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ORIGINAL PAPER

Seroprevalence of IgG and IgM Antibodies against SARS-CoV-2 Infection in Inhabitants of Itanagar Capital Region, Arunachal Pradesh, India

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ABSTRACT

Objectives: *The assessment of novel coronavirus disease 2019 (COVID-19) antibodies is essential to understand the transmission dynamics of contagious disease. The proportion of the population who developed antibodies against the COVID-19 disease can be estimated through population-based serosurveys. This population based cross sectional serosurvey was designed to assess the seroprevalence of IgG and IgM antibodies of COVID-19 infection.*

Material and methods: *A population based cross sectional serosurvey included 1031 residents of Itanagar Capital Complex region (ICR), Arunachal Pradesh, India, aged above five years. Anti-SARS-CoV-2 specific IgG and IgM antibody levels were analyzed by chemiluminescence immunoassay based serological tests.*

Results: *The overall seropositivity for SARS-CoV-2 IgG and IgM was 92.24% and 7.7%, respectively. The seropositivity of IgG and IgM was 97.68% and 2.32%, respectively, in subjects fully vaccinated with*

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two doses, 97.22% and 13.88%, respectively, in those vaccinated with the first dose, and 80% and 11.33%, respectively, in non-vaccinated participants. In contrast to indigenous tribal participants (IgG 90.55% and IgM 8.88%), seroprevalence was high in non-tribal subjects (IgG 94.72% and IgM 6.84%). Age, ethnicity, and area showed a positive correlation, while vaccination status exhibited a negative correlation with IgG levels (Pearson's coefficient -0.535).

Conclusion: *This first monocentric serosurvey following the high rate of infection with Delta variant in ICR found a high seropositivity for IgG. Further state level serosurveys are needed to assess the infection status, immunological response and associated comorbidities of COVID-19 infection. Periodic vaccination campaigns and early administration of booster doses to the general public might be beneficial in preserving immunity and prevent illness.*

Keywords: COVID-19, seropositivity, vaccination, ethnicity, indigenous population.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late 2019 and was transmitted to more than 180 countries, and consequently became a global pandemic (1). The World health Organization (WHO) declared that the novel coronavirus disease 2019 (COVID-19) outbreak was a public health emergency of global concern on the 30th of January 2020 and on the 12th of March 2020, the outbreak was declared as a pandemic (2). Around ~90 million positive cases and two million deaths were reported in 2021 globally (3). India proclaimed its first COVID-19 case on the 27th of January 2020, and then reported a gradual escalation in positive cases (4). Being a novel form, the related history and immune response of infection was idiopathic (5). According to WHO, population based seroprevalence surveys are worthwhile to predict the proportion of infection and immunity in order to comprehend disease burden, a pattern of transmission and associated risk factors. However, such surveys require a representative sample with suitable parameters to draw reliable conclusions (6, 7).

An individual affected by COVID-19 generally develops antibody response after 6-8 days of infection, with a median observed seroconversion time of 11 to 14 days for IgM and IgG, respectively. The antibody response is proportional to the severity of COVID-19, which wanes after three months of infection (8, 9). The serosurveys are qualitative or quantitative assessments of serum antibody response in specific populations. The outcome of serosurveys indicate the proportion of population who has developed an immune response and is susceptible to infection, thus helping to frame strategies for disease prevention (10). Numerous serosurveys conducted in India revealed varying levels of seropositivity and recommended mandatory immunisation. The Indian Council of Medical Research (ICMR), Government of India, recommended that the subsequent rounds of population based serosurveys can determine the disease burden at community level and helpful to develop and implement appropriate containment measures (11).

