



Covid-19 Serosurveillance Positivity in General Population: Comparison at Different Times

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ABSTRACT

Introduction: Serological surveys estimating the cumulative incidence of the disease are powerful and effective tools for monitoring the epidemic and in determining the immunity status.

Objectives: To compare the percentage sero-positivity for IgG antibodies against SARS-CoV2 at two different time period in the same population to understand the pandemic and predict about the immunity status of the population.

Methods: As a part of Covid19 pandemic management, two separate population based sero-survey within a gap of 1½ months were carried in Ahmedabad city to scientifically document the progress of the Covid19 pandemic. Various demographic factors and other parameters from both the survey were compared with seropositivity for valid and precise estimation of disease situation as well as immunity status of the population.

Results: The study documents an increase in seropositivity by 5.32% (from 17.92% to 23.24%). The seropositivity shows increasing trend with increase in the age group and the seropositivity is significantly higher among females. Overall higher seropositivity against the reported cases in the first sero-survey and the narrow increase in the seropositivity during the subsequent sero-survey inspite of high number of cases may indicate temporary status of the antibodies. This may also be due to the difference in the level of stigma, health care service delivery, service utilization and related field level situation affecting the asymptomatic/unreported case positivity.

Conclusion: The result of seropositivity comparison indicates the scopes for further research to confirm and generate greater evidences regarding the factors affecting seropositivity.

Key words: SARS-COV2; Covid-19, Sero-surveillance; IgG Antibody, Immunity

INTRODUCTION

Covid19, the disease caused by SARS-CoV2, spread across the world during 2020.^{1,2} In the absence of past exposure, presence of antibodies in any individ-

ual indicates an immune response against the virus.³ In the presence of asymptomatic individuals, we cannot rely only on the data of symptomatic individuals who are more likely to get tested on account of

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their symptoms.⁴ Sero-surveillance uncover the asymptomatic as well as subclinical infection. It is an effective & powerful tool for monitoring the development and progression of the epidemic and in determining the increase in herd immunity.⁵ It thus helps primary care physicians in managing their patients and the public health experts in planning an appropriate public health response.^{6,7} For a newly identified agent, WHO has also suggested monitoring of sero-prevalence.⁶ Multiple sero-surveillance studies conducted during the pandemic have focused on antibodies against SARS-CoV2 in the general population.^{8,9} ICMR has also suggested periodic sero-surveillance for guiding the policy makers and issued directives to all the state governments to carry out IgG Elisa test for sero-surveys along with ILI & SARI Surveillance.¹⁰

A population based sero-survey when carried out in the same population at two different point of time can give a better idea about the disease trend as well as the factors affecting the immunity. A direct comparison of the seropositivity with the cumulative incidence of the disease can help in predicting the disease situation as well as disease trend. A change in seropositivity over a fixed time period gives a rough estimate of the population additionally affected by disease and protected during that time period through clinical cases or through asymptomatic infections. A comparison of the seropositivity with the number of cases registered during the sero-surveillance time gap helps in estimating proportion of unreported cases during the same time period.

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.¹¹ Two different population based large scale sero-survey were carried out in Ahmedabad during the second half of June and second half of August 2020. Results of these 2 sero-surveys among the general population were compared with the following aims & objectives.

AIM AND OBJECTIVES

The aim of this study was to compare the population based Covid-19 sero-positivity in Ahmedabad city. The study was conducted with objectives to compare the seropositivity from 2 different sero-survey in the General population, to correlate the sero-positivity with various factors for better understanding of the pandemic situation, and to understand the change in the immunity status for valid & precise predictions for the future.

METHODOLOGY

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 such survey is to estimate the cumulative incidence for understanding the proportion of population exposed to SARS-CoV-2 infection.

