Original Article

COVID-19 Serological Survey-3 Prior to Second Wave in Mumbai, India

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Abstract

Background: Subsequent to serosurveys 1 and 2 for COVID-19 carried out in three wards of Mumbai in July and August 2020, Municipal Corporation of Greater Mumbai conducted serosurvey 3 in March 2021. This was to identify the extent of exposure by testing specific IgG antibodies against COVID-19. **Material and Methods:** A cross-sectional study was conducted to find the prevalence of seropositivity in Mumbai, which included 10,197 samples belonging to patients visiting public dispensaries (slum population, 6006) and private (nonslum population, 4191) laboratories of Aapli Chikitsa network for blood investigations for non-COVID illnesses. The ward-wise number of unlinked anonymous samples from 24 wards was predecided by using probability proportionate sampling. The samples were collected using quota sampling technique as per predecided sample for each ward. These samples collected from nonimmunized individuals were tested for IgG antibodies at the Molecular Biology Laboratory of Kasturba Hospital for Infectious Diseases by chemiluminescence assay (CLIA) method. **Results:** The overall seropositivity was found to be 36.3% (41.6% in slum and 28.59% in nonslum population). It was more in city wards (38.28%) followed by western suburb (36.47%) and then eastern suburb wards (34.86%), matching with the proportion of cases in these wards during the study period. There was no significant difference in seropositivity among males and females and in different age groups. **Conclusions:** Seropositivity is higher in slum areas than nonslum areas. It has reduced in slum areas and increased in nonslum areas as compared to findings of serosurveys 1 and 2. This explains the detection of a greater number of cases from nonslum areas in the second wave. The average seropositivity of 36.3% justifies the necessity of immunization on a wider scale in the city. Periodic serosurveys are required at fixed intervals to monitor the trend of infection and level of herd immunity.

Keywords: Chemiluminescence assay, COVID-19, IgG antibodies, seroprevalence, unlinked anonymous

Introduction

The Ministry of Health and Family Welfare and the Indian Council of Medical Research recommend periodic seroprevalence surveys in states and districts to monitor the exposure level of the population from time to time.[1] Surveys 1 and 2 were carried out in Mumbai Municipal Corporation of Greater Mumbai (MCGM) in July and August 2020 which included 12,320 individuals from 3 wards of the city. These two surveys, 1 month apart, conducted during the first wave of COVID-19, revealed the proportion of seropositive individuals as 57% and 41% in slum areas and 16% and 18% in nonslum areas. MCGM supported present serosurvey 3 in March 2021, when cases were declining, to understand seropositivity in relation to serosurveys 1 and 2, and the extent of exposure. Until February 28, 2021, i.e., before initiation of this serosurvey, the city reported 326,770 cases, which were 15.16% of the state (21,55,070) and 2.94% of the country then (1,11,12,241).[2,3]

The Centers for Disease Control and Prevention, USA, conducts periodic seroprevalence surveys to find out the total number of people that have been infected, including those infections that might have been missed. These surveys help estimate uninfected population and which helps public health

Access this article online

Quick Response Code:

Website: www.ijcm.org.in

DOI: 10.4103/ijcm.ijcm_984_21

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How to cite this article: Velhal GD, Shastri JS, Shah D, Agrawal SR, Gomare M, Sadawarte DM. COVID-19 serological survey-3 prior to second wave in Mumbai, India. Indian J Community Med 2022;47:61-5.

Received: 27-06-21, **Accepted:** 30-12-21, **Published:** 16-03-22

officials to plan for future health-care needs. Furthermore, such surveys track how infections progress through the population over time by taking "snapshots" of the percentage of people who have antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[4]

Such serosurveys have been undertaken in several countries such as India,[5] the USA,[6] China,[7] and England[8] as well as several states in India, for example, Telangana,[9] Karnataka,[10] and Tamil Nadu.[11] Cities such as Mumbai,[12] Pune,[13] New Delhi,[14] and Bengaluru[15] have also undertaken serosurveys to detect the level of exposure among the population.

