Population-based seropositivity for IgG antibodies against SARS-CoV-2 in Ahmedabad city

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Abstract

Context: Ahmedabad city with approximately 7 million population was one of the earliest cities to witness the high case load of COVID-19 pandemic in India. A population-based sero-survey was ideally suited in Ahmedabad to guide the public health response for managing COVID-19 pandemic. Objectives: To study the percentage sero-positivity for SARS-CoV-2 to understand the pandemic status and deriving conclusions for guiding the public health measures for managing the COVID-19 pandemic. Settings and Design: Population-based cross-sectional sero-surveillance. Methods and Material: Large scale sero-surveillance with population-based stratified sampling covering more than 10,000 samples from general population of Ahmedabad was carried out during second half of August 2020. The seropositivity was correlated and compared with various demographic factors and other parameters for valid and precise predictions on the immunity status of the population. Results: With 2,396 samples positive for IgG antibodies from a total of 10,310 samples, the seropositivity against COVID-19 in the general population of Ahmedabad is around 23.24%. The seropositivity has increasing trend with increasing age and is significantly higher among females (25.37%) than males (21.81%). The zone wise positivity ranged from 11.74% to 33.14%. This closely correlates with the cases recorded so far, higher for those zones with high current or past cases. Conclusions: Seropositivity of 23.24% in general population indicate the overall current level of protection. Since effective vaccine is not yet available, it is required to continue emphasis on the public health preventive measures for controlling and managing the COVID-19 pandemic.

Keywords: Antibodies, COVID-19, immunity, SARS-COV-2, Sero-surveillance

Introduction

During 2020, COVID-19—the disease caused by SARS-CoV-2—has spread across the world in pandemic proportion. Since the SARS-CoV-2 was a novel virus, the entire human population may be assumed to be fully susceptible to COVID-19. Presence of antibodies in an infected individual during the convalescence period after an infection mark, an immune response against the virus, and the presence of these antibodies may be considered as an evidence of immunity. A serological survey can identify the proportion of individual with presence of antibodies and help in predicting the level of herd immunity. While the presence of antibodies indicates a complete or partial immunity, those following the viral infection. Since the SARS-CoV-2 was a novel virus, the entire human population may be assumed to be fully susceptible to COVID-19. Presence of antibodies in an infected individual during the convalescence period after an infection mark, an immune response against the virus, and the presence of these antibodies may be considered as an evidence of immunity. A serological survey can identify the proportion of individual with presence of antibodies and help in predicting the level of herd immunity. While the presence of antibodies indicates a complete or partial immunity, those
Ahmedabad city with approximately 7 million people was one of the earliest cities to witness the high case load in the initial months of the pandemic in India. Having a high number of confirmed COVID-19 cases during the earlier phase of the pandemic, a population-based sero-surveillance was ideally suited in Ahmedabad. A sero-surveillance in such a population on one hand gives the indirect rough estimate of population already affected by the disease agent while at the same time it gives an indication of the proportion of population still susceptible to the infectious agent, which is extremely important for a primary care physician. The existing level of immunity as well as the effect of factors affecting the level of immunity can be understood in a better way by conducting a sero-survey. So, a population-based sero-survey was planned in the second half of the August 2020 with the following aims and objective.

**Aim**

- To study the COVID-19 sero-positivity among general population in Ahmedabad City.

**Objectives**

- To measure the seropositivity for COVID-19 in the General population of Ahmedabad city
- To correlate the seropositivity in general population with major demographic characteristics
- To derive conclusions for guiding public health measures for managing COVID-19 pandemic.

**Materials and Methods**

Indian Council of Medical Research (ICMR) had issued directives for conducting IgG Antibody-based ELISA test for sero-surveys to the monitor the pandemic, understand its progression, and to take appropriate corrective public health measures. The primary purpose of this was to understand the proportion of population exposed to SARS-CoV-2 infection. Ahmedabad Municipal Corporation (AMC), from the state of Gujarat, India, had already completed one large scale population-based sero-surveillance for IgG antibodies against SARS-CoV-2 Virus with an average positivity of 17.61% at the end of June 2020. During the one and half month after the initial survey, there are additional cases of COVID-19. So, a repeat population-based sero-survey was planned to study the COVID-19 seropositivity. “Covid Kavach” (Anti-SARS CoV-2 IgG Antibody Detection ELISA) kits developed and manufactured by Zyduz Diagnostics, validated by National Institute of Virology, Pune, India and approved for use by the Indian Council of Medical Research (ICMR) were used for the purpose of this study after due approval (17 August 2020).