Ahmedabad Municipal Corporation (AMC), from the state of Gujarat, INDIA, had already conducted one large scale population based sero-surveillance for IgG antibodies against SARS-CoV-2 Virus during the second half of June 2020 with a population-based sample of 28402 people.¹² After a gap of one and half month, during the second half of August 2020, another sero-survey with population based stratified sampling of 10310 individuals from general population was carried out.¹³ Since both these surveys were based on the same general population from the same city and large enough to be representative, a direct comparison the results with the cases as well as population characteristics was possible. This can help in identifying the factors affecting the disease, seropositivity and the immunity. "Covid Kavach" (Anti-SARS CoV-2 IgG Antibody Detection ELISA) kits developed and manufactured by Zydus Diagnostics, validated by National Institute of Virology, Pune, India and approved for use by the Indian Council of Medical Research (ICMR) were used in both the sero-surveys which helped in restricting the bias in the seropositivity results due to differential test kits.

During both the surveys, sample population was selected from the field area of the UPHCs. Individuals were enrolled without any exception and 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. The second sero-survey also had other categories like cases of Covid19, Contacts of confirmed cases as well as Health Care Workers (HCWs) which were covered separately over and above the general population. This article covers and describes only the general population component from the second sero-survey. This article focuses on the findings of the scientific exercise comparing the seropositivity among general population of Ahmedabad from both the studies.

RESULTS

The first sero-survey in Ahmedabad was carried out during the second half of June 2020 and first week of July, with majority of the sample collection completed in June 2020. As a part of this study, a total of 30054 blood samples were collected from the Ahmedabad city. Out of these, 28514 samples were randomly collected from the general population, while the remaining samples were from Covid & non-Covid hospitals. After excluding the rejected samples (112), finally 28402 sample results from general population were available for the comparison. Out of these, 5091 samples tested positive giving a crude positivity of 17.92% (95% Confidence Interval (CI) 17.48%–18.38%).

During the second sero-survey a total of 10339 blood samples were collected from the general population.

Table 1: Comparison of Covid19 sero-survey positivity in General population

Variables	Study 1 (June '20)			Study 2 (August '20)		
	Results 28402	Positive 5091	% Positivity 17.92	Results 10310	Positive 2396	% Positivity 23.24
Age group						
0-9	92	17	18.48	67	13	19.40
10-19	1214	196	16.14	738	191	25.88
20-29	6955	1110	15.96	3024	606	20.04
30-39	6968	1170	16.79	2437	500	20.52
40-49	5781	1175	20.33	1758	401	22.81
50-59	4231	836	19.76	1263	354	28.03
60-69	2223	410	18.44	727	234	32.19
70-79	788	154	19.54	249	85	34.14
80-89	141	21	14.89	43	11	25.58
90-99	9	2	22.22	4	1	25.00
Zone						
CZ	3773	1077	28.54	844	267	31.64
EZ	3669	860	23.44	1849	443	23.96
NWZ	3662	236	6.44	1593	187	11.74
NZ	4798	1315	27.41	1687	559	33.14
SWZ	2983	402	13.48	819	155	18.93
SZ	3213	540	16.81	1690	404	23.91
WZ	6304	661	10.49	1828	381	20.84

Table 2: Comparison of Covid19 sero-survey positivity with reported Covid19 cases

Zone	Study 1 (June '20)		Study 2 (August '20)		Difference	
	Cases	% Positivity	Cases	% Positivity	Additional Case	% Positivity
CZ	3385	28.54	4342	31.64	957	3.10
NZ	1912	27.41	3995	33.14	2083	5.73
EZ	1658	23.44	3866	23.96	2208	0.52
SZ	2590	16.81	4322	23.91	1732	7.10
SWZ	525	13.48	2010	18.93	1485	5.45
WZ	1485	10.49	4531	20.84	3046	10.35
NWZ	388	6.44	2053	11.74	1665	5.30

