

## ORIGINAL ARTICLE

## Serosurveillance among COVID-19 Cases in Ahmedabad Using SARS-COV2 IgG Antibodies

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### Abstract

**Background:** Serosurveillance study focusing on antibodies against SARS-CoV2 among the Covid19 cases can add value in the scientific knowledge & help in formulating valid predictions regarding immunity status in the post-covid period. **Objectives:** To estimate seropositivity among covid19 cases and to identify various factors affecting seropositivity. **Methods:** During second half of October 2020, a population based serosurvey on Covid19 cases was carried out in Ahmedabad. Covid-Kavach test kits were used and estimated seroprevalence was compared with available demographic and covid19 case related parameters to identify factors affecting seropositivity in the post-covid period. Simple proportions and Z-test were used as appropriate. **Results:** As on October 2020, the sero-positivity among Covid19 cases in Ahmedabad was 54.51% [95% Confidence Interval (CI) 52.14-56.86%]. Females have higher positivity (54.78%) as compared to males (54.30%) but the difference was statistically not significant (Z=0.19, P=0.84). Among children and elderly, the positivity is high and from young adults to elderly the seropositivity has an increasing trend. Severity of clinical illness and longer duration of hospitalization are associated with higher seropositivity. **Conclusion:** With 54.51% seropositivity among covid19 cases, it is clear that all the covid19 cases may not have developed IgG antibodies, have undetectable level or might have disappeared during the post-covid period. Comparison of seropositivity with age group and clinical case details clearly suggest close correlation with the severity of clinical symptoms. The seronegative cases indicate the need for further in-depth scientific research to identify the factors affecting immunity and to uncover the reasons behind the same.

### Keywords

COVID-19 cases; Immunity; IgG Antibodies; SARS-CoV2; Seroprevalence

### Introduction

Covid19, the disease caused by SARS-CoV2, spread across the world during 2020.(1,2) In view of the large number of asymptomatic cases, as also suggested by WHO, the indirect estimation of actual cases is crucial in assessing the true extent of the spread of SARS-CoV2.(3,4) Sero-

surveillance identifies the proportion of asymptomatic or subclinical infection and helps in understanding the disease dynamics in a better way.(5,6) This in turn helps in planning an appropriate public health response. Multiple sero-surveillance studies conducted during the pandemic have focused on antibodies against SARS-CoV2 in the general population.(7,8) Sero-surveillance studies among

covid19 cases can give scientific insight. Comparison of seropositivity among cases can add value in the scientific knowledge & help in formulating valid predictions regarding immunity status in the post-covid 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. We carried out a population based sero-surveillance during the second half of October'20. In this sero-surveillance, apart from the general population, covid19 cases were also included as an additional category along with health care workers and contacts of cases. This article covers and describes only the "Cases" component of the sero-surveillance study.

### Aims & Objectives

1. To estimate the seroprevalence among COVID-19 cases
2. To correlate the seropositivity with various demographic and other factors

### Material & Methods

With the objective of estimating the proportion of population exposed to SARS-CoV-2, Indian Council of Medical Research (ICMR) had issued directives for conducting IgG antibody based sero-surveys to monitor the pandemic, understand its progression and to take appropriate corrective public health measures. The authority of the Ahmedabad Municipal Corporation (AMC), from the state of Gujarat, INDIA, already completed two large scale population based sero-surveillance for IgG antibodies against SARS-CoV-2 during June & August 2020. A repeat population based sero-survey, 3rd serosurvey in Ahmedabad was carried out during the second half of October 2020. The sample size for the general population category was calculated based on the results of the previous studies using the population based stratified sampling. Confirm cases of Covid-19 were covered separately along with population based sero-survey. Sample size for the "Case" category was decided as at least 10% of the general population sample targets, and covered separately over and above the general population sample. Thus, the "case" selection was unrelated to the covid19 cases and based on population proportion. To enroll sufficient number of cases from different time period since the pandemic, UPHC medical officers were advised to enroll cases registered during different months since the beginning of the pandemic.

