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Population based Seroprevalence of SARS-CoV-2 Specific IgG Antibodies in Rural Ahmedabad – A Case Control Study

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1. Introduction

Coronavirus disease (COVID-19) is an infectious disease caused by the most recently discovered coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The disease was declared as a Public Health Emergency of International Concern and later as a global pandemic by the World Health Organization. [1]Community serological surveys are aimed at estimating prevalence of infection in a given region and thereby guiding strategies to prevent its spread. They are based on detecting antibodies (generally the category called immunoglobulin G, or IgG) in the population of the region. The presence of antibodies in an individual is indicative of that person having had infection in the recent past (that is, 10-14 days ago). The estimate of the population share with antibodies (or the seropositivity rate) tells one the level of prevalence of infection in the region. Sero-surveys also tell one about the prevalence of asymptomatic infection in the region. The immune system mounts a defence against an infection by producing antibodies specifically against the invading pathogen, with the IgGs, in particular, lasting for fairly long. Recent studies on patients who recovered from COVID-19 suggest that IgGs against SARS-CoV-2 may last up to six to eight months. [2]Antibodies develop against different proteins that are part of the virus. Antibodies against one type of viral protein might neutralise the virus by blocking its entry into cells and preventing it from multiplying, while others might not.

The health authorities started extensive case-detection and contact-tracing activities. Case-detection was based on the testing of nasopharyngeal samples by reverse transcriptase-polymerase chain reaction (RT-PCR) [3].First case of Ahmedabad came on 19th March 2020,with a travel history from U.K.[4]

The seropositivity among those who were COVID-19 infected usually fluctuates from 40% to 60% during the12 weeks period. Since there are few cases from the very early phase of pandemic (march to mid may), there are higher fluctuations for week 20 or more. [5]If it is known that the antibodies detected in the population are of the virus-neutralising kind, then the health authorities can focus their infection control strategies on regions that have low seropositivity rates rather than those that have high seropositivity rates. [3] Detection of infection by the use of SARS-CoV-2 specific IgM and IgG antibodies has several advantages. As compared to RT-PCR based detection of infection, the antibody-based tests are cheaper and faster. They also pose less danger of infection for health workers since patients may disperse the virus during respiratory sampling. Also, blood samples show reduced heterogeneity compared to respiratory specimens [3] [6].Besides, the presence of IgG antibodies gives a clue to the presence of humoral immunity to SARS-CoV-2. However, both B and T cells may provide immune-mediated protection to viral infection. [7]

Seroprevalence studies provide important complementary information to frame evidence-based strategies for SARS-CoV-2 infection prevention.

Similar approach is also explored in understanding the efficacy of vaccination against Covid-19 and serosurveillance for the same.

Here, we present the results of a case control seroprevalence study in rural Ahmedabad, conducted on the patients of months June, august and September 2020, to estimate the prevalence of IgG antibodies against SARS-CoV-2 among adults in all talukas of Ahmedabad. The antibody testing was done in December 2020. [3]

Aim and objectives

1) To know the prevalence of Covid-19 antibodies in age and sex approach in all talukas of Ahmedabad.
2) To know the efficacy of active surveillance in the district.
3) To assess and correlate the seroprevalence with herd immunity.

2. Methods and Materials

Study design, setting, and participants:
We conducted a seroprevalence study in rural part of District Ahmedabad in December 2020. It has an estimated 16, 84,684 population in 2020. The study was retrospective case control study. All nine talukas of Ahmedabad district was covered.

Selection of participants:
Antibody testing was done on three different sets of participants:

1) RT-PCR confirmed Covid -19 patients of months june, august and September 2020(299 cases)
2) Close contacts of those 299 RT-PCR confirmed cases(1430 contacts)
3) RT-PCR negative patients as control(1143 controls)

Thus total of 2872 antibody testing were done.

Definition of Case: RTPCR conformed case of June, August and May 2020 of Ahmedabad District.

