BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-053791
Article Type:	Original research
Date Submitted by the Author:	24-May-2021

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Keywords:	COVID-19, EPIDEMIOLOGY, Epidemiology < INFECTIOUS DISEASES

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Results of a population-based survey to estimate the seroprevalence of SARS-CoV-2 specific IgG antibodies in Kashmir, India, seven months after the appearance of the first COVID-19 case

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Word Count: 2913

ABSTRACT

Objectives: We designed a population-based survey in Kashmir to estimate the seroprevalence of SARS-CoV-2 specific IgG antibodies in the general population aged 18 years and above.

Setting: The survey was conducted among 110 villages and urban wards across ten districts in Kashmir from 17 Oct 2020 to 04 Nov 2020.

Participants: Individuals aged 18 years and above were eligible to be included in the survey. Serum samples were tested for the presence of SARS-CoV-2 specific IgG antibodies using the Abbott SARS-CoV-2 IgG assay.

Primary and secondary outcome measures: We labeled assay results equal to or above the cut-off index value of 1·4 as positive for SARS-CoV-2 specific IgG antibodies. Seroprevalence estimates were adjusted for the sampling design and assay characteristics.

Results: Out of 6397 eligible individuals enumerated, 6315 (98.7%) agreed to participate. The final analysis was done on 6230 participants. Seroprevalence adjusted for the sampling design and assay characteristics was 36.7% (95% CI 34.3%-39.2%). Seroprevalence was higher among the older population. Only one-half of symptomatic individuals reported having been tested. One out of every ten seropositive individuals reported a history of COVID-19 like symptoms. We estimated an infection fatality rate of 342 deaths per million infections.

Conclusions: During the first seven months of the COVID-19 epidemic in Kashmir valley, approximately 37% of individuals were infected. There is still a significant pool of susceptible people in Kashmir. The number of infections will continue to rise unless infection prevention measures are practiced by the population.

ARTICLE SUMMARY

Strengths and limitations of this study

- The findings of our study are based on a representative sample of the population.
- The laboratory test used for the detection of SARS-CoV-2 specific IgG antibodies in serum samples provides valid results.
- We report seroprevalence estimates adjusted for sampling design and test performance.
- Even though we adjusted the weighted seroprevalence estimates for test performance using manufacturer-provided sensitivity and specificity (100% and 99·63% respectively), we did not quantify the test validity in-house.
- Because of lack of age- and gender-specific mortality data we could not estimate age- and gender-specific infection fatality rate.

INTRODUCTION

On 11 Feb 2020, the World Health Organization announced that the disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) will be named coronavirus disease (COVID-19).[1] In Kashmir, the first case of COVID-19 was confirmed in Srinagar city on 18 Mar 2020.[2] The government imposed the first phase of the lockdown in Kashmir on 24 March 2020. During this phase, inter-state travel remained suspended. People were barred from moving outside except in an emergency. Except

for essential services, all government and private offices were advised to work from home. Universal masking was made mandatory. The lockdown was extended till 31 May 2020 and later relaxed in a phased manner.

Mild or asymptomatic infections are common in Coronavirus disease 2019 (COVID-19) and are an important source of infection transmission.[3,4] Such cases are less likely to be detected by a surveillance system based on reverse-transcriptase polymerase chain reaction (RT-PCR) testing. The number of reported RT-PCR positive cases are an underestimate of the true number of infections in a population.

Seroprevalence surveys have been conducted in various countries at different stages of the current epidemic among various population groups.[5–14] Seroprevalence surveys provide a more accurate estimate of past infection, improve understanding of the infection transmission dynamics, and guide public health response.[15]

We designed this survey to estimate the seroprevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) specific IgG antibodies in the adult population of Kashmir valley.

METHODS

We designed a population-based cross-sectional study. The study covered all the ten districts of Kashmir, a valley in northern India. We completed data collection in three weeks, from 17 Oct 2020 to 04 Nov 2020.

