

A longitudinal study depicting persistence of COVID-19 antibodies after half a year using chemiluminescent microparticle immunoassay among healthcare workers and frontline workers in Mumbai, India's largest metropoli

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ABSTRACT

Introduction: Antibodies play a role in herd immunity, and studies estimate that COVID-19 antibody protection lasts approximately 1 to 4 months following COVID-19 vaccination and 6 to 8 months following infection. As healthcare workers and frontline workers were highly exposed to the infection, it is necessary to know if they have developed immunity against COVID-19. **Aim:** To estimate the seroprevalence of SARS-CoV-2 antibodies among health care workers and frontline workers in Mumbai qualitatively and quantitatively and observe the change in antibody levels after 6 months. **Material and Methods:** This longitudinal study was conducted over 9 months in two phases among 1544 healthcare workers (HCWs) and 1555 frontline workers (FLWs) aged above 18 years in Mumbai, Maharashtra, India. Participants' blood samples were analyzed using the SARS-CoV-2 IgG-II Quant assay for the qualitative and quantitative determination of IgG antibodies to SARS-CoV-2. A follow-up was done after 6 months. **Results:** A total of 2733 (88.2%) of the 3099 participants were followed up in Phase II, which included 1370 (50.1%) FLWs and 1363 (49.9%) HCWs. The study found a seropositivity rate of 99.9% with a significantly higher antibody titre in BEST (public transport) workers, those vaccinated with precautionary doses, those vaccinated with precautionary doses with a history of confirmed COVID-19 disease, and participants over 45 years. **Conclusions:** The study findings indicate a widespread seroconversion against COVID-19 and a beneficial effect of precautionary dose in addition to two doses of the COVID-19 vaccine. The role of subclinical infection needs to be explored.

Keywords: COVID-19, immunity, India, Mumbai, serosurvey

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Introduction

Three years after the origin of COVID-19 pandemic, the world is still bracing from its impact. Worldwide, there have

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been 772,838,745 COVID-19 cases and 6,988,679 deaths as of December 17, 2023.^[1] India (population over 1.41 billion)^[2] reported 45,009,248 cases and 533,334 deaths, while its state of Maharashtra reported 8,172,135 cases and 148,564 deaths by December 25, 2023.^[3] As of 24th January 2025, Maharashtra's capital, Mumbai city has achieved extensive vaccination with over 22,200,000 COVID 19 vaccine doses, including precautionary doses.^[4]

Serology helps detect COVID-19 through antibody study, aiding outbreak size estimation.^[5] Seroconversion studies assess if specific antibody levels offer disease protection.^[5] Antibodies, developed through natural infection and/or vaccination, play a role in developing herd immunity, which occurs when a significant proportion of the population develops immunity against a disease, thereby reducing disease transmission.^[6]

Hybrid immunity results from a COVID-19 vaccine dose and a SARS-CoV-2 infection before or after vaccination.^[7] Studies predict that antibody levels from natural infection and vaccination decline over time, reducing immunity and raising infection risk.^[8] In Mumbai, seroprevalence varied over the years as follows: 16.1% (non-slums) and 54.2% (slums) in July 2020, 17.5% (non-slums) and 44.9% (slums) in August 2020, 41.6% (public dispensaries) and 28.6% (private labs) in March 2021, 50% in the pediatric age group in June 2021, and 86.6% in September 2021 in municipal dispensaries and private clinics.^[9-13]

As healthcare workers were early COVID-19 vaccine recipients, an essential workforce in this pandemic, understanding their immunity status from prior infection or vaccination is vital. Therefore, the authors conducted a COVID-19 Serosurvey phase 6 with the aim to quantify IgG antibodies against COVID-19 in healthcare and frontline workers and monitor antibody changes after 6 months.

