140 cases received both doses of vaccination while 3 cases received only a single dose of the vaccination. 25 cases out of 143 gave a positive history of laboratory confirmed COVID 19 infection.

This study also included 8 HCWs who did not receive any vaccination of which 3 was found positive. Interestingly, all 3 were doctors and 2 of them gave a previous history of laboratory confirmed COVID-19 infection.

Table 2: Seropositivity Rates Among Different HCWs According To Their Job Categories

Category of work	N	Seropositive cases (prevalence rate)	Two doses of vaccination taken	Single dose of vaccination taken	No vaccination	History of previous Covid 19 infection	No history of previous COVID 19 infection
Doctors	347	189 (54.5%)	181	5	3	31	158
Nurses	135	92 (68.1%)	86	6	0	8	83
Allied HCWs	193	143 (74.1%)	140	3	0	25	118
Administrative/ Clerical staffs	65	47 (72.3%)	47	0	0	4	42
pre and paraclinical staffs	194	124 (63.9%)	121	3	0	4	120
TOTAL	934	595	575	17	3	72	521

This study included HCWs who were vaccinated with 2 doses, single dose and a few cases with no vaccination. Table 3 shows a comparison in the prevalence rates of these HCWs along with their history of previous COVID 19 infection. HCWs who were vaccinated with both doses contributed to about 901 of the total number of cases of this study followed by 25 cases of single vaccinated cases and 8 cases without any vaccination. Irrespective of their history of previous laboratory confirmed infection, highest seropositivity rate (68%) was noted in HCWs who had taken only a single dose of the vaccination. If, however, previous history of laboratory confirmed infection was taken into account, then, maximum seropositivity was seen in HCWs who had received both doses of the vaccination (56.5%).

Table 3: Comparison Of Prevalence Rates

Total Doses Of Vaccination Given	Total number of cases	(N ₁)	Prevalence rate (P ₁)	History of previous COVID-19 infection	(N ₂)	Prevalence rate (P ₂)
2 doses	901	575	63.8%	66	509	56.5%
Single dose	25	17	68%	6	11	44%
None	8	3	37.5%	3	0	0

N₁= Number of positive cases

N₂= Number of positive cases after exclusion of those with previous laboratory confirmed history of infection

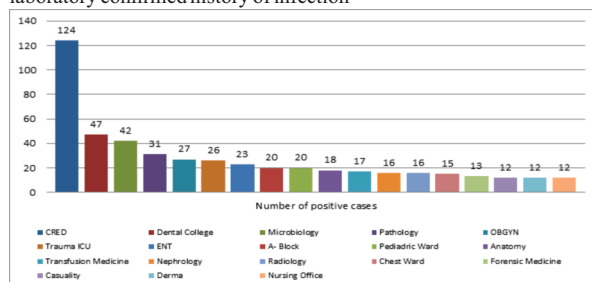


Figure 1: Departmental distribution of positive cases

An attempt was made to include as many samples as possible from all departments and sectors of this hospital. Maximum number of samples were received and also highest seropositivity was seen in CRED (Center for Research on Environmental Development) staffs who were working at the time from an outsource agency with a total of 124 cases and a seropositivity rate of 63.9%. Figure 1 shows the total number of positive cases from each department in descending order in this tertiary centre.

DISCUSSION

The nature and importance of antibody production against SARS-CoV-2 either after infection or vaccination, has been a subject of debate as it holds an important correspondence with the severity of the disease. Routine research studies and analysing seroprevalence in COVID -19 helps give a clear perspective on the impact of this virus over time.[6]

In January 2022, Taoufik B H et al conducted a study on the production of neutralizing antibodies directed against spike Ag of SARS- CoV-2 in fully vaccinated HCWs in Morocco and detected a high seropositivity rate of 97%.[7] Another study done in India by Manoj V et al in 2021 amongst the Indian population to detect the presence of IgG antibodies against S1-RBD and nucleocapsid protein of SARS-CoV-2 using chemiluminescence immunoassay also concluded a high positivity rate (85.2%) amongst its HCWs.[8] These positivity rates were higher than the findings of this study which included 934 HCWs, and detected a 63.7% positivity rate. However, this finding was higher when compared to findings of a similar study done in asymptomatic HCWs in India by Wattal C et al with a positivity rate of 25.8%.[9]

Seropositivity in females were found to be higher than males (64.6%), which was in concordance with other studies done by Shields A et al (females 26.3% > males 18.8%) and Self W H et al (females 58% > males 42%).[10,11] This result could be due to maximum number of samples collected from the female HCWs and does not necessarily prove an association of positivity in accordance with gender.