Ethics

We obtained written informed consent from all study participants. The study was approved by the Institutional Ethics Committee of Government Medical College Srinagar. We used anonymized participant data for analysis.

Patient and Public Involvement

No patients were involved in this study.

Sample size

We calculated the minimum sample size based on an anticipated seroprevalence of 20%, an absolute error of 2%, and a design effect of 2. To obtain precise estimates for district Srinagar, sample size estimation was made for the district separately. We targeted a total sample size of 6400.

Participants

All adults ≥18 years of age were eligible to participate in the study. We selected eligible participants using a three-stage stratified cluster sampling technique. We listed all clusters in the valley using the Census 2011 data.[16] Within each of the ten districts in the valley, clusters were stratified into urban and rural clusters. We selected clusters within each of the 20 strata by probability proportionate to size (PPS) sampling. Except for district Srinagar which we oversampled to obtain precise seroprevalence estimates for, ten clusters were randomly selected from each district. We selected 20 clusters from district Srinagar. Within each selected cluster we identified four random locations and approached consecutive households to enroll at least ten eligible participants. We thus identified a total of 440

random locations within 110 clusters in ten districts. We invited all eligible adults in a household for participation.

Variables

The main outcome variable of interest was SARS-CoV-2 specific IgG antibodies. We obtained information from participants about their age, gender, history of COVID-19 like symptoms in the three months before the interview date, history of contact with a known COVID-19 patient, and history of COVID-19 testing.

Procedure

We informed eligible adults about the purpose and the procedure of the study. Study participation was voluntary. Participants were interviewed by health personnel specifically trained for the interview. Interview responses were recorded in an Epicollect5 form.[17] Once the interview was completed, a trained phlebotomist collected 3-5 mL of venous blood from the antecubital vein under aseptic precautions into a red-top collection tube containing a clot activator. The tube was left standing, undisturbed, for at least 30 minutes for clot formation. The sample was later transported to a central facility for centrifugation. Centrifuged samples were transported to a central laboratory for further processing and analysis. Serum samples were tested for the presence of SARS-CoV-2 specific IgG antibodies using the Abbott SARS-CoV-2 IgG assay. The assay uses chemiluminescence to detect IgG antibodies against the SARS-CoV-2 nucleocapsid protein. The reported sensitivity and specificity of the assay are 100% and 99·63%, respectively.[18] As recommended by the manufacturer, we labeled assay results equal to or above the cut-off index value of 1·4 as positive for SARS-CoV-2 specific IgG antibodies.

Statistical methods

We report unweighted seroprevalence estimates in percentages. We used the Agresti-Coull procedure to calculate a 95% confidence interval (CI) for seroprevalence estimates. A weighted estimate of seroprevalence is provided. To calculate survey weights (inverse of sampling probability) we used the estimated population of the districts. We used the census 2011 data and growth rates from Sample Registration System to estimate the population of the districts in 2020.[16,19] Survey weights so obtained was further adjusted for non-response and age and sex structure (post-stratification weights). We further adjusted the weighted seroprevalence estimates for test performance to calculate "weighted seroprevalence adjusted for test performance". We did this using the formula:
$Weighted\ seroprevalence\ adjusted\ for\ test\ performance =$
$(Weighted\ seroprevalence + Test\ specificity - 1)/(Test\ sensitivity + Test\ specificty - 1)$.[20]

We used the manufacturer-provided sensitivity and specificity in the above formula.[18]

We analyzed the difference in seroprevalence estimates across levels of a categorical variable using a Chi-square test adjusted for the sampling design.

We estimated the number of SARS-CoV-2 infections by multiplying the weighted seroprevalence adjusted for test performance with the estimated population of the valley. To estimate the number of infections per reported case, we divided the estimated number of SARS-CoV-2 infections by the reported number of COVID-19 cases two weeks before the survey date. We calculated the infection

fatality rate by dividing the reported number of deaths by the number of estimated infections, assuming a three-week lag time from infection to death.[21]

We analyzed the data using Stata version 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LP.).