To estimate the required sample size, we referred to the earlier sero-survey in Ahmedabad city carried out in later half of June and First week of July 2020 which showed a seropositivity of 17.61% with a range from 5% to 43% at different Urban Primary Health Centres (UPHCs). Considering the highest positivity of 43% in a population of 70 lakhs with 95% confidence level with 1% margin of error, the minimum required sample size came to around 9,404. Using the available population data, based stratified sampling, ward wise desired sample size was derived. Considering the chances of sample rejection and indeterminate results, the ward wise sample size was rounded up to the next zero and an overall target was set for minimum 10,000 serum samples. There are 75 UPHCs within 48 wards across 7 zones in Ahmedabad city. As the minimum required sample was decided according to ward but the UPHCs were the functional units for sample collection, the concerned zonal officer, based on the proportion of UPHC population in the concerned ward, further divided the ward wise target sample into UPHC wise target.

At the UPHC, random sampling was followed and sample population was selected from the field area of the UPHCs. Willing individual who gives an informed written consent was enrolled in the study irrespective of age, sex, etc. The only exclusion criteria included any contraindication to venipuncture. An effort was made to cover a wide variety of people of different age groups from both the gender and from different localities within the field area of the UPHC. However, an effort was specifically made to cover at least 10% of individuals from the extremes of the age groups, that is, upto 18 years and above 60 years of age. Any one of the co-investigators conducted online virtual meeting with all the medical officers and zonal officers to explain the sampling process and other details of the protocol, including clarifying any doubts and answering their concerns. To standardize the sample collection and testing, a brief demonstration session cum training covering all necessary information was carried out by the concerned health authorities either at zonal/sub-zonal level with appropriate safety and social distancing measures. All personnel involved in the study were trained for standard as well as droplet precautions as part of infection prevention and control procedures particularly with reference to the COVID-19 as per the national guidelines. The biomedical waste generated during the study was managed according to biomedical waste management rules and waste management guidelines specific to COVID-19.

The study was carried out after clearance of the Institutional Ethics Committee. An informed written consent was taken from all the participants before enrollment. In case of children, informed written consent of their parents/guardian was taken for the purpose of the study. To reduce the sample rejection rate, SST-Gel vacutee were used for the collection of blood samples. Strict confidentiality was ensured at all the levels. For the purpose of testing and standardization, only those laboratories attached with medical colleges with all necessary equipment and facilities were considered. To cope-up the need for timely testing of the bulk samples, private laboratories with national level accreditation, state-of-the-art facilities and equipment were invited to participate.
and support. The collected samples were distributed to the designated laboratories for the purpose of testing.

Data of the study participants and their samples was enlisted at the UPHC and compiled at the zonal level before sharing it with the health department on daily basis. The dataset was updated routinely on declaration of the results. The crude positivity in the general population was considered as an indicator for the current level of immunity. An in-depth analysis of available data was carried to find out the factors affecting seropositivity and their correlation with various age-sex groups and for different UPHC/Ward/Zone. The result with estimated seropositivity was discussed personally with the concerned authorities to take appropriate public health measures, as required. The result was shared widely with all concerned including local media and social media for larger benefit of the population. We herewith share the findings of our results for the detailed insight by the scientific community.

Results

A total of 10,339 blood samples were collected from the general population of Ahmedabad city for the purpose of the study. From these, 29 samples were rejected by the laboratories because of various reasons. Results were thus available for the remaining 10,310 samples, from which 7,830 (75.95%) were negative and 84 (0.81%) had indeterminate results. Thus, a total of 2,396 results were positive for the specific antibodies against COVID-19 giving an overall crude positivity of about 23.24% with a confidence interval of 22.43–24.06%.

There were 6,184 males and 4,155 females from the general population who were enrolled in the study and results were available for 6,168 males and 4,142 females. As shown in Table 1, a total of 1,345 from 6,168 males were tested positive giving the positivity rate of 21.81% among males. A total of 1,051 out of 4,142 females were tested positive giving the positivity rate of 25.37% among females. Thus, the percentage positivity is higher among females as compared to the males [Figure 1] and the difference is statistically significant ($P < 0.01$).

The age of the sample population ranges from 2 to 99 years. The age distribution typically follows normal distribution. As the age of the enrolled individuals was collected by verbally asking and not verified with any official documents, it typically shows age-heaping bias at every 5 years (raw data not shown, only grouped data available in Table 1). These peaks are smaller and less marked in younger age groups but shows wider variation as the age increases because of recall bias and tendency to rounding the age to nearest 5 years. For age, the mode was 30 years, median 34 years and the mean was 37.02 ± 14.94 years. Among the sample, the mean age of females was 36.03 ± 14.91 years, whereas the mean age of males is 37.69 ± 14.92 years. Considering the seropositive, the mean age for females was 38.06 ± 15.61 years whereas that of male is 39.66 ± 16.16 years ($P < 0.05$).