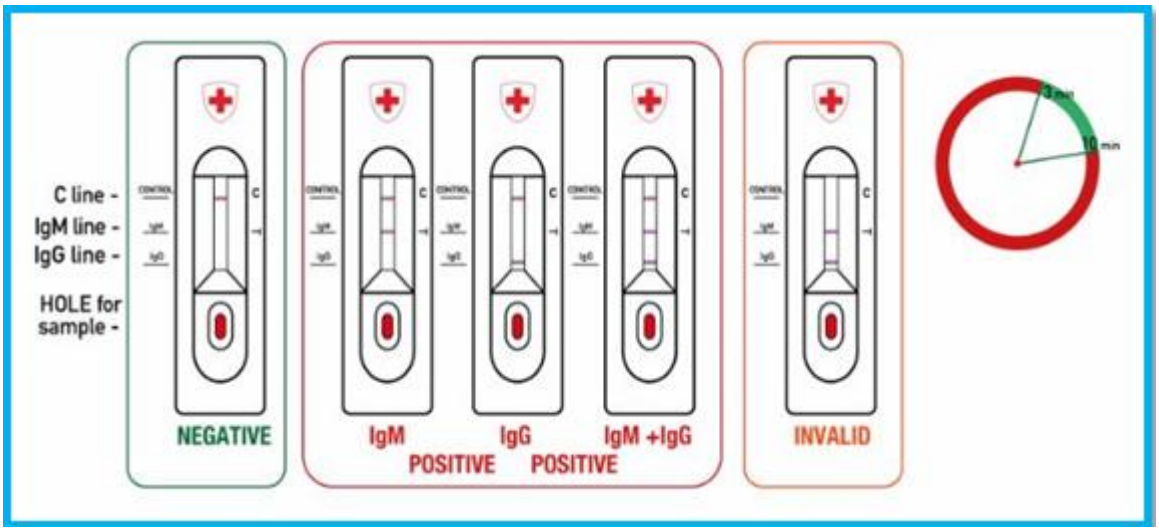
Close contact: Close contact of case may be family contact, Household contact, Office/Workplace Contact, Health Worker Contact

Control of Case: Should have exposed to positive case of significant period of time and negative up till now.

The rapid test for SARS-CoV-2 diagnosis provides qualitative detection of IgG and/or IgM from human serum, whole blood or plasma in approximately 10-15 minutes. The rapid tests are based on the principle of lateral flow immunoassay chromatography and are available in cassette form. The test is based on the separation of the components of a mixture through a medium using capillary force and the specific and rapid binding of an antibody to its antigen.

The level of IgM antibody begins to rise after 1 week after the initial infection, while the IgG appears later than IgM (usually in 14 days after infection) and can last for 6 months or even several years, which means that the IgG serves as an indicator of previous infection. Suspected patients that are infected by SARS-CoV-2 can be rapidly identified by simultaneous monitoring of IgM and IgG.

To be validated this test have to present a positive line for control (C)	
Result	Interpretation
IgM+/IgG+	Recent infection with SARS-CoV-2
IgM+/IgG-	Recent infection with SARS-CoV-2
IgM-/IgG+	Previous infection with SARS-CoV-2
IgM-/IgG-	No infection or not enough detectable antibodies in the early infection



3. Results

Total cases up to 31st January were 4053 and in our study we validated 2301 cases from the month of June, August and September of 2020.

Out of 299 total RT-PCR positive cases of Covid-19 in months of June, august and September, only 232 valid test samples were assessed. Out of these, maximum positive cases were detected in 30-59 years of age group (71 cases) as compared to other age groups. Similarly antibody positive cases found in males were double the number of cases found in females as per 'Table 1'. How so ever no statistical association was found between positive cases and age and gender profile. 'Table 2' shows Taluka wise distribution of positive cases where Sanand taluka has maximum positivity rate (77%); maybe due to close proximity to Ahmedabad city. The second highest positivity of cases being in Bavla and Dholka.

Out of total 1430 samples of close contacts of RT-PCR positive patients, we could get only 1104 valid samples for antibody testing. 'Table 3'showsmaximum antibody positivity was found in younger age group i.e. from 0 to 29 years (104 positive) and minimum in age group above 60 years. Taluka wise distribution shows Mandal taluka with maximum antibody positivity (63%) followed by Sanand taluka as seen in 'Table 4'.

Out of total 1143 RT-PCR negative samples, we could get only 965 valid samples for antibody testing. Out of these maximum antibody positivity was found in age group from 30 to 59 years (52 positive) as seen in 'Table 5'. Among the taluka, maximum antibody positivity was found in Dascroi taluka as depicted in 'Table 6'.

'Graph 1' shows the age and gender wise distribution of antibody positivity rate while 'Graph 2' highlights the gender and taluka wise distribution of antibody positivity cases.