RESULTS

We enumerated 6 397 individuals ≥18 years from 3 077 households and 110 clusters (34 urban clusters and 76 rural clusters) between 17 Oct 2020 and 04 Nov 2020. Out of the 6 397 eligible individuals, 6 315 (98·7%) agreed to participate and were enrolled. The final analysis was done on a sample of 6 230 participants. (Figure 1)

Of the 6 230 participants, 1 513 (24·3%) were between 18 and 30 years of age, 2 672 (42·9%) were aged 30-49 years, 1 643 (26·4%) were aged 50-69 years, and 402 (6·4%) were 70 years and older. (Table 1). There was equal representation from males and females, and 3 364 (54·0%) resided in a rural area. Of the 3 104 females, 56 (1·8%) reported pregnant at the time of the survey. Four hundred seventy-four (7·6%) reported COVID-19 like symptoms in the three months preceding the survey and 439 (7·0%) reported to have ever come in contact with a known COVID-19 case. One thousand ninety-two (17·5%) reported having been tested for COVID-19 using RT-PCR or a Rapid Antigen Test (RAT) previously, of whom 176 (16·2%) reported to have tested positive for the disease.

Table 1: Characteristics of study participants

	Frequency	**Percent**
Total	6230	..
Age, years	..	..
18-29	1513	24·3
30-49	2672	42·9
50-69	1643	26·4
≥70	402	6·5
Gender	..	..
Male	3126	50·2
Female	3104	49·8
Residence	..	..
Urban	2866	46·0
Rural	3364	54·0
Pregnant (n=3104)	56	1·8
Self-reported history of chronic disease	1145	18.4
History of COVID-19 like symptoms	474	7·6
History of contact with a known COVID-19 case	439	7·0
Ever tested for COVID-19 (RT-PCR)	1092	17·5
RT-PCR result (n=1088*)	..	..
Positive	176	16·2
Negative	912	83·8

COVID-19: Coronavirus disease; RT-PCR: Reverse transcriptase polymerase chain reaction

*RT-PCR result not known in four participants

We found an overall unweighted seroprevalence of 38·8% (95% CI 37·6 – 40·0). The seroprevalence ranged from 28·2% in district Kulgam to 41·7% in district Badgam. The overall weighted seroprevalence (adjusted for sampling design) was 36·9% (95% CI 34·5 – 39·4). The weighted seroprevalence adjusted for test performance was 36·7% (95% CI 34·3 – 39·2). (Table 1)

Seroprevalence was lowest among participants aged 18-29 years [33·5% (95% CI 29·8 – 37·4)] and was higher in older age groups. Seroprevalence was highest in those aged 70 years and above [45·1% (95% CI 37·6 – 52·8)]. Seroprevalence was not significantly different among males and females (p=0·34). The seroprevalence among urban residents was 40·0% (95% CI 36·1 – 43·9), slightly but not significantly, higher than rural residents [35·3% (95% CI 32·2 – 38·5), p=0·07]. (Table 2)

Table 2: Seroprevalence of SARS-CoV-2 specific IgG antibodies by participant characteristics