Material and Methods

This was a longitudinal study conducted over 9 months at the primary level (health posts, dispensaries), secondary level (peripheral hospitals, special hospitals), and tertiary health care institutions (municipal teaching hospitals), Bus depots, and Solid Waste Department offices of Mumbai across 24 wards under jurisdiction of Brihanmumbai Municipal Corporation [Figure 1]. Phase I (baseline) of the serosurvey was conducted in March–April 2022, consisting of 3099 participants, while Phase II (follow-up) was conducted after 6 months in September–October, 2022. The study participants were health care workers (HCWs) and frontline workers (FLWs) aged above 18 years residing in Greater Mumbai for at least 6 months [Figure 2].

Sample size calculation

The sample size was calculated based on seroprevalence of 86.6% from the fifth COVID-19 serosurvey conducted in Mumbai by Bansode-Gokhe SS *et al.*^[13] with an absolute precision of 1.25% and a design effect of 1, giving an effective sample size of 2783.

A nonresponse rate of 10% was added; the final sample size was 3092, and 3099 participants were included in phase I. At follow-up (Phase II), 2733 participants remained.

Enrolment of participants

Among the frontline workers, Brihanmumbai Electric Supply and Transport Undertaking (BEST) and Solid Waste Management (SWM) workers were selected equally from BEST depots and Solid waste management offices. The BEST workers are mainly involved in public bus transport services, involving interaction with numerous commuters daily,^[14] whereas the SWM workers are involved in the collection, transport, and disposal of garbage and cleaning of general public areas.^[15] The respective administrative authorities provided a list of the various strata of workers among 1544 healthcare workers and 1555 frontline workers. Then, a list of randomly selected participants was shared with the respective nodal persons [Figure 2].

Administrative approval was taken prior to the commencement of the study. Ethical approval was obtained for the Ethics Committee for Academic Projects (ECARP) [Protocol number: ECARP/2022/31]. All methods followed relevant guidelines and regulations as per the Declaration of Helsinki.

Data collection

Teams received training for data uniformity and quality. The study team supervised data collection using a pretested interview schedule. All study participants provided written informed consent.

Sample collection

For the antibody titer estimation, 5 ml of blood was collected from each participant by venepuncture in a Golden Serum Separator Tube SST II gel vacutainer and transported at 2 to 8°C in a vaccine vial carrier. The samples were then analyzed using the SARS-CoV-2 IgG-II Quant assay, which is an automated, two-step chemiluminescent microparticle immunoassay (CMIA) used for the qualitative and quantitative determination of IgG antibodies to SARS-CoV-2 in human serum and plasma on the Abbott ARCHITECT-i System. The cutoff for seropositivity of COVID-19 antibodies is taken as 50.0 AU/mL and above. Reports were shared individually with the respective participants.

Categorical variables are represented as frequency (percentage), and quantitative variables as mean (standard deviation) for descriptive data. Paired t-tests were employed to compare baseline and 6-month antibody titers, while independent t-tests and one-way ANOVA were used to determine the differences in titers between other categories. Tukey test was utilized for pairwise *post hoc* tests to find differences in mean for the possible pairs in each group for multiple comparisons. Correlation test was applied to explore the association between duration from the last vaccine dose and the antibody levels. All data were analyzed using SPSS (Version 28).

