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Seroprevalence of anti SARS-CoV-2 IgG antibodies among adults in Jammu district, India: A community-based study

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***Background & objectives*: Serology testing is essential for immunological surveillance in the population. This serosurvey was conducted to ascertain the cumulative population immunity against SARS-CoV-2 among adults in Jammu district and to understand the association of seropositivity with sociodemographic and clinical correlates.**

***Methods*: On September 30 and October 1, 2020, a household survey was done in 20 villages/wards chosen from 10 health blocks in district Jammu, India. Demographic, clinical and exposure information was collected from 2000 adults. Serum samples were screened for IgG antibodies using COVID Kavach MERILISA kit. Tests of association were used to identify risk factors associated with IgG positivity. Crude odds ratio with 95 per cent confidence intervals (CIs) was calculated during univariate analysis followed by logistic regression.**

***Results*: Overall adjusted seroprevalence for SARS-CoV-2 was 8.8 per cent (95% CI: 8.78-8.82); it varied from 4.1 per cent in Chauki choura to 16.7 per cent Pallanwalla across 10 blocks in the district. Seropositivity was observed to be comparatively higher in 41-50 and 61-70 yr age groups, among males and in rural areas. Fever, sore throat, cough, dyspnoea, myalgias, anosmia, ageusia, fatigue, seizures, history of exposure, medical consultation, hospitalization and missing work showed significant association with seropositivity on univariate analysis. On logistic regression, only sore throat, myalgia and missing work showed significant adjusted odds of IgG positivity. Extrapolation to adult population suggested that exposure to SARS-CoV-2 was 14.4 times higher than reported cases, translating into Infection fatality rate of 0.08 per cent.**

***Interpretation & conclusions*: Since a major part of population was immunologically naive, all efforts to contain COVID-19 need to be vigorously followed while these baseline results provide an important yardstick to monitor the trends of COVID-19 and guide locally appropriate control strategies in the region.**

Key words: Antibody - COVID-19 - ELISA - IgG - SARS-CoV-2 - seroprevalence - serosurveillance - risk factors

On January 30, 2020, the WHO acknowledged the novel coronavirus outbreak a 'Public Health Emergency of International Concern', following which COVID-19 was declared to be a pandemic in March 2020[1,2]. This

pandemic was the third coronavirus outbreak in the last two decades after the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome (MERS)-CoV. In India, the first case was detected on January 30, 2020[3], and in Jammu and Kashmir, the first case was surfaced on March 18, 2020[4].

SARS-CoV-2 RNA testing is endorsed as the most sensitive diagnostic test for early infection, as viral RNA is detectable even before antibody formation. IgM and IgG antibodies to the virus are mostly produced within 15 days (1-3 wk)[5]. IgG antibodies are generally quantifiable after two to three weeks of onset of infection, and last for many months. Therefore, IgG serosurveys are not suitable for detecting acute infection. However, detection of IgG antibodies for SARS-CoV-2 may be beneficial to estimate the percentage of population already exposed, including asymptomatic individuals[5]. Depending on the seroprevalence, appropriate public health policies and mediations can be planned and implemented for impeding the spread of disease.

In this context, a community survey was conducted in 10 health blocks of Jammu district to evaluate the overall seroprevalence of IgG antibodies to COVID-19 in the general adult population and to determine the association between sociodemographic-clinical correlates and seropositivity.

Material & Methods

There are 10 health blocks in district Jammu namely, Marh, Dansal, Akhnoor, Jammu, Bishnah, Pallanwalla, RS Pura, Kotbalwal, Chauki-Chaura and Sohanjana. These health blocks include both rural and urban population, *i.e.,* there are both villages and wards respectively, in all the 10 health blocks. The present cross-sectional serosurvey was conducted among adults >18 yr of age, on September 30 and October 1, 2020 in all the health blocks after obtaining ethical clearance from Ethics Committee of Government Medical College (GMC), Jammu, India (IEC/GMC/Cat B/2020/152, dated: 28-08-2020). Based on an anticipated prevalence of 10 per cent, an absolute precision of two per cent, a design effect of two for cluster design, and a non-response rate of 15 per cent, the minimum sample size needed at 95 per cent confidence interval (CI) was approximately 2000.