	Number tested, n	Number seropositive, n	Unweighted seroprevalence, % (95% CI)	Weighted seroprevalence, % (95% CI)	Weighted seroprevalence adjusted for test performance, % (95% CI)	Design-based F, p-value
Total	6230	2415	38·8 (37·6-40·0)	36·9 (34·5-39·4)	36·7 (34·3-39·2)	..
Age, years	..	..	..	..	..	..
18-29	1513	538	35·6 (33·2-38·0)	33·7 (30·1-37·6)	33·5 (29·8-37·4)	6·42, 0·0006
30-49	2672	1000	37·4 (35·6-39·3)	36·3 (33·5-39·3)	36·1 (33·3-39·1)	..
50-69	1643	691	42·1 (39·7-44·5)	42·5 938·8-46·2)	42·3 (38·6-46·0)	..
≥70	402	186	46·3 (41·5-51·2)	45·3 937·8-53·0)	45·1 (37·6-52·8)	..
Gender	..	..	..	..	..	..
Male	3126	1166	37·3 (35·6-39·0)	36·1 (33·5-38·9)	35·9 (33·3-38·7)	0·94, 0·34
Female	3104	1249	40·2 (38·5-42·0)	37·8 (34·5-41·30	37·6 (34·3-41·1)	..
Residence	..	..	..	..	..	..
Urban	2866	1180	41·2 (39·4-43·0)	40·2 (36·3-44·1)	40·0 (36·1-43·9)	3·43, 0·07
Rural	3364	1235	36·7 (35·1-38·4)	35·5 (32·5-38·7)	35·3 (32·2-38·5)	..

Self-reported history of chronic disease	..	..	..	..	..	..
Yes	1145	495	43·2 (40·4-46·1)	41·9 (37·4-46·6)	41·7 (37·2-46·4)	6.14, 0.02
No	5085	1920	37·8 (36·4-39·1)	36·2 (33·7-38·9)	36·0 (33·5-38·7)	
History of COVID-19 like symptoms	..	..	..	..	..	..
Yes	474	247	52·1 (47·6-56·6)	47·4 (37·9-57·1)	47·2 (37·7-56·9)	5·53, 0·02
No	5756	2168	37·7 (36·4-38·9)	36·3 (33·9-38·8)	36·1 (33·7-38·6)	..
History of contact with a known COVID-19 case	..	..	..	..	..	..
Yes	439	219	49·9 (45·2-54·5)	45·2 (38·3-52·2)	45·0 (38·1-52·0)	7·13, 0·01
No	5791	2196	37·9 (36·7-39·2)	36·5 (34·1-39·0)	36·3 (33·9-38·8)	..
Ever tested for COVID-19 (RT-PCR)	..	..	..	..	..	..
Yes	1092	485	44·4 (41·5-47·4)	41·0 (35·4-46·9)	40·8 (35·2-46·7)	2·17, 0·14
No	5138	1930	37·6 (36·2-38·9)	36·2 (33·5-39·0)	36·0 (33·3-38·8)	..
RT-PCR result (n=1088*)	..	..	..	..	..	..
Positive	176	140	79·5 (73·0-84·9)	81·8 (74·8-87·1)	81·7 (74·7-87·1)	74·93, <0·0001
Negative	912	345	37·8 (34·7-41·0)	38·8 (33·3-44·7)	38·6 (33·1-44·5)	..

COVID-19: Coronavirus disease; RT-PCR: Reverse transcriptase polymerase chain reaction
*RT-PCR result not known in four participants

One in five participants (1145/6230, 18·4%) self-reported a history of at least one chronic disease (Table 1). Hypertension (815/6230, 13.1%) and diabetes mellitus (314/6230, 5.0%) were the most commonly reported chronic diseases (Supplementary File 1). Seroprevalence was significantly higher in participants who self-reported history of chronic disease (41·7%, 95% CI 37·2 – 46·4) as compared to those who did not report a history of chronic disease (36·0%, 95% CI 33·5 - 38·7) (Table 2).

Among participants who reported a history of COVID-19 like symptoms, seroprevalence was 47·2% (95% CI 37·7 – 56·9) compared with 36·1% (95% CI 33·7 – 38·6) among participants who did not report such symptoms. Seroprevalence was higher among those who reported contact with a known COVID-19 case [45·0% (95% CI 38·1 – 52·0)] than participants who did not report any history of such contact [36·3% (95% CI 33·9 – 38·8)]. (Table 2)

Seroprevalence was not significantly related to being tested for COVID-19 (RT-PCR). However, those who reported a positive RT-PCR COVID-19 test had significantly higher seroprevalence (81·7%, 95% CI 74·7 – 87·1) as compared to those who reported a negative RT-PCR COVID-19 test (38·6%, 95% CI 33·1 – 44·5). (Table 2)

Among 2 415 seropositive individuals, only 247 (10·2%) reported a history of COVID-19 like symptoms. Among 474 who reported a history of COVID-19 like symptoms, 233 (49·2%) were tested for COVID-19 (RT-PCR).