Material and Methods

A cross-sectional seroprevalence study for COVID-19 was conducted on blood samples collected by Aapli Chikitsa network in the city which is a public–private initiative. Aapli Chikitsa is a service outsourced for investigations of patients from peripheral health service delivery points in the MCGM. Samples were collected from patients reporting to municipal dispensaries and private laboratories receiving samples from private sector health service delivery points for investigations as per their non-COVID-19 requirements. Data collection was done for 1 month, i.e., March 2021. To represent the entire city, the strategy of unlinked anonymous seroprevalence survey was used, which included samples for antibody testing from 24 wards.

Sample size

Twelve thousand using

$$n = \frac{z^2 * P * q}{d^2}$$

Z= 2.58 (99 % confidence interval), P= average proportion/ percentage of seropositivity in serosurveys 1 and 2 (16) i.e. 47%, Q= 100-p = 53% d = 2 absolute precision, and to increase the precision of study 1.5 times, sample size was increased by 21.5 i.e by 2.83 times. Hence, final sample size = (6.66 × 47 × 53) × 2.83/4 = 11,731, rounded up to 12,000. However, in a period of 1 month, 10,197 samples were tested for COVID-19 antibodies. The population probability sampling method was used for identifying the number of samples to be collected from each ward, as detailed in Table 1. The samples were collected using the quota sampling technique as per predecided sample for each ward. Samples that could not be processed (insufficient quantity, hemolysis, etc.) or from patients residing outside MCGM limits and who were vaccinated for COVID-19 were excluded.

Antibody testing

Chemiluminescence assay (CLIA) for estimation of IgG antibodies was performed at Molecular Biology Laboratory, Kasturba Hospital for Infectious Diseases, Mumbai. Nucleocapsid (NC) antibodies were detected by ABBOTT ARCHITECT. Samples ≥1.4 were reported as positive and <1.4 as negative. Spike Protein 1 Receptor Binding Domain (S1RBD) (receptor-binding antibodies) antibodies were detected by SIEMENS ATELLICA. Samples ≥1 were considered positive and ≤1 were considered negative.

Table 1: Ward-wise selection of samples

Ward	M.Y.E.P. year 2020 (sample to be selected from ward)
A	191,450 (178)
B	131,718 (123)
C	171,941 (160)
D	358,933 (335)
E	406,967 (379)
F/S	373,529 (348)
F/N	547,438 (510)
G/S	390,890 (364)
G/N	619,878 (578)
City bureau	3,192,744 (2976)
H/E	576,624 (537)
H/W	318,281 (297)
K/E	852,546 (795)
K/W	774,733 (722)
P/S	479,631 (447)
P/N	974,114 (908)
R/S	715,275 (667)
R/C	581,718 (542)
R/N	446,374 (416)
West bureau	5,719,296 (5331)
L	933,611 (870)
M/E	835,819 (779)
M/W	426,222 (397)
N	644,521 (601)
S	769,657 (717)
T	353,343 (329)
East bureau	3,963,173 (3694)

Calibrators and controls were run every day prior to commencement of testing. All samples were first tested for NC antibodies. If tested negative for NC antibodies, then the samples were tested for S1RBD antibodies. Seroprevalence was assessed by IgG antibody testing of these unlinked anonymous samples from 24 wards.

A preformed Microsoft Excel master sheet was made which included the information maintained at Aapli Chikitsa Network Laboratory. Institutional Ethics Committee approval was obtained from Kasturba Hospital, Mumbai. Being unlinked anonymous survey consent was not applicable. Data were analyzed using the Chi-square test in SPSS software (26 version) (IBM SPSS South Asia Version 26, USA) to find any association between gender and area of the sample collected versus seropositivity.

Results

The total number of 10,197 samples was tested from 24 wards of Mumbai city, out of which 6006 belonged to patients from public sector (MCGM) dispensaries (considered as slum population) and 4191 from private sector laboratories (considered as nonslum population) to represent the exposure levels in both areas.

Table 2 shows the age-wise and area-wise reactive samples. The mean age of study subjects was 42 years (range from 17 years to 94 years). Gender was not mentioned for 11 samples all from nonslum areas, and one person was transgender from slum areas. Out of the total number of samples examined from the slum population, 36.67% were male and 63.30% were female and the corresponding figures for nonslum areas were 46.11% and 53.52%, respectively.

