



SEROPREVALENCE OF ANTI-SARS-COV-2 TOTAL ANTIBODIES IN THE RURAL POPULATION OF UDAIPUR DISTRICT

Biochemistry

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ABSTRACT

The Covid19 disease has spread and caused high morbidity and mortality worldwide. Testing for antibodies against COVID-19 has been previously recommended. We conducted a serosurvey in rural population of Udaipur district. We found the seroprevalence 11.61 % in rural population of Udaipur district, which is much higher than RTPCR positive prevalence. So the burden of disease is much higher on population. Our study has some limitation as well, so we suggest for Longitudinal studies involving serial sampling with bigger sample size will be necessary to provide up-dated assessments of seroconversion and actual burden of covid 19 virus in population.

KEYWORDS

Seroprevalence, SARS-CoV-2, SARS-CoV-2 IgG Antibody, Seroconversion.

INTRODUCTION

After the identification of the new Severe Acute Respiratory Syndrome Corona virus 2 (SARS-CoV-2) and the first case of Corona virus Disease 2019 (COVID-19) in Wuhan, China, in November 2019, the World Health Organization declared a pandemic alert to all nations (1). SARS-COV-2 virus belongs to novel corona family resulted in covid-19 infection which causes severe acute respiratory syndrome (2-5). The disease has spread and caused high morbidity and mortality worldwide (1). The spectrum of COVID-19 severity varies widely, from asymptomatic infection to severe outcomes including organ failure and death (4-6). The virus spreads by environment exposure or person to person by both symptomatic and asymptomatic patients (6,7). Data suggest 20% of cases converted in acute respiratory distress syndrome which requires isolation and contact tracing with clinical monitoring and support (8,9).

Covid-19 diagnosis primarily relies on molecular testing for viral RNA by using a swab collection through sputum or throat/nasal secretion (10,11). SARS-COV-2 RNA test is the most sensitive diagnostic test for infection by detecting viral RNA prior to seroconversion (12,13). In most patient antibodies (IgM & IgG) against the virus synthesized within 15 days which is called seroconversion rate (13-17). Combined approach of both RNA testing and serology (serum or plasma sample) defines the highest diagnostic sensitivity (13). Total antibody serology test (combined IgG & IgM detection) may give earlier detection of seroconversion than either IgG or IgM alone (13,14).

Serology testing is essential for covid-19 surveillance along with supporting a diagnostic tool of disease. This serology is helpful to understand viral prevalence, as many patient remain mild symptomatic or asymptomatic. By assessing antibodies against covid-19 virus in the population may help in understanding disease spread (both recovered and current). Most of the confirmed case counts have been based on positive viral detection on the reverse transcriptase-polymerase chain reaction (PCR) test among individuals with symptoms. These case counts are underestimates of the total burden of SARS-CoV-2 infection because of incomplete testing availability and the substantial fraction of asymptomatic individuals with SARS-CoV-2 infection who never receive testing. Therefore, seroprevalence of anti-SARS-

CoV-2 can be used as a complementary tool to measure the total burden of infection in the population.

Thus, in the absence of seroprevalence surveys and an unknown proportion of asymptomatic cases in the country, the true infection rate of severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) the virus that causes COVID-19 remains unclear. The World Health Organization (WHO) therefore recommends conducting sequential seroprevalence surveys to monitor the trends of infection for planning and mounting effective and adequate public health response (15). Consequently, the knowledge gained from seroepidemiological studies is expected to provide key insights in population-level immunity and aid in the implementation of public health interventions towards containing the spread of the COVID-19 pandemic.

MATERIAL AND METHOD

The study was conducted at department of Biochemistry, R.N.T. Medical College, Udaipur in November, 2020. In this population-based cross-sectional study, we used serological testing for anti-SARS-CoV-2 antibodies to assess the prevalence of SARS-CoV-2 infection in 15 tehsils of Udaipur district. The selected tehsils were badgaon, Bhinder, Girwa, Gougunda, Jhalod, Kanor, Kherwada, Kotra, Lasadiya, Mavli, Rishabhdeo, Salumber, Semari, Sarada and Vallabhagar. Total population of Udaipur district is 3068420. The rural population is 2459994, which is 80.19 of total population (18).

We randomly selected and invited study participants from the general population from the all tehsils of Rajasthan. To increase the rate of participation, testing was done at the place of work (eg bank or supermarket) from which individuals were selected. Written informed consent was sought from all individuals before enrolment in the study. There was no predefined sample size. There was no inclusion and exclusion criteria.

Around 3 ml venous blood sample was collected through venepuncture by a trained phlebotomist under all aseptic precautions. The samples were transported to the clinical chemistry laboratory, department of Biochemistry, RNT medical college, Udaipur under standard operating procedures. Data on sociodemographic and clinical

characteristics of the participants were collected through face to face interviews conducted by one of the survey team members using a pretested brief interview schedule.

For qualitative detection of total antibody (IgG & IgM) for SARS-CoV-2 virus in human serum and plasma CV2T assay kit was used using Siemen's Dimension EXL integrated system with LOCI module. Chemiluminescent immunoassay used for total antibody (IgG&IgM) detection in serum or plasma. We have taken sample of 812 individuals from rural population of Udaipur district of rajasthan (southern rajasthan) who had not done RTPCR test before and had no or mild symptoms.