Among 36 participants who reported a positive RT-PCR COVID-19 test but were seronegative, the duration between COVID-19 RT-PCR test and serological testing ranged from 9 days to 101 days. In only four of these 36 participants, the duration between the COVID-19 RT-PCR test and the serological test was 14 days or less. Of the remaining 32 participants, 21 did not report a history of CVOID-19 like symptoms, nine did not report a history of contact with a known COVID-19 case, and eight reported neither a history of COVID-19 like symptoms nor a history of contact with a known COVID-19 case.

We estimated that there were 1 673 484 (95% CI 1 564 047 – 1 787 482) cumulative number of infections among adults aged ≥18 years in the valley by 03 Oct 2020, two weeks before the start of the survey. If we assume that the seroprevalence was similar to the overall seroprevalence in the population not included in our study (<18 years of age) then the estimated cumulative number of infections in the valley by 03 Oct 2020 was 2 791 933 (95% CI 2 609 354 – 2 982 119). Considering that the cumulative number of reported COVID-19 cases was 47 071 by 03 Oct 2020, we estimate the number of infections per reported case as 59·3 (95% CI 55·4 – 63·4). The number of reported COVID-19 deaths after a three-week lag period (on 24 Oct 2020) was 955. Thus, we estimated the infection fatality rate as 342·1 deaths per million infections (95% CI 320·2 – 366·0).

DISCUSSION

The results of our study indicate that by the first week of October 2020, nearly seven months after the appearance of the first laboratory-confirmed COVID-19 case on 18 March 2020, close to 37% of the valley's population aged ≥18 years had been infected. Our results suggest that the cumulative number of SARS-CoV-2 infections by the first week of October 2020 was nearly 2·8 million with an estimated infection fatality rate of 342·1 deaths per million infections. Seroprevalence did not differ by gender but was higher in older age groups.

The findings of our study are based on a representative sample of the population. The laboratory test used for the detection of SARS-CoV-2 specific IgG antibodies in serum samples provides valid results.[18,22] We report seroprevalence estimates adjusted for sampling design and test performance.

The overall adjusted seroprevalence of around 37% indicates that a large proportion of the valley's population has been infected with the virus. Easing of lockdown, being fed up with the restrictions, and non-adherence to prevention norms are the possible reasons. Even though a large proportion of the population has been infected, the transmission of infection is expected to continue till most of the susceptible population becomes immune. Herd immunity in the context of COVID-19 is a matter of debate as reports of a second infection continue to pour in.[23] Several factors potentially influence the seroprevalence rates. These include population density, social and demographic structure of the population, governmental policies and the extent of their implementation, immunity level of the population, time since the start of infection transmission, adherence to infection prevention guidelines, quality of contact tracing and quarantine, and possibly the geography and environment of an area.

The seroprevalence of SARS-CoV-2 IgG antibodies was higher in older age groups. During the early period of the pandemic, people were adherent to social distancing and other non-pharmaceutical interventions because of a fear of the disease and administrative restrictions. With time, administrative restrictions were relaxed, fear of the disease attenuated, and people became sort of fed up with the social restrictions. This not only led to an increase in the number of reported COVID-19 cases but also provided the population, including older age groups, an opportunity to contract the infection. That older people have an increased risk of symptomatic and more severe disease is now well known.[24,25] However, age-based differential susceptibility to SARS-CoV-2 infection antibody response and the reasons thereof are still a grey area and need further understanding. Existing literature might suggest that the young who are more mobile and socially active have a higher risk of infection.[6,7] However, this should not imply that the elderly have a decreased susceptibility to SARS-CoV-2 infection or a decreased antibody response.[26] On the contrary, several studies suggest that the seroprevalence of SARS-CoV-2 specific IgG antibodies is higher in the older age groups and particularly so in more dense population groups.[4,5,8–11,13] Furthermore, SARS-CoV-2 antibody levels have been reported to be higher in older people.[12]