4. Discussion

The results of our study provide an approximate estimate of the prevalence of IgG antibodies against SARS-CoV-2 in Ahmedabad rural. The prevalence estimates depend on the stage of the epidemic in the area at the time of the study and the accuracy of the antibody detection test used. The rapid test for SARS-CoV-2 diagnosis provides qualitative detection of IgG and/or IgM from human serum, whole blood or plasma in approximately 10-15 minutes. [3]In our study we have usedthe rapid tests based on the principle of lateral flow immunoassay chromatography which are available in cassette form. The test is based on the separation of the components of a mixture through a medium using capillary force and the specific and rapid binding of an antibody to its antigen. Anti-SARS-CoV-2 IgM and IgG can

therefore be detected in samples from affected patients. IgG antibodies appear, on an average, 10–11 days after symptoms or two weeks after infection [6]and are maintained at a high level for an extended period [7]Immediately after infection, the IgG titres are negative and thus do not help in the diagnosis of the infection in the early stage. A combination of IgM and IgG antibody tests is more helpful. That is why we selected the samples from month of July, august and September and conducted the antibody test in month of December.

In our sample of RT-PCR positive antibody was detected in 42% of sample (n=107), in RT-PCR negative antibody was detected in 12% of sample (n=113) and among the close contacts of RT-PCR positive patients 21% of sample (n=236) shows antibody.

With 19.8% seropositivity of our total sample(n=2301) we are consistent with other studies showing that even in the areas highly affected by SARS CoV-2 during this pandemic have shown very low level of seropositivity. Several seroprevalence studies conducted across the world have reported prevalence ranging from 1% in California to 23% in Delhi. [10]A national seroprevalence study from India documented the national seroprevalence of just 0.73% while another study by ICMR(during December) found 55% seroprevalence in the containment zone. [11]

Overall among the 232 confirmed Covid-19 cases sampled as part of study, only 46% were detected positive for antibodies as against the 56% positivity found in another seroprevalence study in Ahmedabad. It can be probably due to the association with the time duration from their diagnosis of Covid-19 as they might have lost their antibodies over the long time-gap or it may be on account of the severity of their clinical illness. [12]

We did not find any significant difference in seroprevalence among males and females in all the three age groups. Similar finding have been observed in a study conducted in Srinagar[3]and also outside India in Spain. [13]

People 30-59 years of age in all the three study groups had a higher antibody positivity as compared to the age group of 0-29 years and those above 60 years. This finding is consistent with that of the study conducted in Srinagar [3]. However another study conducted in Ahmedabad showed seropositivity to be highest in children and elderly. [5]The presence of Influenza like illness symptoms in the recent past was the factor most strongly associated with the presence of SARS-CoV-2 specific IgG antibodies. People with a recent history of Influenza like symptoms had a 3.7 times higher chance of showing evidence of SARS-CoV-2 infection as compared to those without such history. [3]

Also the difference in the positivity rate as per the talukas in rural Ahmedabad is statistically not significant but it was found that the taluka in the vicinity of the Ahmedabad city showed greater antibody positivity (41% in Sanand and 24% in Dholka).
Considering 25% of seropositivity in our sample, we estimate that in 16 lacs of rural Ahmedabad [14] population nearly 4 lacs people will have antibody present in them. Up till 31st January 2021 we have total 4053 Covid-19 positive cases in rural Ahmedabad. So with above estimation of antibody we might have missed 3, 95,947 cases in general population.

5. Limitations

The selection of study participants was not completely random, and this could have led to an overestimation of the seroprevalence estimates.

We, hopefully, reduced the bias by providing age- and gender standardized estimates, but could not nullify or estimate the bias. Detection of SARS-CoV-2 specific IgM antibodies and simultaneous RT-PCR could have provided a better estimate of the current infection rate.

6. Conclusions

This is a study to assess the seropositivity during the COVID-19 pandemic from rural Ahmedabad, India which is a population based case control study. In view of these findings with the absence of an evidence of lifelong immunity after COVID-19 infection, it can be concluded that the population of Ahmedabad is still largely susceptible. As of now, we cannot rely on this level of immunity to protect and the preventive measures need to be strongly relied on till an effective vaccine is provided to the people at large. [10]There is no gender difference in seropositivity and there are also indications that these IgG may not be long lasting. Further in-depth scientific studies are required to give more insight for the future predictions.