The Itanagar capital complex region (ICR) measuring approximately 200 km^2 has an estimate population of 0.12 million (12). Arunachal Pradesh registered its first COVID-19 positive case on the 2nd of April 2020. Since its emergence, ICR was claiming the highest numbers of COVID-19 positive cases and highest number of active cases (13). Early April 2021, the trend of positive cases and deaths was 16785 and 56, respectively, which reached 26778 and 114, respectively, by the 30th of May 2021. By the end of June 2021, the state reported 35571 positive cases and 168 deaths, which escalated to 45867 and 226 as of July 2021 and to 52634 and 260 as of August 2021 (14). COVID-19 vaccination was commenced among all health care workers, then expanded to front line workers, followed by people above 60 years of age, citizens above 45 and eventually those under 18. Even though, a sudden surge in the number of positive cases was observed after commencing the vaccination in both the entire country and state. In this regard, with current trends in the positivity rate

among people of ICR, Arunachal Pradesh, India, the present population based cross sectional serosurvey was designed to assess the seroprevalence of COVID-19. ❑

MATERIALS AND METHODS

Study design and setting

The present research was a population based cross sectional serosurveillance study. It was conducted in the area of ICR, Arunachal Pradesh, India, and supported by Tomo Riba Institute of Health and Medical Sciences (TRIHMS), Naharlagun and Government of Arunachal Pradesh, India, between September 2021 and November 2021.

Inclusion criteria

Participants above five years of age who had resided in ICR region for more than a year, subjects regardless of whether or not they had been infected by or vaccinated against SARS-CoV-2 even if partially.

Exclusion criteria

Residents aged under five years and those not willing to participate in the study were excluded.

Informed consent and ethical approval of the study

Informed consent was obtained from all study participants and parents/guardians of subjects aged under 18 years. The study protocol was approved by the scientific research committee and institutional ethics committee (No. TRIHMS/ETHICS/01/2019-20/19) of TRIHMS, Naharlagun.

Sampling strategy

A total sample size of 461 was determined using Cochran's methodology. Following Cochran's, a purposive sample size of 1031 was determined based on the government of Arunachal Pradesh's proposal and the funding provided by TRIHMS.

The multi stage sampling method was employed. Itanagar capital region was divided into three circles – Itanagar, Naharlagun and Banderdewa (as *per* the data available in https://itanagar.nic.in/) (Figure 1) – and each circle has been divided into urban and rural areas. The Itanagar circle has 12 wards in its urban part and 21 villages in the rural part. Naharlagun circle has seven wards in the urban region and 32 villages in the rural one, while Banderdewa has one ward in its urban part and 50 villages in the rural part. The total population of ICR is 1, 22,930, with 77.8% (95,648) residing in the urban area and 22.2% (27,282) in the rural area (Figure 2).

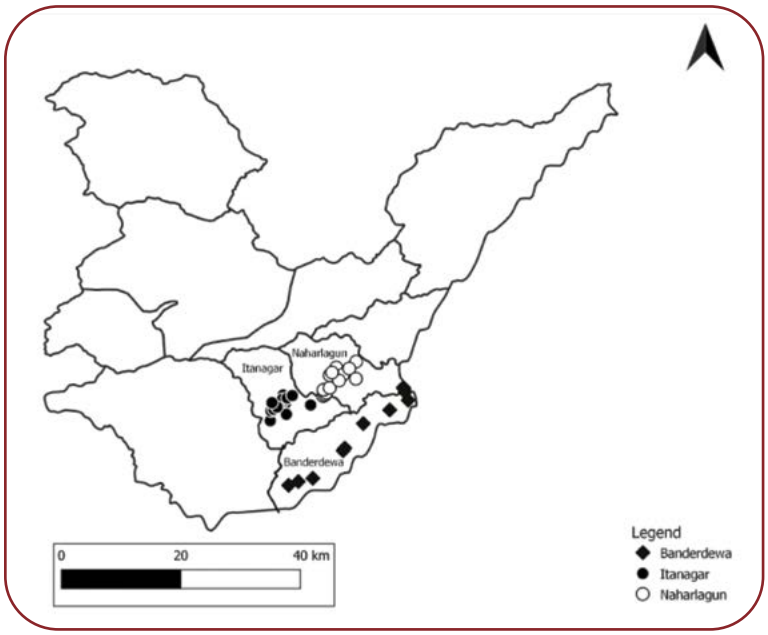

FIGURE 1. Sample collection areas of the study representing geographic spread across Itanagar capital complex region, Papum pare district, Arunachal Pradesh, India

Multistage sampling

Stage 1: Three circles of capital complex, including Itanagar, Naharlagun and Banderdewa, were selected (Figure 1).