For the purpose of this survey, each block was treated as a single cluster and then from each cluster one village representing rural population and one ward representing urban population were randomly selected. Two teams; one from GMC, Jammu, and the other from the office of the respective Block Medical Officer (BMO) were deputed in each health block. Accredited Social Health Activists (ASHA), Auxiliary nurse midwives (ANM) and pharmacists underwent rigorous two-hour training and sensitization in the department of Community Medicine, GMC, before proceeding for field work. ASHA and ANM sensitized the locals from the selected villages and wards in every block, one day ahead of sampling. On the day of sampling, an arbitrary starting spot was chosen in the selected villages on day one and in wards on day two and all adjoining households were contacted until 100 eligible adults were recruited per block per day. Samples were taken, irrespective of acute or prior COVID-19 infection from individuals >18 yr of age.

Each participant was asked to complete a questionnaire/schedule with the help of a postgraduate medical student from the department of Community Medicine, GMC, or a medical officer from BMO office. This questionnaire covered demographic, clinical and exposure information of the past six months[6]. Trained phlebotomists from each of the survey teams obtained 3-5 ml of venous blood in a serum separator vacutainer from each participant. All the samples were transported in cold-chain on same day along with the sample transportation sheet bearing a unique ID for each sample to the department of Blood Transfusion, GMC, where serum was stored at 2-8°C. The samples were later tested in batches on ELISA reader and washer (BioRad, USA) for the detection of IgG antibodies against SARS-CoV-2 using ICMR-NIV Anti-SARS CoV-2 Human IgG ELISA COVID KAVACH – MERILISA, Code CKMELI-01 (M/s Meril Diagnostics Pvt. Ltd, India). Sensitivity and specificity of the MERILISA kit were 93.3 and 100 per cent, respectively and manufacturer's instructions were strictly followed during testing[7].

Serum samples with indeterminate reports underwent repeat testing with MERILISA kit. If the results came out to be indeterminate on repeat testing also, those samples were labelled as negative. For the purpose of quality assurance, one per cent of serum samples that tested negative, were randomly chosen from each plate and re-tested.

Statistical analysis: Data were collected on a preformed structured questionnaire and entered into Microsoft Excel. Quantitative variables were reported as mean and standard deviation (SD) and qualitative

variables as percentages and proportions. Selected socio-demographic variables were compared in seropositive and seronegative adults to identify factors associated with IgG positivity. Chi-square/Fischer's exact/Mid *P* exact tests were used as required. Crude odds ratio with 95 per cent confidence intervals (CIs) was calculated during univariate analysis followed by logistic regression wherein adjusted odds ratio (OR) with 95 per cent CIs were calculated. All statistical analyses were done using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp. Armonk, NY, USA), STATA software package version 16 (Stata Corp, College Station, Texas, USA) and Open Epi version 3.01. Cluster sampling design was accounted for while analyzing the data by using survey data analysis module for STATA (STATA syntax svyset). Adjusted prevalence was calculated using the following formula[8]:

$$AD = [CP+SP-1] / [SN+SP-1],$$

where AD is the adjusted prevalence; CP is the crude prevalence; SP is the specificity and SN is the sensitivity. Crude prevalence was the total number of positive tests using the test kit, and sensitivity and specificity were used as per manufacturers' estimates. The SE for this test adjusted rate was calculated by the formula[9]:

$$SE = \sqrt{[p(1-p) \times D/n]},$$

where p is the test adjusted rate, D is the design effect and n is the number[9]. Ninety-five per cent CIs were then calculated using the formula: 95 per cent CI = $p \pm 1.96 \times SE$[8,9].

Results

A total of 2010 adults were sampled during the survey, of whom 10 samples were rejected; six due to haemolysis and four due to insufficient quantity, so the total samples tested were 2000. Of these, 22 results were indeterminate and were repeat tested. On repeated testing, all the 22 indeterminate samples tested negative. In addition, for quality assurance, one per cent negatives were re-tested from each plate (Pack size = 96T) *i.e.* 21 negative samples were re-tested and all turned out to be negative on re-testing.

Mean age of the participants was 40.17 (±13.41) yr, ranging from 18 to 98 years. The mean age of males was 40.28 (±13.49) yr while mean age of females was 39.96 (±13.26) yr with no significant difference between the two groups. Maximum surveyed individuals (28.6%) belonged to the age group of 18-30 yr with 55.9 per cent being <40 yr of age. Among the surveyed individuals, 44.9 per cent belonged to rural areas and 55.1 per cent were urban residents. As shown in Table I, crude seroprevalence was observed to be 8.2 per cent (95% CI: 7.07-9.48) while adjusted overall seropositivity was 8.8 per cent (95% CI: 8.78-8.82). Hence, majority of population remained unexposed to the COVID-19 infection. The prevalence of infection/exposure was observed to be comparatively higher in 41-50 and 61-70 yr age groups which were 10.7 and 11.6 per cent, respectively. Adjusted seroprevalence was higher in males and residents of rural areas but these odds were not significant.