The seroprevalence of SARS-CoV-2 specific IgG antibodies did not differ significantly by gender, though the figure was slightly higher for females. These findings are consistent with the available literature.[6,13] Difference in seroprevalence by gender has been suggested by some studies and females have been reported to have lower antibody levels.[5,7,9,11,12,14,27]

Urban areas are more densely populated as compared to rural areas which accelerate the transmission of infections in the population. Seroprevalence of SARS-CoV-2 specific IgG antibodies is thus expected to be higher in urban areas especially during the early phases of an epidemic. As the epidemic progresses the seroprevalence gap between urban and rural areas will wane off. We estimated an adjusted seroprevalence of 40·0% (95% CI 36·1 – 43·9) in urban areas as compared to 35·3% (95% CI 32·2 – 38·5) in rural areas (p=0·07).

People with a chronic disease experience more severe COVID-19 symptoms and are more likely to die when compared to people with no chronic disease.[28] We found a higher proportion of symptomatic infection among participants with a self-reported history of chronic disease (78/1145, 6·8%) as compared to participants with no chronic disease (169/5085, 3·3%) (Supplementary File 2). Little is,

however, known about the risk of infection in chronic disease patients. We found a significantly higher seroprevalence among participants with a self-reported history of chronic disease (Table 2). This finding needs further research for corroboration and possible explanations.

People with a self-reported history of COVID-19 related symptoms, contact with a known COVID-19 case, or a positive COVID-19 RT-PCR had a higher seroprevalence as compared to their complement. Among seropositive individuals, only 10·2% reported being symptomatic. The percentage of asymptomatic infections, thus, was 90%. However, only 49% of individuals with a history of COVID-19 like symptoms were tested using RT-PCR. We also estimated that only one out of almost 59 infections gets reported. This reflects the necessity of improving the efficiency of RT-PCR testing so that more symptomatic individuals receive the test. Not all individuals with a known RT-PCR positive result showed the presence of IgG antibodies. Around 20% of RT-PCR positive individuals were seronegative and in a large majority of them (32 out of 36) the duration since RT-PCR positivity was more than two weeks. This may be attributed to a poor B cell response or a false negative antibody test.[29] Around 38% of RT-PCR negative individuals were seropositive suggesting a false-negative RT-PCR or infection acquisition at a date later than the RT-PCR test.

We estimated an infection fatality rate of 342·1 per million infections (95% CI 320·2 – 366·0). In developed nations like the United States and many European countries, a higher infection fatality rate has been reported.[24,30] The infection fatality rate is, however, known to be lower in developing nations.[24,31]

One important limitation of our study is that even though we adjusted the weighted seroprevalence estimates for test performance using manufacturer-provided sensitivity and specificity (100% and 99·63% respectively), we did not quantify the test validity in-house. Another limitation of our study estimates is that we excluded people <18 years of age. The results of our study may not thus be generalizable to this group of the population. Further, because of lack of age- and gender-specific mortality data we could not estimate age- and gender-specific infection fatality rate.

CONCLUSIONS

We conclude that nearly 37% of individuals aged 18 years and above were infected with SARS-CoV-2 in Kashmir by October 2020. The infection fatality rate in the valley is around 342 deaths per million infections. A majority of cases go unreported. Given the current adherence to COVID-19 prevention measures in the valley, the seroprevalence is going to increase. Since almost half of the symptomatic individuals go unreported, testing of symptomatic individuals and effective contact tracing needs to continue. We further recommend that adherence to COVID-19 prevention measures should be ensured at least till a large proportion of the population gets vaccinated.