Stage 2: From the urban area of each circle, all wards were included in the gazette, *i.e.*, the Arunachal Pradesh Gazette, dated the 21st of February 2020. For the rural area, each circle/block, different villages were selected through proportionality based simple random sampling technique.

Stage 3: The systematic random sample approach was used to select an equal number of households out of each ward/village.

Stage 4: Through a simple random procedure, eligible residents above five years of age were selected from each dwelling. If either the chosen residence was unoccupied at the time or the chosen individual refused to participate in the survey, the next house (adjacent) was selected as a replacement.

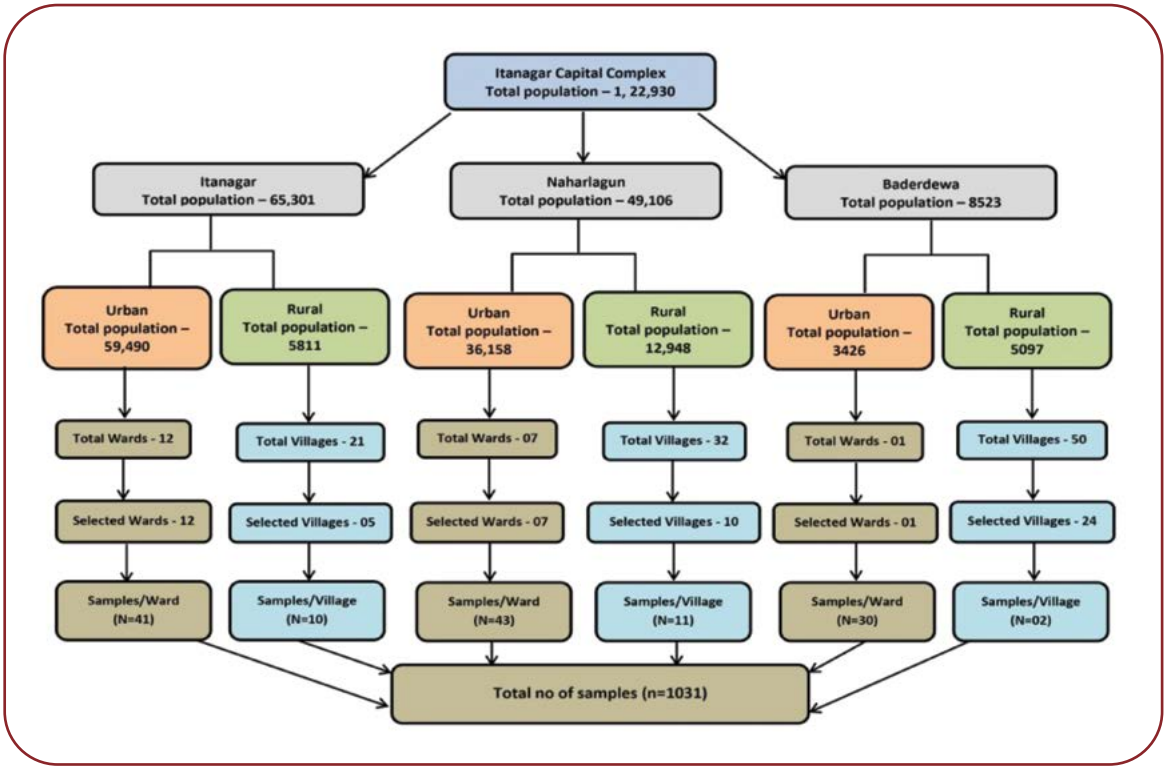

FIGURE 2. Schematic representation of participants' recruitment

Laboratory analysis

Four teams of skilled phlebotomists drew 3-5 mL of peripheral venous blood into a simple container. After centrifuging the blood at 2000-3500 rpm for 10-15 minutes, the serum/plasma was pipetted into an aliquot and stored at 2-80 °C for up to seven days. Anti-COVID-19 IgG and IgM concentrations in serum/plasma were determined using a chemiluminescence immunoassay employing the Maglumi 800 according to the manufacturer's protocol at the Central Laboratory, Dedicated COVID Hospital, Chimpu, Itanagar, under TRIHMS.