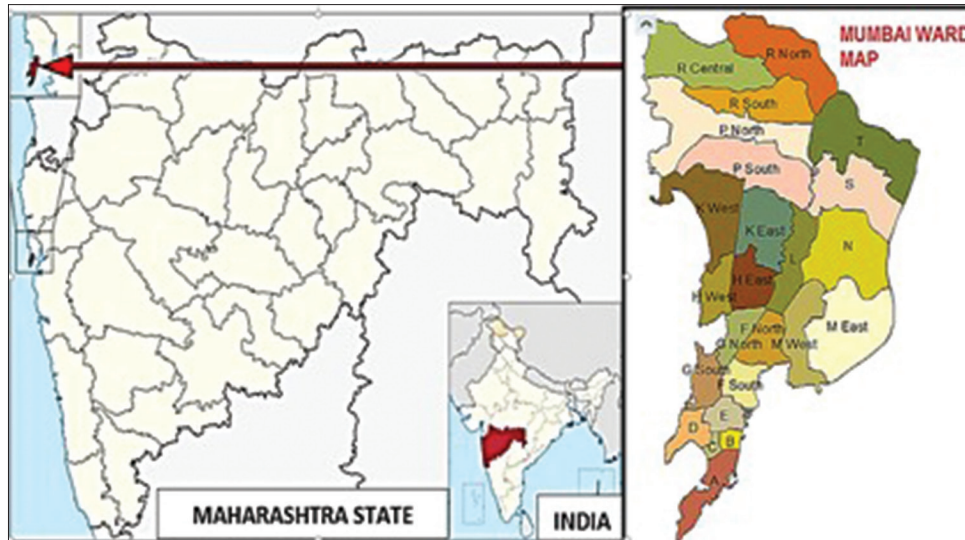


Figure 1: Map of the study site showing Mumbai district in the state of Maharashtra, India [Source: Modi S. Econ in the Bar. 2018 Feb 28. Internet. Available from: medium.com/econinthebar]

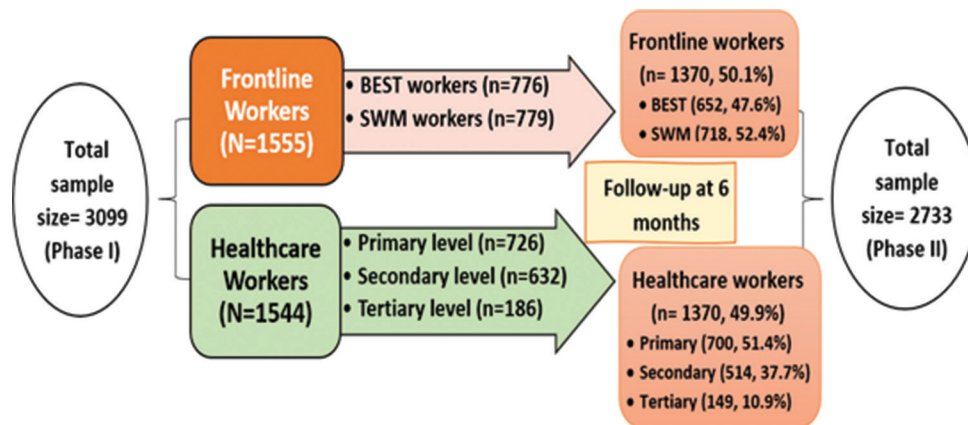


Figure 2: Flowchart of methodology [Source: Original]

Results

Out of the 3099 participants enrolled, 2733 (88.2%) participants remained in the study after 6 months, giving an attrition rate of 11.8%. Of the 2733 participants, 49 (1.8%) were aged 18 to 25 years, 556 (20.3%) were aged 25 to 35 years, and 1002 (36.7%) belonged to the more than 35 to 45 years age group, followed by 891 (32.6%) aged >45 to 55 years and 235 (8.6%) aged >55 to 65 years. The sample consisted of 1567 (57.3%) male and 1166 (42.7%) female participants.

Only one dose of the COVID-19 vaccine was taken by 32 (1.2%), two doses by 1498 (54.8%), and 1185 (43.4%) participants took two doses with precautionary doses. Eighteen (0.7%) participants did not receive any dose of COVID-19 vaccine.

Out of 2733 participants, 446 (16.3%) out of 2733 had tested positive for COVID-19 disease.

Out of a total of 2733 participants, 621 (22.7%) had some comorbidity, the most common being hypertension (12.8%), diabetes (10.8%), thyroid disorder (2.4%), asthma (0.5%), and heart disease (0.4%). Many participants (4.6%) also suffered from multiple comorbidities.