'WHO' has given the definition of a case of covid19, including the suspected, probable and confirmed case.(9) We used the confirmed case definition of covid19 for the purpose of our study. "Covid Kavach" (Anti-SARS CoV-2 IgG Antibody Detection capture ELISA) kit developed and manufactured by Zydus Diagnostics, validated by National Institute of Virology, Pune, India and approved for use by ICMR was used for the purpose of this study. Covid-Kavach has sensitivity of 92.37% and specificity of 97.9% as per

the validation reports.(10) Thus, with a high level of sensitivity and specificity, the results received through this testing kit is quite reliable. The manufacturer reported no cross-reactivity with other viruses in the serum from RTPCR confirmed patients of various other infections. Testing procedures were followed as per the manufacturer's instructions. To reduce the sample rejection rate, SST-Gel Vacutec were used for the collection of blood samples.

The primary health care network in Ahmedabad city includes 75 Urban Primary Health Centres (UPHCs) within 48 wards, across 7 zones. Since the UPHCs are functional units for the covid19 case management, these 75 UPHCs have the details of all the reported cases from their area. Based on the calculated sample size, COVID-19 cases of either sex diagnosed at any point of time, who gave informed written consent, were enrolled as 'case'. However, so far as possible, an effort was specifically made to cover a wide variety of cases from different time period since the beginning of the pandemic, of different age-group (with at least 10% of cases from <18 years and >60 years age) from different localities within the field area of the UPHC.

The study was carried out after the approval of ethics committee of AMC MET Medical College. A written informed consent was taken from all the participants before enrollment & in case of minors, an assent with informed written consent of their parent/guardian was taken for the purpose of the study. Strict confidentiality was ensured at all the levels. For the purpose of testing and standardization, only those laboratories with national level accreditation and state of the art facilities and equipment were approved for testing the samples. Microsoft Excel and Epi-Info was used for the purpose of data management. In-depth analysis of the data was carried out with focus on the seropositivity and its correlation among cases with various demographic and other factors. Simple proportions and appropriate statistical tests were used wherever required.

### Results

A total of 1711 (745 female, 966 male) samples were collected out of which 3 samples were rejected (2 Female & 1 Male). Results were thus available for 1708 cases. From these results 758 (44.38%) were negative and 19 (1.11%) had indeterminate results. Thus, a total of 931 results were positive for the IgG antibodies giving an overall positivity of 54.51% [95% Confidence Interval (CI) 52.14-56.86%].

Detailed analysis of 1708 case ([Table-1](#)) show that there were 743 females and 965 males for whom results were available. A total of 407 samples were positive among female giving a positivity rate of 54.78% [95%CI 51.18–58.32%] whereas 524 samples were positive among male giving a positivity of 54.30% [95%CI 51.15–57.42%] This

difference between two sex group is statistically not significant ( $Z=0.19$ ,  $P=0.84$ ).

The age distribution of the cases typically follows age-heaping bias at 5 years gap (data not shown, only grouped data shown in data table) as the age of the enrolled individual cases were not verified with any official document. The age of the cases ranged from 3-93 years with a mode of 40, median of 40 and an average of 41.67+16.23 years. Considering the sero-positive cases, the mean age for females is 44.82+15.80 years where as that of male is 44.76+17.06 years.

The age group wise analysis of positivity among cases ([Figure-1](#)) shows that the lowest positivity is for 20–29 years i.e., 39.14%. Children had higher seropositivity than young adults. From young adults to elderly the seropositivity gradually increases from around 40% to around 70%. On both the extremes of age range, i.e., in children and elderly, the positivity is high as compared to the young adults. When the age group wise positivity is further bifurcated according to sex, the trend almost remains similar with variations at both the extremes of the age. Analyzing the zone wise seropositivity, the highest positivity was recorded in South Zone (71.67%) and the lowest in the South West Zone (40.15%). All the other zones recorded seroprevalence among cases in a narrow range of 51.88% to 58.55%.

Looking into details of patient management ([Figure-2](#)) cases requiring hospital admission had higher seropositivity (72.31%) as compared to cases managed at Covid Care Centre (CCC) (52.38%) and cases managed at home had the lowest seropositivity (49.41%). Among the hospitalised cases, patients on ventilator had the highest seropositivity (100%) as compared to patients requiring Oxygen (81.58%) with cases not requiring oxygen had the lowest seropositivity (70.91%). According to duration of hospitalization the seropositivity for <1 week, 1-2 week, 2-3 week & >3 week were 62.35%, 73.30%, 75.76% & 100% respectively.