Statistical analysis

Descriptive statistics and seropositivity corelation was used to analyse demographic and categorical variables by employing SPSS V26. Furthermore, the Chi-square test was performed to determine the significance of variations in seroprevalence, with a significance level of $p<0.05$ deemed as statistically significant. ❑

RESULTS

The majority of study participants (69.26%) were aged 18-44 years, followed by those aged 45-65 years (19.6%), under 18 years (10.8%) and above 65 years (0.4%). The proportion of male subjects (63.43%) was higher than that of female participants (36.25%). Around 79.28% of all participants were residing in urban areas and 20.28% in rural areas of ICR. The majority of participants belonged to indigenous tribes (59.56%) and 44.44% of subjects were non-tribal participants. Around 99.70% of participants received COVISHIELD™ (manufactured by Serum Institute of India Pvt Ltd) and only 0.30% of subjects received COVAXIN® vaccine (Bharat Biotech in collaboration with the ICMR-NIV). Regarding the vaccination status, 71.19% of participants were fully vaccinated with two doses, only 3.49% had a single dose, 15.51% were not vaccinated and 10.76% of participants were under 18 years of age, with the latter having no permission for vaccination until the 2nd of January 2022. The seropositivity rate was 98%, 97.5% and 97.42%, with an interval of 28, 45 and 84 days between two successive vaccine doses, respectively.

Among the IgG positive participants (92.2%), 65.6% were between 18-44 years of age, which were 69.3% among study participants. The majority of indigenous and non-indigenous partici-

TABLE 1. Overall results of the objectives according to the different age groups

Parameters		Age wise distribution of study participants (years)									
		5-17		18-44		45-65		>65		Total	
		Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Area	Urban	58	5.62%	585	56.74%	175	16.98%	04	0.4%	822	79.72%
	Rural	53	5.14%	129	12.5%	27	2.61%	00	0.0%	209	20.27%
	Total	111	10.8%	714	69.3%	202	19.6%	04	0.4%	1031	100.0%
IgG	Negative	32	3.1%	38	3.7%	09	0.9%	01	0.1%	80	7.8%
	Positive	79	7.7%	676	65.6%	193	18.7%	03	0.3%	951	92.2%
	Total	111	10.8%	714	69.3%	202	19.6%	04	0.4%	1031	100.0%
IgM	Negative	105	10.2%	653	63.3%	190	18.4%	04	0.4%	952	92.3%
	Positive	06	0.6%	61	5.9%	12	1.2%	00	0.0%	79	7.7%
	Total	111	10.8%	714	69.3%	202	19.6%	04	0.4%	1031	100.0%
Ethnicity	Non-tribal	24	2.3%	295	28.6%	95	9.2%	03	0.3%	417	40.4%
	Tribal	87	8.4%	419	40.6%	107	10.4%	01	0.1%	614	59.6%
	Total	111	10.8%	714	69.3%	202	19.6%	04	0.4%	1031	100.0%
Vaccination status	Vaccinated	00	0.0%	546	53.0%	187	18.1%	01	0.1%	734	71.2%
	First dose	00	0.0%	33	3.2%	03	0.3%	00	0.0%	36	3.5%
	Not vaccinated	00	0.0%	135	13.1%	12	1.2%	03	0.3%	150	14.5%
	Under age	111	10.8%	00	0.0%	00	0.0%	00	0.0%	111	10.8%
	Total	111	10.8%	714	69.3%	202	19.6%	04	0.4%	1031	100.0%

TABLE 2. Seropositivity among tribal and non-tribal participants (N=1031)