Only 213 individuals (10.7%) of the total sampled reported any COVID-19 like symptoms in the past six months. Among the seropositives, almost 75 per cent of individuals were asymptomatic *i.e.* only a quarter of individuals reported a history of fever or cough or fatigue which was significantly more than the percentage of seronegative (9.3%) who reported any of these symptoms (Table II).

Approximately, one-fifths of the patients with fever (n=22), sore throat (n=18) and fatigue (n=7) were seropositive for COVID-19 antibodies, while two-fifths with anosmia (n=3), ageusia (n=3), and seizures (n=3) had antibodies. Fever, sore throat, cough, dyspnoea, myalgias, anosmia, ageusia, fatigue and seizures showed significant association with seropositivity on univariate analysis as shown in Table III. It is evident that only 95 adults (4.8%) in the total sample reported history of contact with suspected/confirmed case of COVID-19. However, 19 of 95 (20.0%) adults among those who reported contact, were tested seropositive for IgG antibodies. Medical consultation, hospitalization and absence from work were significantly associated with seropositivity on univariate analysis.

On performing multivariate analysis (binary logistic regression) with all variables in Tables I and II, it was noted that only three variables namely, sore throat, myalgia and missing work were associated with significant adjusted odds of IgG positivity (Table IV). Dis-aggregated analysis in Table V reveals uneven distribution of COVID-19 exposure with minimum adjusted prevalence of 4.1 per cent in Chowki Chaura to maximum of 16.7 per cent in Pallanwala.

Discussion

Union territory of Jammu and Kashmir has a population of 13 million and Jammu district has a

Table I. Crude and adjusted seropositivity in the surveyed population according to demographic variables

Variable	Crude seropositivity, n (%)	Total	*P*	Crude OR (95% CI)	Seropositivity (95% CI) further adjusted for test performance
Age (yr)					
18-30	43 (7.5)	572	0.300*	Reference	8.0 (7.96-8.04)
31-40	38 (7.0)	545		0.92 (0.58-1.45)	7.5 (7.47-7.53)
41-50	45 (10.0)	449		1.37 (0.88-2.12)	10.7 (10.66-10.74)
51-60	25 (8.2)	306		1.09 (0.65-1.83)	8.8 (8.75-8.85)
61-70	10 (10.8)	93		1.48 (0.71-3.06)	11.6 (11.51-11.69)
>70	2 (6.3)	32		0.82 (0.19-3.54)	8.9 (8.76-9.04)
Missing		3		-	-
Area of residence					
Rural	77 (8.6)	898	0.582	1.09 (0.79-1.50)	9.2 (8.84-9.36)
Urban	87 (7.9)	1102		Reference	8.5 (8.48-8.52)
Gender					
Males	114 (9.0)	1271	0.098	1.33 (0.94-1.89)	9.6 (9.58-9.62)
Females	50 (6.9)	729		Reference	7.4 (7.39-7.41)
Total	164 (8.2)	2000			8.8 (8.78-8.82)

**P* value of Chi square for trend is reported. OR, odds ratio; CI, confidence interval

projected population (2020) of 17,44,152[10,11]. As of November 25, 2020, Jammu and Kashmir recorded a little over 100,000 positive cases of COVID-19 (8,166 cases per million inhabitants versus national figure of 6,952.9 cases per million) with 20,092 people registering positive for the infection in Jammu district. As many as 292 people died due to the infection in the district of the total 1,663 deaths in the Union territory till November 25, 2020[4]. In the present survey, overall adjusted seroprevalence for SARS-CoV-2 was 8.8 per cent (95% CI: 8.78-8.82); it varied from 4.1 to 16.7 per cent in 10 blocks across the district. Seropositivity was observed to be comparatively higher in 41-50 and 61-70 yr age groups, among males and in rural areas. Symptoms reported by individuals in the past six months were studied, but on multivariate analysis, only sore throat, myalgia and missing work showed significant adjusted odds of IgG positivity.