We also analyzed the time gap between the date of sero sample collection to the date of diagnosis of Covid19. While comparing the time since diagnosis in months, ([Figure-3](#)), IgG antibodies were detected in the range of 50-55% during the first 4 months of time-gap. For 5 months and 6 months it was 69.70% & 61.54%.

## Discussion

General immune response after any viral infection has already been documented. However, as Covid19 is a novel viral infection, the immune response during and after covid19 infection is still largely evolving.(11,12) The present study on the seropositivity among cases is among the few serological studies from India, exclusively covering the cases of covid-19 cases with a large sample.

Seropositive covid19 cases are the laboratory confirmed cases, who demonstrate IgG antibodies after the infection. This is a direct evidence of immune response as

a result of their infection. While seropositive cases directly indicate proportion of cases who have acquired immune response, the seronegative cases indicate the proportion of cases who did not demonstrate IgG antibodies inspite of having a confirmed infection status in the past. “What are the factors affecting this seropositivity?” is the real question which the experts are trying to answer with scientific evidences. The present study also tries to highlight the proportion of cases with IgG antibodies and its correlation with the duration, severity and time-gap since diagnosis, if any.

It is quite obvious that one would expect all the confirmed cases of Covid19 to have IgG antibodies against the disease agent, SARS-CoV2. However, based on our findings with an average seropositivity of 54.51% [95%CI 52.14-56.86%] among cases, it can be said that majority of the cases demonstrate presence of IgG antibodies after the infection with SARS-CoV2. However, it also implies that the remaining 45.49% have either not developed the antibodies, have antibodies but in undetectable proportion or the antibodies have disappeared, after their development, during the post-covid period. The reasons for seronegative cases require further in-depth scientific research to identify the factors affecting immunity and to uncover the reasons behind the same.

Among the cases, females have higher positivity (54.78%) as compared to males (54.30%) but the difference was statistically not significant ( $Z=0.19$ ,  $P=0.84$ ). This finding signifies that the difference is by chance and the factors affecting immunity seems to be affecting both the biological groups equally. Similar is the finding by other studies, where the difference between the two sex groups is statistically not significant.(13,14)

Since the sample size for cases was calculated based on the population proportion (independent of the cases recorded from each UPHC/Ward), the proportion of seropositive cases should not differ much. Although most zones have seropositive proportion between 51.88-58.55%, the reasons for low seropositivity in West Zone (40.15%) and high seropositivity in South Zone (71.67%) are not clear. This may indicate that there may be multiple other factors affecting positivity in covid19 cases & require further analysis for other factors affecting seropositivity.

Contrary to the general belief that any case of viral disease will have antibodies against the virus in the immediate post-infection period, as per our study the seropositivity among covid19 cases was not 100%. So, it was important to identify the reasons for the same and analyse data to identify the relationship of the factors affecting the seropositivity. There are already documented evidences that the severity of the clinical symptoms affects the immune response. Duration of hospital stay, need for Oxygen / Bipap / Ventilator as part of case management as well as place of case management (home / Covid Care Centre (CCC) / hospitalization) are all directly related to

the severity of clinical symptoms. We tried to analyse available details of the covid19 cases on these parameters to check this association.

Looking at the age-group wise seropositivity (Figure-1), children and adolescent have the higher seropositivity. Most children and young adolescents are more likely to have mild clinical illness which may be due to their strong immune response which leads to development of IgG antibodies in the immediate post-covid period. On the other hand, the seropositivity of about 40% in 20-29 years age group adults indicate that less than half from the young adult age group demonstrate IgG antibodies in the post-covid period. It also shows that the positivity has increasing trend as the age group increases from young adults to elderly. There are also scientific evidences which show that the elderly people are more likely to have symptoms, have more severe symptoms and their period of clinical symptoms stays for longer duration as compared to the young adults.(15,16,17,18,19,20) These differences in the clinical symptoms may be the reason behind the higher seropositivity among elderly as compared to the young adults.

Available evidences suggest that the percent seroconversion in asymptomatic cases is low.(21,22) On verifying this fact with our data, it was found that those requiring hospital admission demonstrated higher seropositivity than those cases who were isolated at home. The patient management principle required for these cases also showed that as the severity of case increase (from not requiring oxygen, requiring oxygen, Bipap to ventilator) the proportion of seropositive cases increases. Even the duration of hospitalization showed that as the duration of hospitalization increases the proportion of seropositive cases increase and all cases requiring hospital admission beyond 3 weeks were seropositive.