Ethnicity)	IgG		IgM	
	Positive	Negative	Positive	Negative
Tribal (N=614)	556 (90.55%)	58 (9.45%)	42 (6.84%)	572 (93.16%)
Non-tribal (N=417)	395 (94.72%)	22 (5.28%)	37 (8.88%)	380 (91.12%)
Total (N=1031)	951 (92.24%)	80 (7.76%)	79 (7.7%)	952 (92.3%)

TABLE 3. Overall results of seropositivity according to the various vaccination status

Vaccination status	IgG	IgM
	Positive	Positive
Fully vaccinated	717 (97.68%)	51 (2.32%)
First dose only	35 (97.22%)	05 (13.88%)
Not vaccinated	120 (80%)	17 (11.33%)
Under 18 years of age	79 (71.18%)	06 (5.40%)
Total	951 (92.24%)	79 (7.7%)

TABLE 4. Correlation between seropositivity and demographic variables

Variables	Correlation Coefficient		Sig. (two-tailed)	
	IgG	IgM	IgG	IgM
Age	.259**	-.060	.000	.053
Ethnicity	.169**	.051	.000	.103
Area	.185**	.146**	.000	.000
Vaccination status	-.535**	-.058	.000	.064

** Correlation is significant at the 0.01 level (two-tailed).

pants were between 18-44 years of age, followed by those aged 45-65. Among all participants, 71.2% were vaccinated with two doses and 14.5% not vaccinated (Table 1).

The levels of seropositivity for IgG were higher in non-tribal subjects (94/72%) than tribal ones (90.55%) (Table 2). The overall seropositivity for IgG was high in participants who had received two vaccine doses (97.68%), followed by those who had a single dose (97.22%), non-vaccinated subjects (80%) and under age participants (71.18%) (Table 3).

IgG levels were positively correlated with ethnicity, region and age groups (Pearson's coefficient was 0.169, 0.185, and 0.259, respectively), and negatively correlated with the vaccination status (Pearson's coefficient was -0.535), which was significant at the 0.01 level (Table 4). However, limited positive instances were observed and no significant correlations with IgM have been found. ❑

DISCUSSION

The current study reported a high seroprevalence (IgG 92.2% and IgM 7.7%) of COVID-19 infection in ICR, Arunachal Pradesh, India, between September–November 2021. A nationwide serosurvey by the Indian Council for Medical Research reported a 67.6% of seroprevalence in the country's population, including children above six years of age, during June and July 2021, with Madhya Pradesh having the highest rate (79%) and Kerala 44.4% (15). Several serosurveys from different geographical areas of India reported various levels of seropositivity during different timelines of 2020 and 2021 (16-22). A systemic review and meta-analysis reported a three-fold raise in seroprevalence between the first and second surge of COVID-19 infection, and more than 2/3 of Indian population had IgG antibodies by August 2021 (23). Factors like representative sample size, current disease burden, duration from disease inception and population density might influence these diverse levels of seroprevalence. The current study indicates a high seroprevalence in a region suffering from a high number of COVID-19 cases. While the study did not record the presence of current or past SARS-CoV-2 infection, the high seroprevalence could occur due to a large number of asymptomatic cases.

The ethnicity of participants and their parents is determined by their birthplace (24). COVID-19 infection and severity were idiopathically affected by ethnicity. According to the US Center for Disease Control and Prevention (CDC), COVID-19 affects certain ethnic groups more than others (25). However, several confounding factors influence the distinction in the humoral immune response among various ethnic groups (26). A systemic review and meta- analysis reported that people of Black and Asian ethnicity are twice and 1.5 times, respectively, more likely to develop COVID-19 compared with persons of white ethnicity (27).