Out of the 5 categories of HCWs' classification, seropositivity was highest in allied group of HCWs (74.1%) which included pre and paraclinical staffs, pharmacists and technicians amongst others. This is in contrast with other similar studies performed by Ko JH et al and Lumley S F et al where nurses or health care assistants were found to have the highest seropositivity amongst HCWs.[12,13] This could be due to negligence or ignorance on the part of the staffs towards maintaining standard Covid appropriate behaviours such as proper usage of face masks and hand hygiene compared to other categories of HCWs. They are also exposed to the common public like asymptomatic SARS-CoV-2 infected patients who come to the hospital before being diagnosed or from asymptomatic patient attendants.

Higher seropositivity was seen in HCWs who had taken either one or two doses of vaccination irrespective of their history of laboratory confirmed infection. However, only 3 unvaccinated HCWs were found to be seropositive (N=3/8, 37.5%). Association between the seropositivity of the vaccinated and the unvaccinated is found to be insignificant. The insignificance can be due to the large difference in the number of vaccinated and unvaccinated HCWs. Two of the three unvaccinated HCWs also gave a previous history of SARS-CoV-2 infection that could have resulted in their seropositivity. One of the HCWs (50 years old, doctor) who had neither taken the vaccine nor had previous SARS-COV-2 infection was detected to be seropositive. This could be because the HCW may have been asymptomatic for SARS-CoV-2 infection and was not tested.

According to CDC, fully vaccinated individuals are six times less likely to get infection compared to unvaccinated individuals. Infection offers longer immunity than vaccination. In a study conducted by Israel et al on SARS-CoV-2 kinetics in fully vaccinated study population and convalescent patients, they witnessed early decay of antibody titres in vaccinated population (40% decrease in each subsequent month) compared to convalescent population (less than 5% per month).[14] Also, infection extends 80-93% protection and decreased risk of subsequent infection by SARS-CoV-2 for at least 6-9 months which is higher compared to the 84-96% provided by full vaccination for up to 6 months. Ig M for SARS-COV2 starts rising within first few weeks of onset of symptoms and persists for 2-3 months. But Ig G levels are detectable up to 12 months post infection. This is longer compared to immune response post vaccination where Ig

G levels wane in 6-9 months.[15] This finding is congruent with our study where HCWs who had previous history of COVID were found to have higher seropositivity than the uninfected demonstrating that infection with the virus gives longer immunity.

Protection against subsequent infection depends on the development of memory B cells which in turn depends on the cell mediated and T cell immunity of the individual. Acquiring antibodies from both vaccination and infection (hybrid immunity) is found to be more effective than with vaccination only. Researches done by Kojima N et al in 2021 and Ewen Callaway also in 2021 found that getting the first dose of COVID-19 vaccination in a previously infected individual is equal to getting the second dose of vaccination in an uninfected individual.[16,17]

Further, infection with the virus recognizes vast variants of corona virus by identifying diverse antigens of spike protein. This enables protection against various strains of the virus providing a broad spectrum of immunity. The memory B cells acquired after infection continues to multiply against various strains for over a year whereas, the growth and diversification stops in weeks after the second dose of vaccination. Research done by Ewen Callaway in 2021 claims that getting a third dose of vaccination can get the benefits of hybrid immunity in individuals with no previous history of infection.[17]

Limitation Of The Study: This study is a single centre study and could include only HCWs as the study population which makes it less generalizable to common population.

CONCLUSION

The results of this study strongly conclude the need of vaccination and booster dose although it offers shorter immunity than infection since the latter has high risk of causing morbidity and mortality. The need for strictly following infection control measures and maintaining Covid appropriate behaviours both in hospital and social gatherings is still desirable. This study provides better understanding of the pandemic and paves the way for a larger population based serosurveillance study which could help in assessing herd immunity.

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Conflicts Of Interest: There are no conflicts of interest

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