The findings of this survey corroborated with the estimates of the ICMR National survey which concluded that seroprevalence in the adult population in the surveyed districts had risen from 0.73 per cent in May-June to nearly 7.1 per cent in August-September 2020[12]. Drawing parallels with the studies conducted in the other parts of world, especially Switzerland with 4.8-10.9 per cent seroprevalence between April 6 and May 9, 2020[13]; 1.0-6.9 per cent in 10 different sites across the United States during March 23-May 12[14]; 6.7 per cent in France between May 4-June 23[15]; this study showed that herd immunity appeared like an infeasible goal but there was widespread infection. However, with regard to nationwide comparisons, our estimates were higher than Kerala which reported 0.8 per cent seropositivity in August[16], and lower than high prevalence States/Union Territories (UT) such as Delhi, Pune, Ahmedabad and Mumbai which recorded 29.1 per cent (between August 1 and 7), 51.5 per cent (August, 2020), 23.2 per cent (September, 2020) and 33.4 per cent (September, 2020) seropositivity, respectively[17,18].

The ICMR survey also observed that the chances of testing positive for COVID-19 in urban zones and urban slums were two to four-times higher than in rural areas which was contrary to our findings[19]. Our study showed that the virus seemed to have taken its roots in rural areas of Jammu district and behavioural attributes like wearing a mask by urban versus rural population might have contributed to the differences in seroprevalence.

Of all those who tested positive, only 11.6 per cent (19 of 164) reported contact with suspected/ confirmed case of COVID-19 which implied that approximately 90 per cent adults did not know about their infection status, and they could have inadvertently passed on the infection to others. Sore

Table II. Distribution of clinical symptoms and exposure factors across study participants in the past six months

Symptoms/exposure factors	Seronegative, n (%)	Seropositive, n (%)	*P**	Crude OR (95% CI)
Fever	81 (78.6)	22 (21.4)	<0.001	3.4 (1.9-5.4)
Sore throat	56 (75.7)	18 (24.3)	<0.001	3.9 (2.2-6.8)
Runny nose	43 (87.8)	6 (12.2)	0.296	1.5 (0.6-3.7)
Cough	64 (83.1)	13 (16.9)	0.005	2.3 (1.2-4.4)
Dyspnea	7 (53.8)	6 (46.2)	<0.001	9.9 (3.2-29.8)
Chills	13 (81.3)	3 (18.8)	0.138	2.6 (0.7-9.2)
Vomiting	1 (50.0)	1 (50.0)	0.157	11.3 (0.7-180)
Nausea	3 (100.0)	0	0.604	-
Diarrhea	5 (71.4)	2 (28.6)	0.107	4.5 (0.8-23.5)
Headache	22 (100.0)	0	0.250	-
Rash	2 (100.0)	0	1.000	-
Conjunctivitis	2 (100.0)	0	1.000	-
Myalgia	79 (83.2)	16 (16.8)	0.002	2.4 (1.3-4.2)
Anosmia	4 (57.1)	3 (42.9)	0.015	8.5 (1.9-38.5)
Ageusia	4 (57.1)	3 (42.9)	0.015	8.5 (1.9-38.5)
Fatigue	26 (78.8)	7 (21.2)	0.015	3.1 (1.3-7.3)
Seizures	5 (62.5)	3 (37.5)	0.022	6.8 (1.6-28.8)
History of Contact	76 (80.0)	19 (20.0)	<0.001	3.03 (1.8-5.2)
Medical consultation	18 (64.3)	10 (35.7)	<0.001	6.5 (2.9-14.5)
Hospitalization	1 (20.0)	4 (80.0)	<0.001	45.8 (5.1-412.4)
Missed work**	15 (55.6)	12 (44.4)	<0.001	9.5 (4.4-20.8)

*P values of Mid *P* exact/Fischer exact test are reported in cases where Chi square test is not applicable; **Missed work was operationally defined as any person reporting absence from work for ≥2 days due to any of the above health related symptoms. OR, odds ratio; CI, confidence interval

Table III. Distribution of the three most common symptoms of COVID-19 among sampled individuals in the past six months

Fever/dry cough/fatigue	Seropositive, n (%)	Seronegative, n (%)	*P*	Crude OR (95% CI)
Reported	42 (25.6)	171 (9.3)	<0.001	3.4 (2.3-4.9)
Not Reported	122 (74.4)	1665 (90.7)		

OR, odds ratio; CI, confidence interval

Table IV. Binary logistic regression for risk factors for seropositivity

Variable	β	SE	*P*	AOR (exp β)	95% CI for AOR*	
					Lower	Upper
Sore throat	1.234	0.518	0.017	3.435	1.244	9.484
Myalgia	0.763	0.361	0.034	2.144	1.058	4.346
Missed work	2.025	0.794	0.011	7.575	1.598	35.899

*Adjusted for all other variables in Tables I and II. OR, odds ratio; SE, standard error; AOR, adjusted OR

throat and myalgia emerged as significant predictors of seropositivity in the present study but these are listed as less-common symptoms of COVID-19 by the WHO[20].