The age group wise analysis of positivity [Figure 2] shows that the positivity in various age-groups is between 20 and 35%. The percent positivity is seen between 19.40 and 34.14%. Children <20 years, and adolescents in particular, have higher positivity as compared to young adults. For adults, from 20 to <80 years, the seropositivity increases with increase in the age group. Individuals with age >80 have lower seropositivity. When the same comparison of age group and positivity is done for both the sex groups [Figure 3], it justifies the earlier findings

| Table 1: Analysis of Covid19 sero-survey positivity in General population |
|---------------------------------------------------------------|
| **Gender** | **Female** | **Male** | **Total** | **Confidence Interval** |
| Age group | Results | Positive | % Positivity | Results | Positive | % Positivity | Results | Positive | % Positivity | Interval |
| 0-9 | 21 | 4 | 19.05 | 46 | 9 | 19.57 | 67 | 13 | 19.40 | 10.76–30.89 |
| 10-19 | 317 | 82 | 25.87 | 421 | 109 | 25.89 | 738 | 191 | 25.88 | 22.85–29.16 |
| 20-29 | 1378 | 295 | 21.41 | 1646 | 311 | 18.89 | 3024 | 606 | 20.04 | 18.65–21.50 |
| 30-39 | 898 | 217 | 24.16 | 1539 | 283 | 18.39 | 2437 | 500 | 20.52 | 18.96–22.17 |
| 40-49 | 642 | 176 | 27.41 | 1116 | 225 | 20.16 | 1758 | 401 | 22.81 | 20.91–24.83 |
| 50-59 | 467 | 133 | 28.48 | 796 | 221 | 27.76 | 1263 | 354 | 28.03 | 25.62–30.37 |
| 60-69 | 319 | 107 | 33.54 | 408 | 127 | 31.13 | 727 | 234 | 32.19 | 28.89–35.67 |
| 70-79 | 87 | 33 | 37.93 | 162 | 52 | 32.10 | 249 | 85 | 34.14 | 28.27–40.39 |
| 80-89 | 11 | 3 | 27.27 | 32 | 8 | 25.00 | 43 | 11 | 25.58 | 13.52–41.17 |
| 90-99 | 2 | 1 | 50.00 | 2 | 0 | 0.00 | 4 | 1 | 25.00 | 00.63–80.59 |
| Zone | CZ | 415 | 133 | 32.05 | 429 | 134 | 31.24 | 844 | 267 | 31.64 | 28.59–34.85 |
| EZ | 763 | 196 | 25.69 | 1086 | 247 | 22.74 | 1849 | 443 | 23.96 | 22.07–25.96 |
| NWZ | 483 | 52 | 10.77 | 1110 | 135 | 12.16 | 1593 | 187 | 11.74 | 10.25–13.41 |
| NZ | 713 | 259 | 36.33 | 974 | 300 | 30.80 | 1687 | 559 | 33.14 | 30.93–35.42 |
| SWZ | 232 | 46 | 19.83 | 587 | 109 | 18.57 | 819 | 155 | 18.93 | 16.39–21.75 |
| SZ | 787 | 212 | 26.94 | 903 | 192 | 21.26 | 1690 | 404 | 23.91 | 21.93–26.00 |
| WZ | 749 | 153 | 20.43 | 1079 | 228 | 21.13 | 1828 | 381 | 20.84 | 19.04–22.76 |
of having a significantly higher seropositivity among females and increasing seropositivity with increase in the age. Among the children, the seropositivity among both the sex group is almost similar but for the adults, the seropositivity increases with age and females have higher seropositivity as compared to males for all the age group.

The zone wise analysis of total tests and positive tests when compared to calculate percent positivity shows that the positivity in various zones varies widely. The zone positivity ranges from 11.74% to 33.14%. The zone wise positivity shows that the North Zone (33.14%) had the highest seropositivity followed by Central Zone (31.64%), whereas South West Zone (18.93%) and the North West Zone (11.74%) were the zones with lowest seropositivity.

Scientific studies have documented that the antibodies do take some time to develop after an infection, approximately 1–3 weeks, with an average of 2 weeks (14 days). So, we can say that the rate of antibody positivity reflects the case scenario about 14 days prior to the study. Accordingly, considering the reported COVID-19 cases and seropositivity for each zone [Figure 4] show some correlation. Most zone shows wide gap between cases and seropositivity with Central zone showing the widest gap, but, North and East Zone show very little gap between the two. The comparison of cases can be compared one step further with wards and UPHCs. Accordingly, the analysis [Table 2] shows that the positivity for the top 10 UPHC/Ward correlate closely with the cases. UPHCs/wards with very high number of COVID-19 cases have higher seropositivity. Such UPHCs/wards include areas with higher number of cases in the recent past or in the earlier months at the beginning of the pandemic in the city.