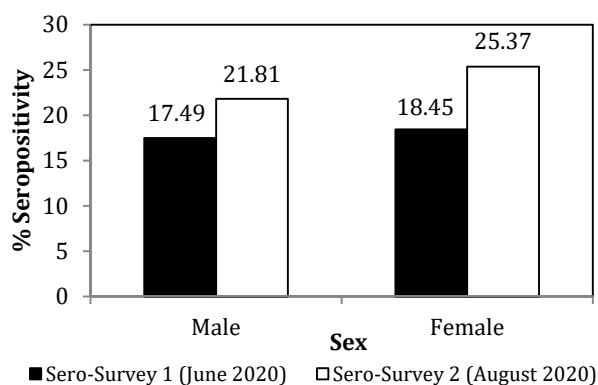
From these, 29 samples were rejected by the laboratories due to various reasons. Results were thus available for the remaining 10310 samples. From these, a total of 2396 results were positive for the specific antibodies against Covid19 giving an overall crude positivity of about 23.24% (95%CI 22.43%–24.06%)

With approximately 2 month's gap between the starting points of these surveys, (i.e. June 16, 2020 to August 16, 2020) the second sero-survey was carried out during the second half of August 2020. The comparison of the results of these sero-survey in general population (Table-1) shows that the seropositivity increased from 17.92% as per our earlier study to 23.24% with an average increase of about 5.32% in population seropositivity.

Since both the sero-survey were population based, carried out in the same general population with the same testing kit, a direct comparison of seropositivity and its correlation with various factor was possible. Sex wise comparison of the results of these sero-surveys (Figure-1) shows that among females the seropositivity increased from 18.45% to 25.37% with an average increase in seropositivity by 6.92%. Similarly, the comparison of the results of these sero-survey among males shows that the seropositivity increased from 17.49% to 21.81% with an average increase in seropositivity by 4.32%.

Thus, the trend of higher seropositivity among females have continued with increase in the gap of seropositivity between the two biological groups.

Comparing the Age-group wise positivity, the first sero-survey had positivity for various age group ranging from 14.89% to 22.22%. During the second sero-survey it ranged from 19.40% to 34.14%. The linear trend of both the sero-survey (Figure-2) showed increasing trend in seropositivity with increase in the age group. For the first sero-survey the linear trend had R^2 of 0.1429 whereas during the subsequent survey it had R^2 of 0.3405.