Table 1: AGE Wise Distribution of Antibody Development RT-PCR Positive

Age	Male				Female				Total			
	Total Test	Vaild Test	Positive	Positivity	Total Test	Valid Test	Positive	Positivity	Total Test	Valid Test	Positive	Positivity
0-09	3	1	0	0	1	0	0	NA	4	1	0	0
10-19	4	2	0	0	3	3	1	33.3	7	5	1	20
20-29	33	20	6	30	14	9	1	11	47	29	7	24
30-39	62	50	26	52	24	18	8	44.4	86	68	34	50
40-49	38	32	15	47	14	11	3	27	52	43	18	42
50-59	31	25	9	36	16	13	10	77	47	38	19	50
60-69	22	18	13	72	18	15	9	60	40	33	22	67
70-79	12	11	5	45	2	2	0	0	14	13	5	38
80-89	1	1	0	0	1	1	1	100	2	2	1	50
90-99	0	0	0	NA	0	0	0	NA	0	0	0	NA
Total	206	160	74	46	93	72	33	46	299	232	107	46

Table 2: Taluka & Gender Wise Distribution of Antibody Development RTPCR Positive

Taluka Name	Male				Female				Total			
	Total Test	Valid Test	Positive	Positivity	Total Test	Valid Test	Positive	Positivity	Total Test	Valid Test	Positive	Positivity
Bavla	43	29	15	52%	16	10	3	30%	59	39	18	46%
Sanand	51	39	27	69%	26	21	15	71%	77	60	42	70%
Dholka	40	30	13	43%	15	13	8	62%	55	43	21	49%
Virmgam	25	25	4	16%	13	11	3	27%	38	36	7	19%
Detroj	7	4	1	25%	0	0	0	0	7	4	1	25%
Mandal	4	3	1	33%	0	0	0	0	4	3	1	33%
Dhandhuka	20	19	5	26%	11	10	0	0	31	29	5	17%
Dholera	2	0	0	NA	0	0	0	NA	2	0	0	0
Daskroi	14	11	8	73%	12	7	4	57%	26	18	12	67%
Total	206	160	74	46%	93	72	33	46%	299	232	107	46%

Table 3: Age Wise Distribution of Antibody Development RTPCR Positive Close Contact MALE

Age	Male				Female				Total			
	Total Test	Valid Test	Positive	Positivity	Total Test	Valid Test	Positive	Positivity	Total Test	Valid Test	Positive	Positivity
0-09	126	91	22	24	88	64	13	20	214	155	35	23
10-19	97	73	16	22	88	72	15	21	185	145	31	21
20-29	170	131	23	18	127	95	15	16	297	226	38	17
30-39	151	112	24	21	123	97	20	21	274	209	44	21
40-49	79	66	17	26	76	65	12	18	155	131	29	22
50-59	65	52	5	10	91	71	23	32	156	123	28	23
60-69	58	44	11	25	50	41	9	22	108	85	20	24
70-79	14	10	2	20	15	11	5	45	29	21	7	33.3
80-89	4	3	2	67	4	2	1	50	8	5	3	60
90-99	0	0	0	NA	4	4	1	25	4	4	1	25
Total	764	582	122	21	666	522	114	22	1430	1104	236	21

Table 4: Taluka & Gender Wise Distribution of Antibody Development RTPCR Positive Close Contact

Taluka Name	Male				Female				Total			
	Total Test	Valid Test	Positive	Positivity	Total Test	Valid Test	Positive	Positivity	Total Test	Valid Test	Positive	Positivity
Bavla	188	153	15	10%	161	143	12	8%	349	296	27	9%
Sanand	149	91	51	56%	137	93	46	49%	286	184	97	53%
Dholka	187	159	25	16%	146	118	34	29%	333	277	59	21%
Virmgam	84	65	5	8%	87	68	5	7%	171	133	10	8%
Detroj	18	14	4	29%	18	14	2	14%	36	28	6	21%
Mandal	21	14	11	79%	15	10	4	40%	36	24	15	63%
Dhandhuka	43	42	2	5%	37	34	1	3%	80	76	3	4%
Dholera	8	3	0	0%	10	2	0	0%	18	5	0	0%
Daskroi	66	41	9	22%	55	40	10	25%	121	81	19	23%
Total	764	582	122	21%	666	522	114	22%	1430	1104	236	21%