**Discussion**

The seroprevalence varies markedly because of a variety of factors. For the same reason, scientific studies have recommended continued surveillance through seroprevalence studies to estimate and monitor the growing burden of COVID-19. Multiple sero-surveillance studies which have focused on antibodies against SARS-CoV-2 have been found to be extremely useful in understanding the progress of the pandemic. The present study on the seropositivity among general population from Ahmedabad is probably one of the few large scale serological studies from India with a sample size beyond 10,000. As on August 2020, the seropositivity for IgG antibodies against COVID-19 in Ahmedabad is approximately 23.24%. This level of seropositivity in general population indicates the overall level of immunity protection as of August 2020, which is important to know for a primary care physician and public health experts for better understanding and proper management of the pandemic. Available results suggest that a majority of the total population has not yet suffered from the disease and has not yet developed the immunity and may still be largely susceptible. Our results of seropositivity are also consistent with other studies showing that even in the areas highly affected by SARS-CoV-2 during this COVID-19 pandemic, have shown very low level of seropositivity.

The seropositivity is significantly higher among females as compared to males. This is in contrast to findings in other similar studies where it is not found to be significant. The reasons for higher seropositivity among females need to be studied in detail to find out the scientific reasons or explanations for the same.

The statistic of mode < median < mean typically shows skewed distribution. This indicates that the distribution had many young adults as compared to elderly and the mean is deviated on the right because of very high values of comparatively small number of elderly contacts with age more than double of the mean age. The seropositivity in the general population for various age group, is seen between 20 and 35% and shows an increasing trend. The linear trend-line suggests that the higher positivity is seen with increase in the age group. This may be because of higher proportion of asymptomatic/mild symptomatic individuals among young adults as compared to the elderly who are more likely to be symptomatic, have severe symptoms and symptomatic phase lasting for a longer duration. This difference may be the reason behind the increasing trend in positivity with increasing age.

The zone wise positivity ranged from 11.74% to 33.14%. This closely correlates with the cases recorded so far, higher for those zones with high current or past cases. Seropositivity for the

**Table 2: Top ten UPHC & Ward with highest seropositivity in General population**

| UPHC of AMC     | Total | Positive | Positivity % | Ward of AMC     | Total | Positive | Positivity % |
|-----------------|-------|----------|--------------|-----------------|-------|----------|--------------|
| JAMALPUR (CZ)   | 87    | 52       | 59.77        | BAPUNAGAR (NZ)  | 109   | 53       | 48.62        |
| NARODA ROAD (NZ)| 94    | 50       | 53.19        | JAMALPUR (CZ)   | 169   | 75       | 44.38        |
| KALUPUR (CZ)    | 71    | 37       | 52.11        | INDIA COLONY (NZ)| 221   | 98       | 44.34        |
| BAPUNAGAR (NZ)  | 109   | 53       | 48.62        | AMRAIWADI (EZ)  | 174   | 75       | 43.10        |
| NEW BEHRRUPURA (SZ)| 105  | 47       | 44.76        | THAKKARNAGAR (NZ)| 70    | 30       | 42.86        |
| AMRAIWADI (EZ)  | 174   | 75       | 43.10        | KHADIA (CZ)     | 146   | 62       | 42.47        |
| SAIJPUR (NZ)    | 91    | 39       | 42.86        | ASARWA (CZ)     | 88    | 34       | 38.64        |
| THAKKARNAGAR (NZ)| 70   | 30       | 42.86        | SARASPUR-RAKHIAL (NZ)| 433  | 161      | 37.18        |
| RAKHIAL (EZ)    | 230   | 98       | 42.61        | DANILIMDA (SZ)  | 130   | 45       | 34.62        |
| ASARWA (NZ)     | 88    | 34       | 38.64        | SAIJPUR BOGHA (NZ)| 196  | 67       | 34.18        |
earliest and worst affected zone (Central zone) has lower level of seropositivity as compared to the zones affected recently. This might be pointing toward the fact that this immunity may not be long lasting and this need further research to cross verify with scientific evidences to prove this observation.

**Conclusion**

As of August 2020, the overall level of IgG antibodies against SARS-CoV-2 virus in the general population of Ahmedabad is 23.24%. This proportion of people having IgG antibodies against SARS-CoV-2 indicate that the majority of the population may still be susceptible. We cannot rely on the present level of immunity to extend the protection to the general population. Preventive measures still need to be strongly emphasized again and again till an effective vaccine is provided to the people at large.

The seropositivity is seen higher among females. The age group wise positivity shows an increasing trend with the increase in the age group. The seropositivity is seen The Zone wise, ward wise and UPHC wise positivity correlates closely with the current or past cases from that area.

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**Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

**Key Messages**

The crude seropositivity of 23.24% suggests that the population is still largely susceptible. We still need to apply public health preventive measures for effective control of the COVID-19 pandemic. The results also indicate the scopes for further research to confirm and generate greater evidences regarding the factors affecting seropositivity.
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Conflicts of interest
There are no conflicts of interest.

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