ACKNOWLEDGMENTS

We thank Prof. Samia Rashid for her support. We thank the following coordinating center teams and the laboratory support team for their support and coordination:

Coordinating center teams: Dr. Sabira Aalia Dkhar, Dr. Kouser Sideeq, Dr. Uruj Qureshi, Dr. Mehraj u Din Sofi, Dr. Yasmin Kango, Dr. Suhail Manzoor Shah, Dr. Bashir Ahmad Khan, Dr. Nabeel Ishtiyaq, Dr. Tajamul Hussain Khan, Dr. Lahoot Hassan Khan, Dr. Muzaffer Ahmad, Dr. Ramesh Kumar Malla, Dr. Ruqaya Rasool Kapta, Dr. Mohammad Yousuf Naikoo, Dr. Khalid Abbass, Dr. Zarar Ahmad Bhat, Dr.

Sumair Rashid, Dr. Zubair Ahmad, Dr. Shalender Singh Bali, Dr. Abdul Rouf, Dr. Abdul Hamid, Dr. Khalid Bashir, Dr. Qazi Najeeb Ahmad, Dr. Syed Irtiza, Dr. P Sharik Shaheen Khaki, Dr. Sheikh Mohammad Shafi, Dr. Mohammad Yousuf Zagoo, Dr. Mukhtar Ahmad Shah, Dr. Imtiyaz-ur-Rehman Bhat, Dr. Showkat Parray, Dr. Fazil Ali Kochak, Dr. Muzaffar Ahmad Zargar, Dr. Abdul Rashid Shah, Dr. Shagufta Saleem, Dr. Abdul Gani, Dr. Nighat Nasreen, Dr. Asima Nazeer, Dr. Zahoor Ahmad Lone.

Laboratory support team: Ishtiyaq Ahmad Handoo, Rahiba Makhdoomi, Iyman Wani, Romana Rasool, Asiya Rehman, Insha Rehman, Zahoor Ahmad, Gulzar Ahmad Wani, Javid Ahmad Bhat, M Saleem Malik, Naveed Manzoor, Mubeena.

COMPETING INTERESTS

We declare no competing interests, financial or otherwise.

FUNDING

The National Health Mission Jammu & Kashmir funded the study. The funding agency was not involved in study designing, implementation, analysis, or interpretation of the study findings.

CONTRIBUTORS

S Muhammad Salim Khan: Conceptualization, Funding acquisition, Methodology, Writing-review & editing.

Mariya Amin Qurieshi, Inaamul Haq, Sabhiya Majid: Formal analysis, Data curation, Methodology, Project administration, Writing-original draft, Writing-review & editing.

Javid Ahmad, Taha Ayub, Anjum Bashir, Ashfaq Ahmad Bhat, Abdul Majeed Ganai, Yasmeen Jan, Rauf-ur-Rashid Kaul, Zahid Ali Khan, Muneer Ahmad Masoodi, Beenish Mushtaq, Fouzia Nazir, Muzamil Nazir, Malik Waseem Raja, Mahbooba Rasool: Project administration, Supervision, Investigation, Writing-review & editing.

Anjum Asma, Munazza Aziz, Shifana Ayoub, Arif Akbar Bhat, Iqra Nisar Chowdri, Shaista Ismail, Misbah Ferooz Kawoosa, Mehvish Afzal Khan, Mosin Saleem Khan, Rafiya Kousar, Ab Aziz Lone, Shahroz Nabi, Mohammad Obaid, Tanzeela Bashir Qazi, Iram Sabah, Ishtiyaq Ahmad Sumji: Supervision, Investigation, Writing-review & editing.

S Muhammad Salim Khan, Mariya Amin Qurieshi, Inaamul Haq, and Sabhiya Majid have verified the underlying data.

DATA SHARING

Anonymized data collected for the study, including individual participant data and a data dictionary defining each field in the set, will be made available to interested researchers on request by Inaamul Haq (haqinaam@yahoo.co.in) for two years from publication. The study protocol, statistical analysis plan, and informed consent forms are also available from Inaamul Haq.