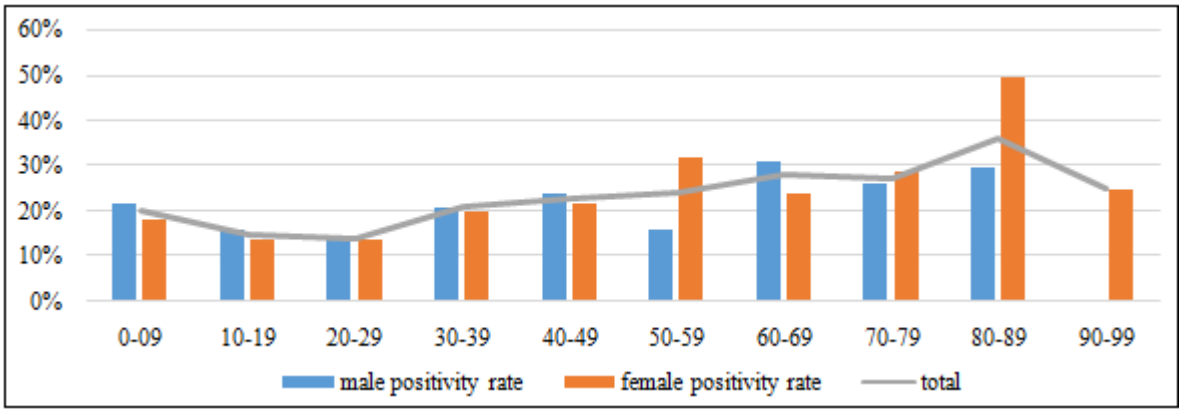
Table 5: Age Wise Distribution of Antibody Development RTPCR Negative

Age	Male				Female				Total			
	Total Test	Valid Test	Positive	Positivity	Total Test	Valid Test	Positive	Positivity	Total Test	Valid Test	Positive	Positivity
0-09	14	12	1	8.3	8	8	0	0	22	20	1	5
10-19	74	65	6	9	51	43	1	2	125	108	7	6
20-29	241	192	20	10	164	147	20	14	405	339	40	12
30-39	150	125	10	8	75	63	8	13	225	188	18	10
40-49	92	76	9	12	52	48	12	25	144	124	21	17
50-59	66	55	7	13	43	38	6	16	109	93	13	14
60-69	43	35	6	17	37	30	3	10	80	65	9	14
70-79	11	10	1	10	13	11	2	18	24	21	3	14
80-89	8	6	1	17	1	1	0	0	9	7	1	14
90-99	0	0	0	NA	0	0	0	NA	0	0	0	NA
Total	699	576	61	11	444	389	52	13	1143	965	113	12

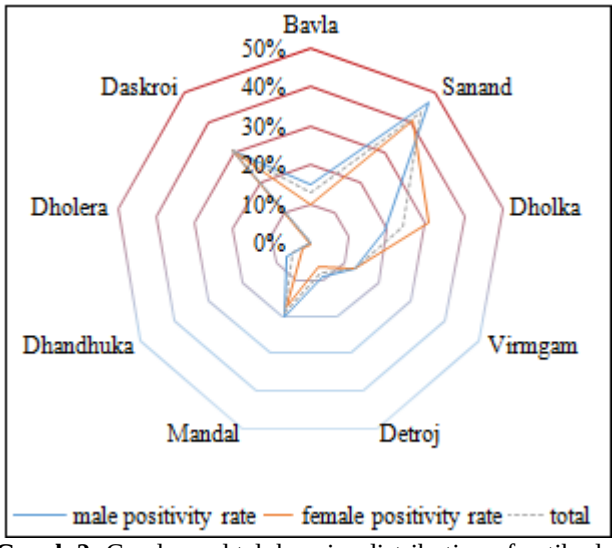
Table 6: Taluka & Gender Wise Distribution of Antibody Development RTPCR Negative

Taluka Name	Male				Female				Total			
	Total Test	Valid Test	Positive	Positivity	Total Test	Valid Test	Positive	Positivity	Total Test	Valid Test	Positive	Positivity
Bavla	113	112	13	12%	61	60	7	12%	174	172	20	12%
Sanand	62	54	9	17%	78	71	14	20%	140	125	23	18%

Dholka	16	15	0	0%	9	3	0	0%	25	18	0	0%
Virmgam	115	90	15	17%	58	55	10	18%	173	145	25	17%
Detroj	125	99	6	6%	86	68	3	4%	211	167	9	5%
Mandal	118	84	8	10%	56	54	7	13%	174	138	15	11%
Dhandhuka	48	38	0	0%	26	19	0	0%	74	57	0	0%
Dholera	57	48	0	0%	28	25	0	0%	85	73	0	0%
Daskroi	45	36	10	28%	42	34	11	32%	87	70	21	30%
Total	**699**	**576**	**61**	**11%**	**444**	**389**	**52**	**13%**	**1143**	**965**	**113**	**12%**



Graph 1: Age and gender wise distribution of antibody positivity rate



Graph 2: Gender and taluka wise distribution of antibody positivity

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