An individual with an antibody titer of 50 AU/mL (antibody Units/mL) and above was considered seropositive, and those with an antibody below 50 AU/mL were considered seronegative for COVID-19 antibodies as per the test-kit specification. Out of 2733 participants, 2732, that is, all except one participant, had protective antibodies, giving the overall study seropositivity rate of 99.9%; the same person was seronegative in both phases, while all the rest were seropositive.

The antibody titer of almost one-third, that is, 864 (31.6%) participants, was observed in the range of 5000 to 10000 AU/mL, followed by 793 (29.0%) with an antibody titer below 5000 AU/mL, 743 (27.2%) between 10000 and 20000 AU/mL, 200 (7.3%) between 20000 and 30000 AU/ml, and 133 (4.9%) between 30000 and 40000 AU/mL.

The mean antibody titer of the study participants was 10586.46 ± 8578.90 AU/mL, which is lower by 1553.58 ± 9209.03 AU/mL than the baseline mean titer of 12140.04 ± 9826.90 AU/mL. The minimum titer was 8.8 AU/ml, and the maximum was 40000.00 AU/ml, with the median of the antibody titer at follow-up being 8210.7 ± 9099.70 AU/ml. There was a significant change in the mean antibody titer from baseline till the follow-up for all participants ($P < 0.01$) [Table 1].

The mean difference in antibody titer at baseline and follow-up was significant for frontline workers ($P < 0.01$) and healthcare workers ($P < 0.01$) [Table 2].

The mean antibody titer of BEST workers was 12837.99 ± 9894.18 AU/ml, and that of SWM workers was 9498.68 ± 8064.26 AU/ml. There was a significant difference in the antibody titer of the BEST and SWM workers at 6-month follow-up ($P < 0.05$).

Among all participant categories, the mean antibody titer of BEST workers was the highest, while that of SWM workers was the lowest at the 6-month follow-up. On *post hoc* analysis using Tukey's HSD comparison test, the mean antibody titer of BEST workers was significantly higher than that of the other category of workers ($P < 0.01$) [Table 3].

Those who received no vaccine had the lowest mean antibody titer, followed by those who took only one dose of the COVID-19 vaccine. Those who received a precautionary dose had significantly higher mean antibody titer levels than those who received two doses of the COVID-19 vaccine at both baseline and follow-up ($P < 0.01$) [Table 4].

The mean antibody titer of those who received a precautionary dose with a known history of COVID-19 was significantly higher than that of those who received a precautionary dose without a history of COVID-19 and

Table 1: Mean antibody titer of the study sample at baseline and at 6-month follow-up

Category of participants	Mean antibody titer at baseline	Mean antibody titer at follow-up	Change in titer from baseline to follow-up	<i>t</i>	<i>P</i>
All participants (<i>n</i> =2733)	12140.04±9826.90	10586.46±8578.90	1553.58±9209.03	8.82	<0.01

Table 2: Change in antibody titer of FLWs and HCWs from baseline to 6-month follow-up

Category of participants	Mean antibody titer at baseline	Mean antibody titer at follow-up	Change in titer from baseline to follow-up	<i>t</i>	<i>P</i>
Frontline workers (<i>n</i> =1370)	12149.59±9770.42	11087.90±9132.10	1061.69±8520.85	4.61	<0.01
Healthcare workers (<i>n</i> =1363)	12130.44±9886.92	10082.45±7955.91	2047.99±9830.79	7.691	<0.01

Table 3: Category-wise mean antibody titer of study participants at baseline, 6-month follow-up, and baseline to 6 months change in antibody titer (*n*=2733)

Participant category	Antibody titer at baseline: Phase I [AU/mL] (Mean±SD)	Antibody titer at follow-up: Phase II [AU/mL] (Mean±SD)	Difference in antibody titer from baseline to follow-up [AU/mL] (Mean±SD)
BEST workers (<i>n</i> =652)	13497.30±10518.35	12837.99±9894.18	-659.31±8974.87
SWM workers (<i>n</i> =718)	10925.76±8869.32	9498.68±8064.26	-1427.08±8075.46
Healthcare workers (<i>n</i> =1363)	12130.44±9886.92	10082.45±7955.91	2047.99±9830.79
Combined (<i>n</i> =2733)	12140.04±9826.90	10586.46±8578.90	-1553.58±9209.03
ANOVA test: <i>F</i> -statistic	7.30	16.33	3.19
<i>P</i>	<0.001	<0.001	<0.001