CV2T assay on Dimension EXL is a sandwich chemiluminescent immunoassay technique based on LOCI technology. In LOCI reagent there are two synthetic bead reagent and a biotinylated SIRBD antigen. First bead reagent is coated with streptavidin and contain photosensitizer dye. Second bead reagent is coated with anti-FITC (fluorescein isothiocyanate) antibody and contain chemiluminescent dye. The anti-FITC antibody coated chemibeads are predecorated with fluoresceinated SIRBD antigen. The sample is incubated with chemibeads and after 1 min biotinylated antigen is added to form bead-cov2-antibody biotinylated antigen sandwich. Sensibeads are added and bind to biotin after incubation to form bead pair immunocomplexes. Illution occurs at 680 nm which generates singlet oxygen from sensibeads who diffuses into the chemibeads and chemiluminescence reaction occurs. Result is measured at 612 nm and directly proportional to concentration in the sample.

RESULT AND DISCUSSION

Among 812 individuals who were contacted across 15 tehsils of Udaipur districts. All individuals initially agreed to participate in the study. However 98 individuals refused to provide blood samples and were excluded. 42 individuals did not provide their demographic information, including age, and were also excluded. So finally 672 subject went under study. 75 persons out of 672 found SARS-CoV-2 antibody positive. So seroprevalence is 11.16% in rural population. Seroprevalence is much more than cases found positive on RTPCR tests (1.82%) in Udaipur district till date 02.06.2021.

Table 1 indicate that the maximum number of participants 151 (22.47 %) were in age group of 50-59 years, followed by 129 (19.19 %) cases in 20-29 age group. It was observed that maximum seroprevalence 22 (29.33%) was in age group 50-59 years followed by 16 (18.67 %) cases in 40-49 age group.

Table 2 shows this study also indicates that majority of our patients were female (379 out of 672). The female population constituted about 56.39 % of the total. While male patients were 293 and constituted about 53.61 % of the total case. The seroprevalence in female is 12.66 % while in male 9.21 %. Since women on average have more B cells and produces more antibodies (19). Higher antibody titres in men, only observed during the acute stage, correlates well with men showing more severe symptoms and increased fatality, as reported (20-21).

In our study Kherwada tehsil seroprevalence was highest (12.47 %) among the all tehsils, and lowest in girwa (9.21 %). The cause of high serorevalence in Kherwada might be due to migrant population, most local people migrate to gujrat and maharashtra for work.

Table-1. Age Wise Distribution Of COVID Antibodies

| Age distribution | SARS-CoV-2 antibody positive(N=75) | SARS-CoV-2 antibody negative (N=597) |
|------------------|------------------------------------|--------------------------------------|
| <20 years | 07 (09.34 %) | 58 (09.71 %) |
| 20-29 years | 12 (16.00 %) | 116 (19.43 %) |
| 30-39 years | 10 (13.33 %) | 83 (13.90 %) |
| 40-49 years | 16 (18.67 %) | 96 (16.08 %) |
| 50-59 years | 22 (29.33 %) | 129 (21.60 %) |
| >60 years | 10 (13.33 %) | 115 (19.26 %) |
| Total | 75 (11.16 %) | 597 (88.84 %) |

Table-2. Gender Wise Distribution Of COVID Antibodies

| Sex | All participants | SARS-CoV-2 antibody positive | SARS-CoV-2 antibody negative | Seroprevalence (%) |
|--------|------------------|------------------------------|------------------------------|--------------------|
| female | 379 | 48 | 331 | 12.66 |
| male | 293 | 27 | 266 | 9.21 |
| Total | 672 | 75 | 597 | 11.16% |

CONCLUSION

We present a cross-sectional study of rural population of Udaipur district of Rajasthan (southern rajasthan). In our study seroprevalence is 11.16% in rural population, which is much higher than the cases reported in rural areas of Udaipur district (0.4%).

We cannot exclude the possibility that this study underestimates SARS-CoV-2 infections in population by failing to capture more recent infections that had not led to seroconversion. We observe that the risk of seroconversion appears variable depending on exposure to viral load and either person symptomatic or asymptomatic. Individuals with prior symptomatic illnesses were significantly more likely to seroconvert than asymptomatic individuals. Our testing methodology may therefore underestimate the true seroconversion prevalence. So actual burden of covid 19 virus is more in population than we get in our study.

Our findings also corroborate the evidence from early serosurvey reports of other studies, which had found the transmission of infection within communities was several times higher as most of the asymptomatic cases of SARS-CoV-2 were not screened using molecular methods (22-24).

Our study findings are in agreement with previous serosurvey reports across India which also observed significantly higher odds of antibody positivity in slums and overcrowded households (25-27).

So we suggest for Longitudinal studies involving serial sampling with bigger sample size will be necessary to provide up-dated assessments of seroconversion and actual burden of covid 19 virus in population.

Limitations

1. Studies show that antibody titer increased over a period of time and thereafter decline so optimum time to measure antibody test is 15 days to 2.5 month.
2. Total antibody test measured in EXL dimension by CV2T kit is a qualitative test i.e if antibody titer is above 1000 QUAL units result came positive and below 1000 QUAL units negative result will come. Means antibody titer either zero or 999 UL units result will be measured by machine as negative.
3. The positive test result does not exclude present or past infection by other corona virus i.e. MERS-COV, HKU1, SARS-COV-1, NL63 or OC43 or due to cross reactivity from pre-existing antibodies or other possible causes.
4. A negative test result does not exclude the possibility of exposure to infection with SARS-COV-2. Patient sample may be negative if collected early (before seroconversion) phase of disease or due to decline in titre overtime. Additionally the immune response may be depressed in immunocompromised patients.
5. Patient sample may contain heterophilic antibodies that could react in immunoassay cause falsely elevated or depressed result.
6. sample containing biotin at a concentration of 300 ng/ml demonstrate a less than or equal to 10 % change in results.

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