The overall seropositivity (IgG antibodies) was 36.30% which was 41.61% in samples collected from slum areas and 28.59% in samples collected from nonslum areas.

The seropositivity in the age group of <18 years was 39.40%. The corresponding figures for age groups of 18–30 years, 31–40 years, 41–50 years, 51–60 years, and more than 60 years were 32.96%, 34.43%, 37.88%, 39.51%, and 37.85%, respectively. There was no statistically significant difference in the prevalence of seropositivity in different age groups (Chi-square = 8.893, df = 1, $P > 0.05$)

The seropositivity among males and females (slum + no-slum) was 35.02% and 37.12%, respectively. No statistically significant difference was revealed in the prevalence of seropositivity in males and females (Chi-square = 1.992, df = 1, $P > 0.05$).

The ward-wise seroprevalence is depicted in Table 3. The seropositivity was significantly less in eastern suburb wards (L, M/E, M/W, N, S, and T) as compared to city area (A, B, C, D, E, F/N, F/S, G/N, and G/S) and western suburb areas (H/E, H/W, K/E, K/W, P/N, P/S, R/C, R/N, and R/S) (Chi-square = 7.635, df = 2, $P < 0.05$). The maximum seropositivity was seen in five wards, i.e., D (47.62%), H/E (45.06%), E (42.24%), C (42.05%), and H/W (41.97%). The lowest prevalence was in S ward (32.17%). The seropositivity was seen more in slum areas as compared to nonslum areas. One thousand six hundred and thirty-three samples were not subjected to S1 RBD IgG testing, as they were found positive by NC IgG testing.

Graph 1 depicts age-wise reactive samples from the slum population. Exposure was seen to be more in the age group of 18–30 years in the slum areas. The NC IgG report for two males from the slum population in the age group of 18–30 years was not available due to insufficient sample. Graph 2 depicts age-wise reactive samples from the nonslum population. The presence of NC antibodies indicated a recent infection (<2 months). Hence, it can be seen that in nonslum areas, recent infection is more.

Discussion

After two serosurveys in July and August 2020, a third serosurvey was undertaken by MCGM in March 2021 to assess the current status (in March 2021) of exposure to COVID-19 among the general population. The previous two surveys were population-based and adopted random sampling methodology from slum and nonslum areas of three wards of Mumbai (F/N, M/W, and R/N). The present survey was done adopting an unlinked anonymous survey using a probability proportionate sampling method from 24 wards, thus making it more representative of the entire population of Mumbai city. A total of 10,197 samples were tested from 24 wards of the city, and the seropositivity was found to be 36.30%.

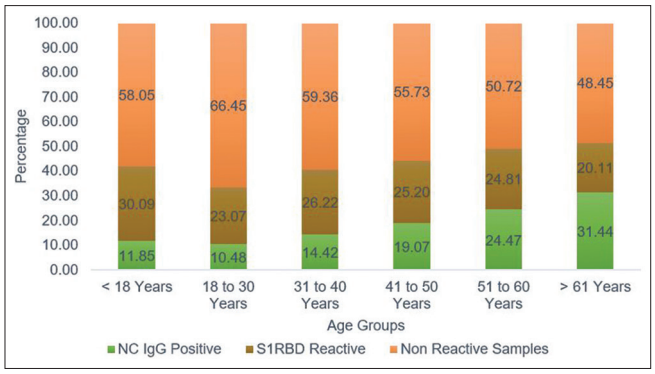
Table 2: Age-wise and area-wise reactive samples

Age category (years)	Total slum area samples	Reactive samples (%)	Total nonslum area samples	Reactive samples (%)	Total sample-slum + nonslum areas	Reactive samples (%)
<18	329	138 (41.95)	138	46 (33.33)	467	184 (39.40)
18-30	1994	669 (33.55)	697	218 (31.28)	2691	887 (32.96)
31-40	1026	417 (40.64)	879	239 (27.19)	1905	656 (34.44)
41-50	1012	448 (44.27)	746	218 (29.22)	1758	666 (37.88)
51-60	903	445 (49.28)	770	216 (28.05)	1673	760 (45.43)
>61	741	382 (51.55)	950	258 (27.16)	1691	640 (37.85)
Total	6005	2499 (41.62)	4180	1195 (28.59)	10,185	3694 (36.27)