The antibody titer of BEST workers was significantly higher than other categories at the 6-month follow-up ($P < 0.05$)

Table 4: Mean antibody titer level at baseline and at 6-month follow-up with respect to vaccination status of participants

Number of doses of COVID-19 vaccine	Antibody titer at baseline: Phase I [AU/mL] (Mean±SD)	Antibody titer at follow-up: Phase II [AU/mL] (Mean±SD)	Difference in antibody titer from baseline to follow-up [AU/mL] (Mean±SD)
None (<i>n</i> =18)	6953.28±7191.78	7044.38±9163.12	91.11±11133.07
One dose (<i>n</i> =32)	12072.52±9877.04	9684.56±9672.01	-2387.96±10719.42
Two doses (<i>n</i> =1498)	11584.00±9646.60	10157.56±8148.08	-1426.44±9067.46
Precaution dose (<i>n</i> =1185)	12923.56±10022.73	11206.81±9021.84	-1716.75±9318.55
ANOVA test: <i>F</i> -statistic	5.8	4.5	0.5
<i>P</i>	<0.01	<0.01	0.68

Those who received a precaution dose had significantly higher mean antibody titer level than those who received two doses of COVID-19 vaccine at both baseline and follow-up. ($P < 0.05$)

those who received two vaccine doses without a COVID-19 history ($P < 0.01$).

At 6 months from baseline, the mean antibody titer was 12213.29 ± 9637.41 AU/mL for those who ever tested positive for COVID-19, as compared to 10269.21 ± 8322.21 AU/mL in those who never tested positive for COVID-19. Thus, the mean titer was significantly higher by 1944.08 AU/mL in those who tested positive for COVID-19 compared to those who never tested COVID-19-positive.

The mean antibody titer in those with any comorbidity was 12034.60 ± 9682.13 AU/mL at follow-up, significantly higher than those without comorbidity. The mean antibody titer of the participants over 45 years was significantly higher than that of other age groups ($P < 0.05$).

The 6-month titer of females was found to be significantly lower than that of males, and the change in titer was significantly more in females than in males ($P < 0.01$) [Table 5].

The mean duration of days from the last dose of vaccine till antibody measurement was 362.7 ± 150.2 days. The minimum and maximum number of days from the last dose of vaccine till antibody testing were 7 and 650 days, respectively. The correlation between duration from the last vaccine dose and antibody titer at follow-up showed that the antibody titer decreased as the duration from the last vaccine dose increased. However, the correlation was weak ($R = -0.03$) and not statistically significant ($P = 0.14$) [Figure 3].

The reasons for attrition (11.81%) were as follows: 250 (8.1%) participants did not respond to 3 contact attempts, 30 (1.0%) were

on extended leave of absence from work, 23 (0.7%) were out of station, 23 (0.7%) were on other duty shift and did not report not on given alternative days twice, 8 (0.3%) were hospitalized for conditions unrelated to COVID19, 8 (0.3%) refused to follow-up, 7 (0.2%) had some other medical appointment, 5 (0.2%) were transferred to another workplace and died of myocardial infarct unavailable for data collection at the transfer site on the given alternate day, one (0.03%) retired, 1 (0.03%) had died, and 5 (0.2%) had other reasons such as multiple prick or missed appointment.

Eight participants were hospitalized for various diseases such as CVA stroke, dengue, malaria, and fracture. Two participants were hospitalized for treatment of dengue fever, two for cerebrovascular stroke, one for bone fracture, and one for malaria. One female participant was hospitalized following surgery unrelated to COVID-19.