Block-wise adjusted seropositivity ranged from 4.1 per cent in Chowki Chaura to maximum of 16.7 per cent in Pallanwala. The age group wise adjusted seropositivity ranged between 7.5

Table V. Crude and adjusted seropositivity in the ten health blocks in Jammu district

Name of block	Crude seropositivity, n (%)	Total samples	Seropositivity (95% CI) further adjusted for test performance
Marh	24 (12.0)	200	12.9 (12.83-12.97)
Dansal	9 (4.6)	194	4.9 (4.77-5.03)
Akhnoor	19 (9.2)	206	9.7 (9.64-9.76)
Jammu	15 (7.5)	199	8.0 (7.95-8.05)
Bishnah	25 (12.7)	197	13.6 (13.53-13.67)
Pallanwalla	31 (15.6)	199	16.7 (16.63-16.77)
RS Pura	16 (7.8)	206	8.3 (8.25-8.35)
Kotbalwal	10 (5.0)	200	5.3 (5.16-5.44)
Chauki Chaura	7 (3.5)	200	4.1 (3.97-4.23)
Sohanjana	8 (4.0)	199	4.3 (4.26-4.34)
Total	164 (8.2)	2000	8.8 (8.78-8.82)

CI, confidence interval

and 11.6 per cent. While a little more than half of the surveyed adults were <40 yr of age, maximum seropositivity was reported in the geriatric age-group of 61-70 yr followed by 41-50 yr which was in agreement with other serosurveys[17,18]. Pre-existing humoral immunity due to prior human coronavirus (HCoV) infections may to some extent, account for age related COVID-19 susceptibility where higher HCoV infection rates in younger age groups coincides with relative protection from COVID-19[21]. The wide range of seropositivity in the 10 blocks and in different age-groups emphasizes the need to boost testing of both suspected and vulnerable populations in all areas.

The adjusted seroprevalence estimate of the present study was extrapolated to >18 yr old population in Jammu district using estimated population projection data[11], to assess the total number of COVID 19 infections in the district. Forty per cent of the population in the UT of Jammu and Kashmir is <18 yr of age[10,11]. Excluding this fraction (0.4 × 17,44,152 = 6,97,660) from the 17,44,152 projected population of Jammu district, it was observed that adjusted seropositivity of 8.8 per cent in Jammu district, translates into 92,091 cases (1046,491 × 0.088) in adult population which was 14.4 times higher than that the 6396 reverse transcription (RT)-PCR confirmed COVID-19 cases among adult population on September 9, 2020[4].

According to official records, 73 deaths had occurred in Jammu district by September 9, 2020. The infection fatality rate was estimated by dividing this number by the total estimated infections *i.e.* 92,091 cases, which worked out to be 0.08 per cent which was lower than the case fatality rate of 1.5 per cent for UT of Jammu and Kashmir according to official records[4].

The present serosurvey had some limitations. Such serosurveys do not provide reliable data on community immunity as ELISA detects binding antibodies which are known to wane and even disappear from detection over weeks or months. This study does not show that those with antibodies are immune to the virus as there is poor correlation of binding antibodies to virus neutralizing antibodies[22]. We might have underestimated seropositivity by unintentionally skipping high prevalence clusters. By selecting more than one individual per household, we might have overestimated positivity in general population as spread is likely to be higher within households. Hence, selection bias as well as recall bias might also have affected the results. People <18 yr of age were not tested, and the positive serum samples were not re-tested. Novel coronaviruses may encounter pre-existing immunity in humans hence distinguishing pre-existing humoral immunity is critical to interpreting the results of the present study[21]. Moreover, IgG serostudies may underestimate the true level of immunity to COVID-19 in the populace as actual immunity may be higher due to immunity conferred by T cells[23].

In conclusion, the present survey has brought forth the fact that even if it is assumed that antibodies confer protection against re-infection, herd immunity was not achieved as major proportion of the population was still immunologically naïve. Hence, continued surveillance of seroprevalence may be mandatory to

monitor the trends and dynamics of COVID-19 with regard to the degree, speed and severity of spread and to gauge the possible effect of containment policies over time. These preliminary results may provide an important benchmark to monitor the state of the COVID-19 epidemic and guide locally appropriate control strategies in the region.

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***Conflicts of Interest*:** None.

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