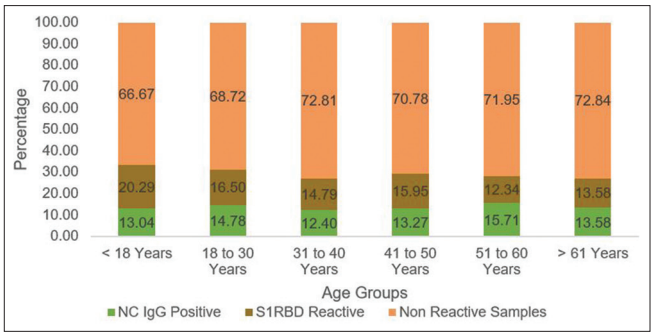
Gender was not mentioned for 11 samples all from nonslum areas and one person was transgender from slum area. Hence, total samples collected were 10,185+12=10,197

Table 3: Area-wise distribution of seropositive (either nucleocapsid immunoglobulin or S1 receptor-binding domain immunoglobulin) study subjects

Area	Total samples from slum area	Reactive samples (%)	Total samples from nonslum area	Reactive samples (%)	Total samples	Reactive samples (%)
1. City bureau (A, B, C, D, E, F/N, F/S, G/N, and G/S)	1489	631 (42.38)	862	251 (29.12)	2351	900 (38.28)
2. Western suburbs (H/E, H/W, K/E, K/W, P/N, P/S, R/C, R/N, and R/S)	2668	1103 (41.34)	1480	410 (27.70)	4148	1513 (36.48)
3. Eastern suburbs (L, M/E, M/W, N, S, and T)	1849	765 (41.37)	1849	524 (28.34)	3698	1289 (34.86)
4. Grand total	6006	2499 (41.61)	4191	1203 (28.70)	10197	3702 (36.30)



Graph 1: Age-wise reactive samples from slum population



Graph 2: Age-wise reactive samples from nonslum population

As compared to the previous two serosurveys, the positivity in slum areas seems to have reduced from 57% in the first round to 45% in the second round and 41% in the current round. However, it is still higher than nonslum areas. The seropositivity in nonslum areas has apparently risen from 16% in the first round to 18% in the second round and 28.5% in the current round.[16,17] This could be because of inevitable community transmission in nonslum societies because of resumption of routine activities and relaxation in observance of COVID discipline.

The previous serosurvey conducted in July–August 2020 across three wards of Mumbai city revealed that the proportion of positive tests by age and sex in different sites shows markedly higher proportions in slums than in nonslum areas. The positive proportions were higher among women than among men in both slum and nonslum areas, which was not seen in the current survey. In the previous survey, the positivity seen among individuals older than 60 years was lower in nonslum areas compared to slums, whereas in the current survey, there was no significant difference in age-wise seropositivity in slum and nonslum areas.[16]

ICMR conducted two population-based serial serosurveys in 70 districts of India by ADVIA Centaur immunoassay system using the Siemens SARS-CoV-2 IgG assay (Siemens Healthineers, Mumbai, India) and Abbott Architect i2000SR automated analyzer using the Abbott SARS-CoV-2 IgG assay (Abbott Park, IL, USA). The Siemens assay detects IgG antibodies against the spike protein of the receptor-binding domain (S1-RBD), and the Abbott assay detects IgG antibodies against the NC protein of SARS-CoV-2, which indicated that the seroprevalence to SARS-CoV-2 infection among adults had increased from 0.73% in May–June 2020 to 7.1% in August–September 2020.[18,19] The third nationwide serosurvey was conducted in December 2020–January 2021, wherein the 28,598 serum samples from the general population were tested, out of which 4585 (16%) had IgG antibodies against the N protein, 6647 (23.2%) had IgG antibodies against the S1-RBD protein, and 7436 (26%) had IgG antibodies against either the N protein or the S1-RBD protein. The seroprevalence against either of the antibodies was 24.1% (95% CI: 23.0%–25.3%). Among 7385 health-care workers, the seroprevalence of anti-S1-RBD IgG antibodies was 25.6% (95% CI: 23.5%–27.8%).[20] This could be due to increased exposure of HCWs to COVID-affected individuals.