Discussion

This was the only study conducted in Mumbai that performed quantitative antibody estimation with a follow-up 6 months after baseline. The seropositivity for COVID-19 IgG antibodies found in the current study was 99.9% among the participants, that is, healthcare workers and frontline workers.

The initial Mumbai serosurveys in July and August 2020 showed seropositivity rates of 54.2% and 16.1% in slum and nonslum areas, respectively.^[9] Healthcare workers exhibited a 27.3% seropositivity in August 2020, notably lower than the 99.9% found in a subsequent study, possibly due to the spread of the Omicron variant during the latter research period.^[10] The March 2021 survey among unvaccinated patients reported seropositivity rates of 41.6% and 28.6% in the public and private sectors, respectively, with an overall rate of 36.3%.^[11] The June 2021 survey in the pediatric age group recorded a 50% seroprevalence, which then rose to 86.6% by the fifth survey encompassing municipal dispensaries and private clinics.^[12,13] Subsequent surveys in September 2021 reported 11.1% seroprevalence among 801 healthcare workers and a 10% rate among 1005 healthcare workers from December 2020 to February 2021, with a higher rate (23.3%) in those with a history of COVID-19.^[16,17]

The first national serosurvey, conducted during the initial COVID-19 wave, revealed a 0.73% seroprevalence among 28,000 individuals, reflecting an underestimated infection extent.^[18]

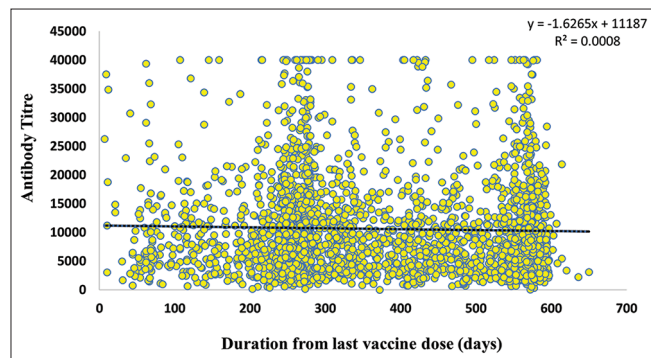


Figure 3: Correlation between duration from last vaccine dose and antibody titer at follow-up (Phase II) [Source: Original]

Table 5: Gender-wise mean antibody titer at baseline and at 6-month follow-up (n=2733)

Gender of participant	Antibody titer at baseline: Phase I [AU/mL] (Mean±SD)	Antibody titer at follow-up: Phase II [AU/mL] (Mean±SD)	Difference in antibody titer from baseline to follow-up [AU/mL] (Mean±SD)
Female (n=1166)	12309.19±9838.74	10208.69±7876.13	-2100.50±9836.68
Male (n=1567)	12014.18±9819.33	10867.56±9058.82	-1146.62±8693.61
Independent samples test (t)	0.78	-2.03	-2.63
P	0.44	0.04	0.01

The mean difference in antibody titer from baseline to follow-up of female and male participants was statistically significant with a higher fall in females than males ($P < 0.05$)

Subsequent serosurveys displayed escalating figures, and by the third survey (December 2020–January 2021),^[19] the overall seroprevalence reached 24.1%, in which healthcare workers exhibited 25.6% seropositivity. This rose to 67.6% in the general population and 85.2% among healthcare workers in the fourth national serosurvey.^[20] These findings signify a steady increase in COVID-19 IgG antibody seroprevalence, suggesting the evolving landscape of infection and vaccine-induced immunity.