It has been documented that onset of symptoms should be preferred rather than the date of diagnosis for consideration of this time-gap.(16) However, due to higher reliability of data, we preferred comparing the date of diagnosis over the onset of symptoms. Comparing the proportion of seropositive cases with time since diagnosis (in months) (Figure-3), we observed that the seropositivity stays between 50-55% during the first 4 months of time since diagnosis. The higher seropositivity for 5 & 6 months from diagnosis coincides with the first peak of cases in the city. The relationship of seropositivity with the time since diagnosis is not very clear and require detailed scientific inquiry to understand the dynamics of immune reaction in the post covid period.

## Conclusion

As on October 2020, with 54.51% seropositivity among covid19 cases, it is clear that many covid19 cases may not have developed IgG antibodies, have undetectable level or might have disappeared during the post-covid period.

Sex wise difference in seropositivity is statistically not significant. Both the extremes of age show higher seropositivity and from young adults to elderly the proportion of positivity shows increasing trend. The severity of clinical symptom is clearly related with more pronounced immune response. The seronegative cases indicate the need for further in-depth scientific research to identify the factors affecting immunity and to uncover the reasons behind the same.

## Recommendation

All the confirm cases of Covid19 may not have demonstrable IgG Antibodies. Seronegative cases indicate the need for further scientific research

## Limitation of the study

We have collected limited demographic details of the enrolled Covid19 cases so that only limited analysis on serosurveillance result was possible. The limitations of the testing kit "Covid-Kavach" automatically applies to the findings of our study.

## Authors Contribution

OP, JS, SV & AS conceptualized the study. BS, DM and their team under the guidance of OP planned and carried out the serosurvey. Testing of the samples and reporting was managed by MK and her team. Data analysis was carried out by JS, SV, AS. The statistical analysis was done by HT. Primary manuscript was prepared by OP and JS and equally contributed by all the other coauthors. All authors contributed to the interpretation of data and approved the final manuscript after critical review.