**Figure 1: Sex wise comparison of sero-surveillance positivity**

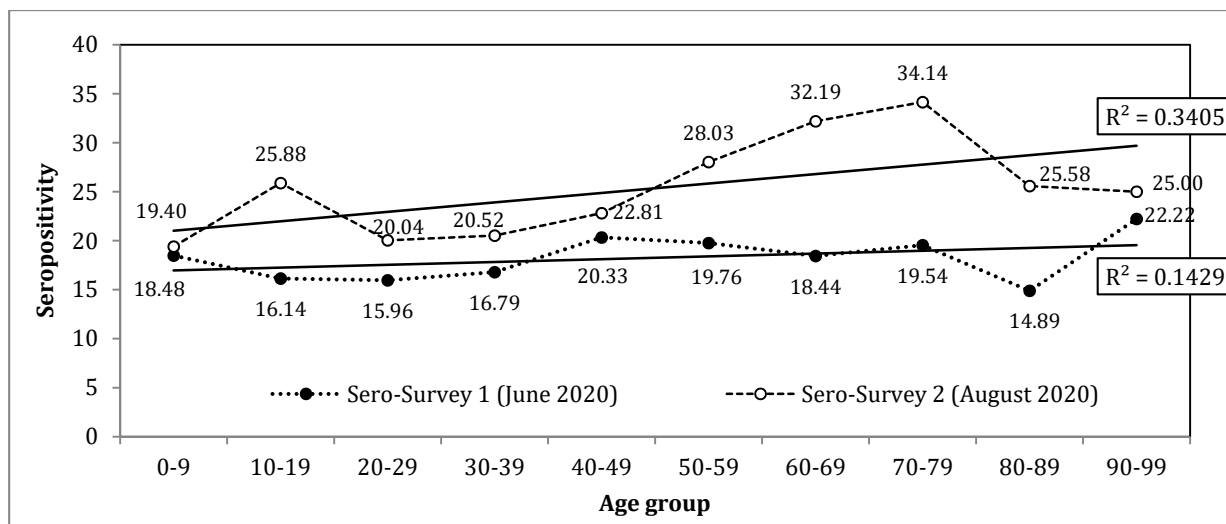


Figure 2: Age-group wise sero-surveillance positivity

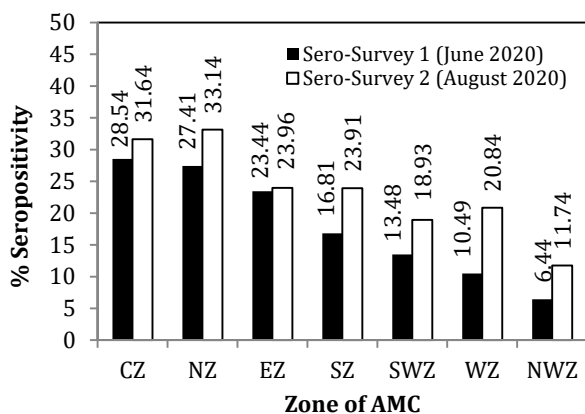


Figure 3: Comparison of zone wise seropositivity in the general population

Comparing the Zone wise positivity, the first sero-survey had positivity for various zone ranging from 6.44% to 28.54%. During the second sero-survey it ranged from 11.74% to 33.14%. The comparison of the zone wise seropositivity (Figure-3) showed that the sequence of zone according to seropositivity level almost remained same with minor changes and confirmed a close association between the findings of the two sero-survey.

During the intervening period between the two sero-survey, addition in confirmed cases of Covid-19 is likely to affect the seropositivity. Since the IgG take some time to appear after an infection, approximately 1 to 3 weeks, with an average of 2 weeks (14 days), the antibody positivity reflects the case scenario about 14 days prior to the study.^{14,15} So, the seropositivity for the first & second sero-survey correlate with cases by May-end and July-end respectively. Ahmedabad had reported 11943 Covid-19 cases by May end whereas the total reported cases by July end were 25119. The comparison of cases with seropositivity (Table-2) shows that the zone wise and total increase in percentage seropositivity

correlates very well with the increase in reported cases during the intervening time.

DISCUSSION

The seroprevalence varies markedly due to a variety of factors.¹⁶ So, it is important to know these factors before concluding disease trend on the basis of surveillance results. Scientific studies have also recommended repeated surveillance through seroprevalence studies to estimate and monitor the growing burden of Covid-19.¹⁷ Multiple sero-surveillance studies which have focused on antibodies against SARS-CoV2 have been found to be extremely useful in understanding the progress of the pandemic.^{9,18,19,20} Comparing the seroprevalence in the same general population at different times can add scientific value to its interpretations. For better understanding and proper management of the pandemic, it is important that the primary care physician and public health experts understand these factors and carefully interpret the trends.

The present study on the comparison of seropositivity among general population from Ahmedabad is probably one of the first few largescale serological studies from India with a sample size beyond 10000 and where population based seropositivity is compared and reported in the same general population. While many other sero-studies carried out during the similar time period in general population generally reported their seropositivity in single digit percentage, the seropositivity of 17.92% in the first sero-survey in June 2020 is an indirect evidence of large number of Covid-19 cases during the first half of 2020 early in the pandemic in Ahmedabad and justifies our statement that it was one of the leading cities from India reporting high number of Covid-19 cases. The increase in the average seropositivity by 5.32% during the subsequent sero-survey is justified with increase in cases during the

intervening time and corresponding increase in population with IgG antibodies against Covid-19.