Various serological studies from other parts of India have reported a seroprevalence ranging from 3.9% to 25.6% in healthcare workers.^[21–25] There is evidence of global asymptomatic infections too, suggesting subclinical infection, which can produce antibodies in the population.^[26–28]

The 99.9% seropositivity observed in our study at baseline and at follow-up indicates a consistent antibody response among the participants. This high rate can be attributed to widespread infections by 2022 and extensive vaccination coverage in Mumbai. Research shows sustained antibody responses for over 6 months after two COVID-19 vaccine doses.^[29] Recent clinical trials also affirm robust immune responses post COVISHIELD and COVAXIN, the two primary COVID-19 vaccines used in India.^[30]

COVID-19 vaccination commenced in India from January 2021, primarily for healthcare and frontline workers (HCWs and FLWs), with initial administration of the Covishield and Covaxin vaccines. The national vaccination program recommended two COVID 19 vaccine doses 4–6 weeks apart, followed by a precautionary dose.^[31,32] In a Delhi study (2022) reporting 88% seroprevalence, vaccinated individuals showed four times higher seropositivity than unvaccinated individuals, suggesting enhanced immunity conferred by vaccination.^[33] This aligns with this study's findings, where higher antibody levels were observed in those receiving a precautionary dose alongside two vaccine doses.

Our study revealed 100% seroprevalence among frontline workers, including BEST (public transport) and Solid Waste Management workers. Bhartiya S *et al.* (2022)^[34] reported a 74.1% seroprevalence among Mumbai police in January 2021 when vaccines were introduced. Their study highlighted higher seroprevalence in individuals with a history of PCR positivity (96.4%), reported COVID-19 history (95.5%), and close contact with COVID-19-positive individuals (89.6%). These groups might exhibit short-lived antibodies after natural infection.

Police personnel, akin to BEST and Waste Management workers, are nonhealthcare frontline essential workers vulnerable during the pandemic. Further serosurveys are warranted to explore antibody presence, infection history, and vaccination status. Studies across India reported healthcare worker seroprevalence ranging from 10% to 85%.^[17,21]

Our study noted significantly higher antibody levels in those with a precautionary dose and a confirmed COVID-19 history than

in those with a precautionary dose alone or two vaccine doses without a confirmed COVID-19 history. Research evidence shows robust immune responses post COVID-19 infection or vaccination, particularly with hybrid immunity, involving both vaccination and infection.^[35,36]

In participants aged 45 years and above, a group with a higher proportion of vaccinated individuals, antibody levels were found to be higher. Advanced age is a crucial risk factor for severe COVID-19.^[37] These higher antibody levels among older age groups may suggest hybrid immunity's influence, stemming from infection history and vaccination within this demographic.^[38]

Though the COVID pandemic has subsided, COVID strains are still circulating and mutating. It is essential that the general practitioners and family physicians be updated on the evolving epidemiology of COVID and keep the endemicity in mind while dealing with patients suffering from respiratory tract infections. It also helps in resolving queries by patients concerned about vaccination and booster dose requirement.

Strengths and limitations

The study sample was representative of Mumbai's healthcare workers and frontline workers with a robust sample size. A sizable number (88.2%) of participants remained till the follow-up, giving an effective attrition rate of 1.8%, as an extra 10% sample was collected to ensure the study's validity. This study included the quantitative estimation of SARS-CoV-2 antibodies. One of the limitations of this study is that the role of subclinical infection could not be studied.

Conclusion

The study found a 99.9% seropositivity rate among participants, which remained the same after 6 months. Those who received a precautionary dose and those with a history of COVID-19 infection and a precautionary dose (hybrid immunity) showed higher antibody titers. These findings indicate a widespread seropositivity for COVID-19, which may provide herd immunity and prevent further outbreaks. Further research is warranted to estimate the protection duration that antibody levels offer. Near 100% seropositivity is likely to protect against deaths and severe infections due to COVID-19. Precautionary doses likely offer higher antibody levels, and the role of subclinical infections may be studied further.

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Ethics

Ethical approval was obtained for the Ethics Committee for Academic Projects (ECARP), TN Medical College and BYL Nair Hospital, Mumbai- 400008 [Protocol number: ECARP/2022/31].

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Conflicts of interest

There are no conflicts of interest.

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