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**Tables**

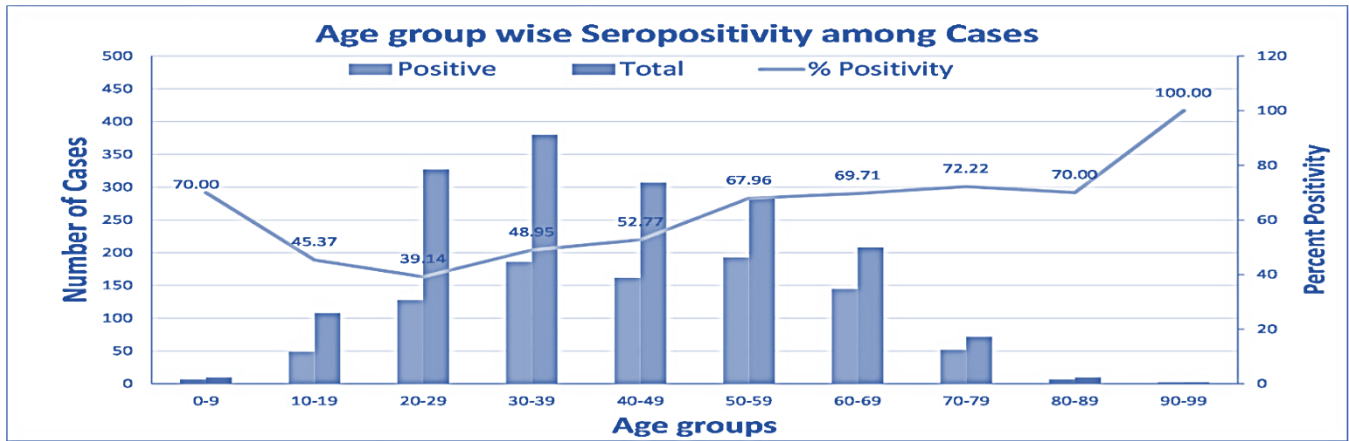
**TABLE 1 ANALYSIS OF COVID19 SERO-SURVEY POSITIVITY AMONG CASES**

	Female			Male			Total			95% CI
	Results	Positive	% Positivity	Results	Positive	% Positivity	Results	Positive	% Positivity	
<b>Total</b>	743	407	54.78	965	524	54.30	1708	931	54.51	52.14 – 56.86
<b>Age group</b>										
0-9	4	2	50.00	6	5	83.33	10	7	70.00	34.75 – 93.33
10-19	49	23	46.94	59	26	44.07	108	49	45.37	35.76 – 55.24
20-29	145	50	34.48	182	78	42.86	327	128	39.14	34.01 – 44.53
30-39	154	78	50.65	226	108	47.79	380	186	48.95	43.96 – 53.96
40-49	137	78	56.93	170	84	49.41	307	162	52.77	47.18 – 58.28
50-59	130	92	70.77	154	101	65.58	284	193	67.96	62.19 – 73.35
60-69	96	67	69.79	112	78	69.64	208	145	69.71	62.98 – 75.87
70-79	23	13	56.52	49	39	79.59	72	52	72.22	60.41 – 82.14
80-89	3	2	66.67	7	5	71.43	10	7	70.00	34.75 – 93.33
90-99	2	2	100.00	0	0		2	2	100.00	15.81 – 100.0
<b>Zone</b>										
CZ	85	48	56.47	96	53	55.21	181	101	55.80	48.25 – 63.16
EZ	119	61	51.26	108	60	55.56	227	121	53.30	46.59 – 59.93

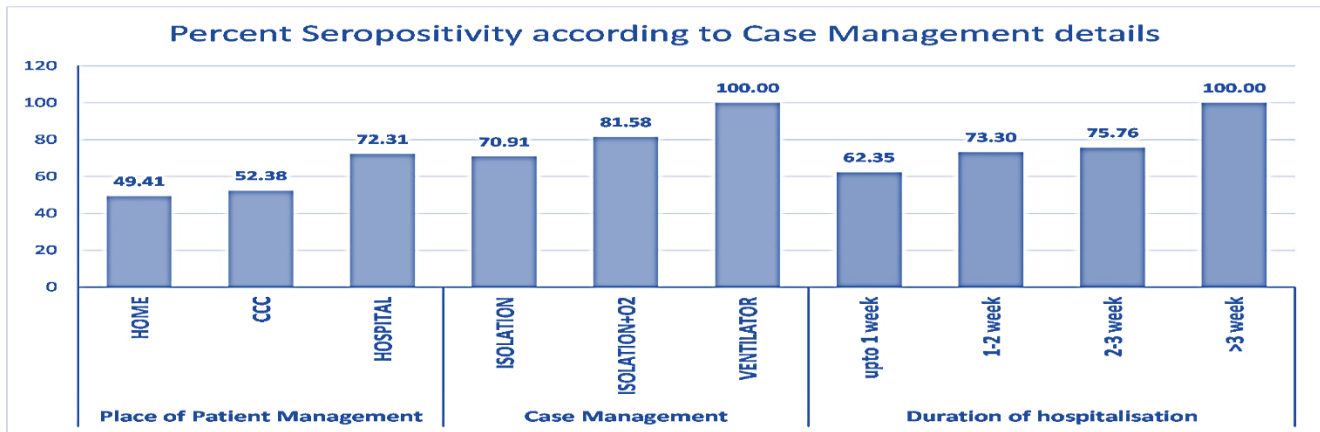
	Female			Male			Total			95% CI
	Results	Positive	% Positivity	Results	Positive	% Positivity	Results	Positive	% Positivity	
NWZ	35	23	65.71	98	46	46.94	133	69	51.88	43.05 – 60.62
NZ	170	95	55.88	151	85	56.29	321	180	56.07	50.61 – 61.40
SWZ	64	32	50.00	88	57	64.77	152	89	58.55	50.29 – 66.48
SZ	123	84	68.29	170	126	74.12	293	210	71.67	66.14 – 76.76
WZ	147	64	43.54	254	97	38.19	401	161	40.15	35.47 – 45.02

**Figures**

**FIGURE 1 AGE GROUP WISE SEROPOSITIVITY AMONG CASES**



**FIGURE 2 SEROPOSITIVITY ACCORDING TO CASE MANAGEMENT DETAILS**



**FIGURE 3 SEROPOSITIVITY AMONG CASES ACCORDING TO TIME SINCE DIAGNOSIS IN MONTHS**