With an average increase in seropositivity by 6.92% among females and an average increase in seropositivity by 4.32% among males, the result shows a consistent trend where females have significantly higher seropositivity than males, but also shows that the rate of increase is higher among females. The gap between the seropositivity among both the sex-groups widens further. The result differs from other sero-surveillance studies where both the sex groups have shown no significant difference.^{21,22} With large and representative samples in both the serosurvey and consistent results of significant difference between the two sex groups, indicates a detailed situational analysis to find out possible reasons behind this significantly higher seropositivity among females in Ahmedabad.

Considering the age group wise comparison, the seropositivity for all the age groups have higher seropositivity for the second sero-survey. With increase in the cases in the meantime, this is quite obvious. However, for various age groups the gap in seropositivity is small to very large. The seropositivity in the general population for various age group in both the sero-survey, particularly for the young adults to elderly, shows increasing trend with the increase in the age group. Comparing the linear trend-line and the R^2 values, the steep rise in positivity is more marked in the second sero-survey. This may be due to 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.^{23,24,25,26,27}

With around 12000 cases by end of May, the seropositivity in the first serosurveillance was reported to be 17.92% (95%CI 17.48%–18.38%). Considering this level of seropositivity in a city with around 70 Lakh population, approximately 12,00,000 individuals already have antibodies against SARS-CoV2. This indicates that a large proportion of asymptomatic/mildly symptomatic individuals developing the IgG antibodies. This finding is quite similar to other studies where large percentage of people infected with SARS-CoV-2 presented either no symptoms or relatively mild disease and so the infection was found to be much more widespread than indicated by the number of confirmed cases.^{28,29} There are also studies where for 1 symptomatic case as high as 95 asymptomatic individuals are assumed to be infected with the virus and subsequently developing the immunity.³⁰ However, the reasons were not very clear & satisfactory for the first sero-surveillance, where the proportion of undiagnosed (asymptomatic, pre-symptomatic or hidden infected) as against the reported cases has been very high.

The reason soon became clear with the 2nd Sero-surveillance. From first to second sero-survey, in spite of doubling the confirmed cases, the

seropositivity increased by a meagre 5.32%. This may be due to the temporary nature of the antibodies which disappear over a period of time.^{31,32} However, this may also be partly attributed to the earlier situation where large number of people were in lockdown, avoiding testing & clinical checkup, refused doorstep medical services and preferred to stay at home in spite of having symptoms, infecting many more in and around themselves. The second sero-surveillance represent the much-improved version of ground reality where testing was increased, medical facilities and services were more accessible than ever before, and proactive health seeking behaviour was observed among majority of the population along with reduced level of stigma. So, the accessibility of most of the symptomatic cases have improved and lead to a situation where large number of cases are reported with little improvement in the seropositivity.

The comparison of the zone-wise seropositivity ranged from 11.74%-33.14%. This closely correlates with the reported cases, higher for those zones with high current or past cases. Barring some exception, the increase in the seropositivity is closely related with the increase in the number of Covid-19 cases reported in the meantime. The reason of a relatively small increase in positivity against high number of additional cases in the East Zone is not clear and may simply be by chance.

CONCLUSION

This is probably the first-ever study representing the comparison of the sero-surveillance positivity from the same general population with a sample size above 10000 in both the surveys. The study documents a modest increase in seropositivity by 5.32% from 17.92% to 23.24%. The seropositivity among females is significantly higher and the gap has increased in the subsequent sero-survey. The seropositivity shows increasing trend with increase in the age group and the increasing trend is more marked in the subsequent sero-survey. The zone wise comparison is well correlated with the number of cases reported so far. Overall higher seropositivity against the reported cases in the first sero-survey and the narrow increase in the seropositivity during the subsequent sero-survey in spite of high number of cases may be due to temporary nature of the antibodies or it may be due to the difference in the level of stigma, health care service delivery, service utilization and related field level situation. The narrow increase in seropositivity indicate continued further emphasis on the public health preventive measures. A subsequent sero-survey positivity and its correlation may give more light on the factors affecting seropositivity. The results also indicate the scopes for further research to confirm and generate greater evidences regarding the factors affecting seropositivity.

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