In India, the first surge of COVID-19 affected mostly the urban inhabitants, while the second surge devastated the rural part, which claimed 52% of deaths and 53% of new cases by May 2021 (28). In March 2020, Nuzrath Jahan *et al* reported a higher seroprevalence among urban residents than rural inhabitants during both waves, possibly due to the high density of population (23). Murhekar *et al* reported a higher seroprevalence in urban areas than rural ones during the first surge of infection. However, in the course of the second surges (June-July 2021), seroprevalence levels were comparable between urban and rural areas (29). In our study, the seropositivity for IgG was 93.55% in urban population and 87.08% in rural population. A systemic review and meta-analysis reported an overall pooled seroprevalence during the second surge (March-August 2021) of COVID-19 was 69.2%. The seroprevalence was higher among subjects aged 41-60 years (81.2%) than those under 20 (63.1%) (23). Consistently, our study reported 7.7% among participants aged 5-17 years, 65.6% among those aged 18-44, 18.7% among subjects aged 45-65 years and 0.3% in those above 65. The higher rate of seropositivity could be possibly due to vaccine prioritization in the specified age group.

Arunachal Pradesh reported daily average of 248 cases during September 2020, which declined to zero by the end of January 2021. The second surge was considered from mid-May 2021 to September 2021, with a highest daily average of 443 cases. In the meantime, vaccination became available for the frontline workers and elderly people. Even though, the second surge affected predominantly by the delta variant of COVID-19 (30, 31). Murhekar *et al* reported a significantly higher seroprevalence in people who received the first vaccine dose (81.0%) and two doses (89.8%) than non-vaccinated ones (62.3%) (32). Similarly, our study showed higher rates of seropositivity among vaccinated participants (single dose 97.22% and two doses 97.68%) than non-vaccinated ones (80%). The Indian government strategies helped to improve awareness in the general population towards vaccination drives, which allowed herd immunity and became a promising measure to control the further spread of SARS-CoV-2 infec-

tion (33). Even after vaccination, people should be instructed to strictly adhere to the COVID-19 appropriate behaviour.

There are multiple factors that influence the seroprevalence at every point of time, which need to be reassessed consecutively to approximate the burden of COVID-19 in the community (34, 35). The information yielded from serosurveys across India depicted the pandemic progression and helped to frame novel strategies for disease control. Assessment of IgG and IgM antibody tests has distinct benefits, because they are most affordable and expeditious investigations than RT-PCR based diagnosis (36, 37).

Study strengths and limitations

The strength of our study was represented by the fact that data on previous infection in sampling area was not available till the study period and our research was the earliest population based serosurveillance study focusing on people of different age groups, ethnic background and vaccination status in the course of the second surge of COVID-19 infection in Arunachal Pradesh, India, with good sampling strategies (multistage sampling and household visits). Our study had also limitations, as it did not include cases with active infection and hospitalization.

Recommendations

The continuous vaccination drives and early implementation of booster doses to the general population could be beneficent to conserve immunity. Additionally, continued use of facemasks, face shields, hand washing, maintaining social distancing, avoiding social gathering and maintaining government framed COVID-19 appropriate protocols can prevent the transmission of the SARS-CoV-2 infection. ❑

CONCLUSIONS

The ICR showed the highest number of COVID-19 positive cases in the course of the second surge of the pandemic, and our seroprevalence study was one of the earliest seroprevalence studies following the infection with Delta variant. We found a high overall seropositivity for IgG (92.2%) of COVID-19 infection. The seropositivity for IgG was 93.55% in urban population and 87.05% in rural population, and 90.55% in indigenous tribal rural population and 94.2% in non-tribal population. A consecutive cohort study is further required to assess the immunity status of the population, effectiveness of vaccines, and immunological response against COVID-19 infection in young people aged under 18 years. ❑

Ethical approval and consent to participate: Yes (No. TRIHMS/ETHICS/01/2019-20/19).

Informed consent: All subjects were informed about the scope of the project and clarified their queries.

Conflicts of interest: none declared.

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Authors' contributions: JK, HN, MJ, SC, YT – conceptualization; HN, JK – data acquisition; JK, MMK, HN, MJ, SC, YT – data analysis and interpretation, manuscript preparation and revision; JK, MMK, HN, MJ, SC, YT – approval of final version of the manuscript.

Availability of data and material: Available upon reasonable request.

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