In the present study, there was no significant difference seen in overall seropositivity among males and females, which was also found in the nationwide serosurvey conducted by ICMR in December 2020–January 2021.[20]

Surveys are being undertaken in different parts of the country to plan the strategies for prevention and control. Repeated cross-sectional surveys in the same geographical areas will provide estimates to monitor the trends over a period of time.[21] Periodic serosurveys will be required at an interval of 6–12 months to understand the trends of infection. Such serosurveys are carried out in various parts of the country, especially major cities.

A survey done in April 2021 by the Tamil Nadu Government revealed overall seroprevalence as 23%, wherein the blood samples were collected from a total of 22,904 participants and tested for the presence of SARS-CoV-2 IgG antibodies using chemiluminescence-based immunoassay (CLIA) which is the same method used in the present survey.[11]

Another population-based seroprevalence study conducted in Pimpri-Chinchwad, Pune, among 5000 residents in October 2020 showed overall seropositivity as 34.04% where the kit from Abbott (SARS-CoV-2 IgG), which employs chemiluminescent microparticle immunoassay technology, was used.[13]

Delhi's fifth serological survey conducted in January 2021 showed 56.13% of the 28,000 people to have developed antibodies against the SARS-CoV-2 virus by CLIA technology, a similar method used in the present study. In total, the seroprevalence in Delhi was ranging from 49%–62%, with an average of 56.13%.[14] Seroprevalence was found to be very high in Delhi mostly due to noncompliance to the strict COVID appropriate behavior.

Bengaluru's serosurveillance study done in 24 slums in May 2021 has shown a seropositivity rate of 20.3%. The study suggests that a large proportion of the population was susceptible to SARS-CoV-2, or might have waning antibodies from previous infections. The survey showed an infection-to-case ratio of 3:1.[15]

All the above serosurveys done in various cities imply one thing that seroprevalence studies help in understanding the level of infection among population, based on which

appropriate public health measures can be undertaken. This will help in earmarking areas of high transmission and help in formulating appropriate control measures.

The present survey was conducted on the unlinked anonymous blood samples and hence, it was not possible to trace, track and treat patients. Also, Sample bias was one of the limitations as the information obtained in the current survey was only from those individuals who requisitioned a blood test for some other purpose. In this survey the information available was only about age, sex and residential area of the patient whose sample was collected by the laboratory. These were the only epidemiological factors assessed in the present study. Also, without knowledge of the level of exposure in the community it is difficult to comment on the level of protective herd immunity.

We recommend that preventive measures for control of transmission and behavior change need to be strengthened with strict observance of social distancing, use of mask, frequent washing of hands and cough etiquettes, and avoiding crowded places, as community transmission is apparent. As the infection is spreading fast, the protective level of herd immunity cannot be estimated at this stage unless we resort to serial surveys. With the detection of variants with increased transmissibility both in India and the rest of the world, it is imperative to resort to serial serosurveys. Immunization should get the necessary impetus as a preventive measure and should reach vulnerable population who are not yet exposed to the virus.

Conclusion

In the present survey, overall seropositivity was found to be 36.3% and was seen higher in slum areas than non-slum areas. As compared to earlier two surveys the seropositivity is found to have increased in non-slum areas due to detection of more cases from non-slum areas in the beginning of second wave.

Acknowledgment

The authors would like to acknowledge MCGM authorities for the financial support of this project. Support extended by functionaries of Aapli Chikitsa Network Laboratories (Thyrocare and Metropolis) is highly appreciated.

Financial support and sponsorship

This study was financially supported by Municipal Corporation of Greater Mumbai.

Conflicts of interest

There are no conflicts of interest.

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