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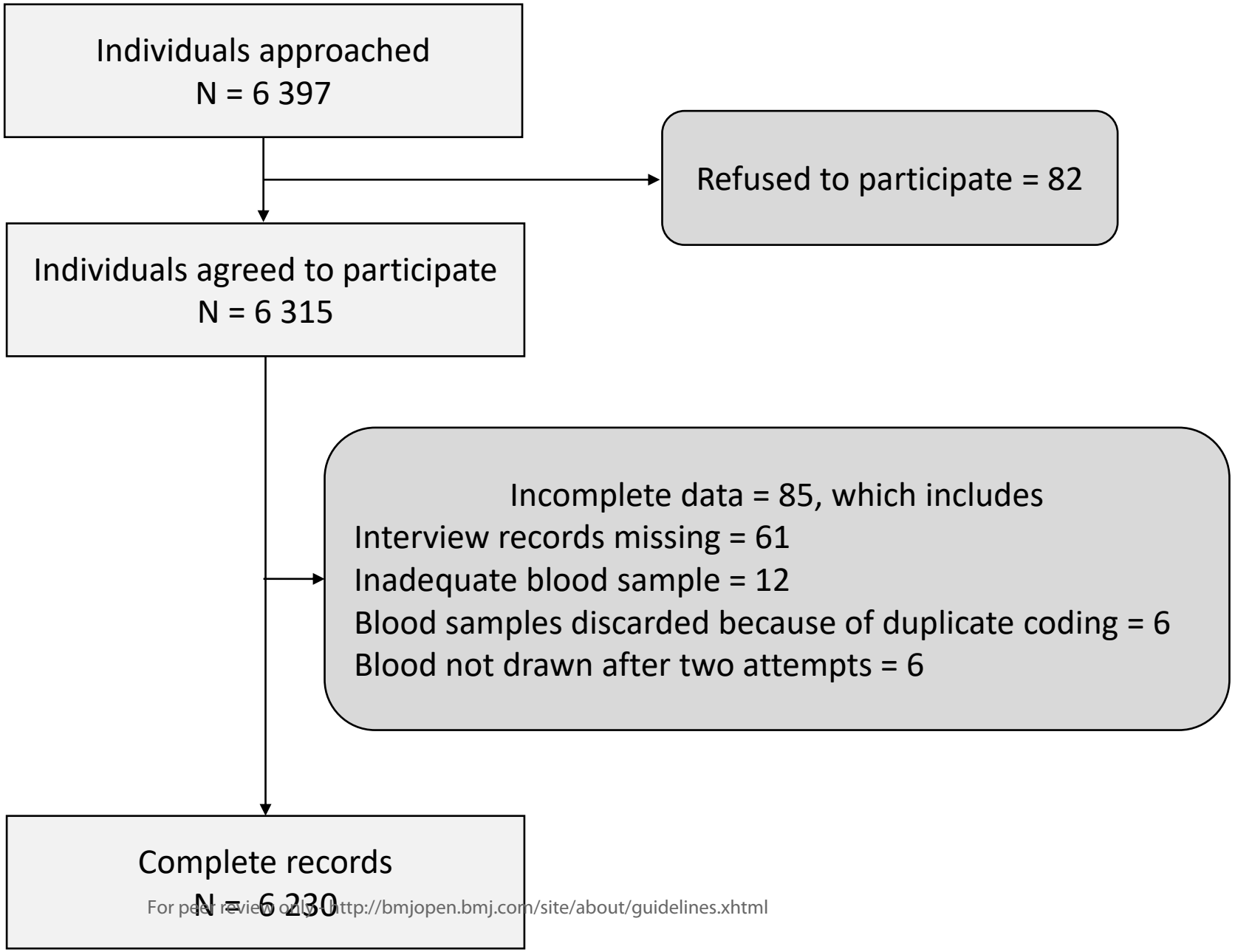
Figure 1 legend:

Figure 1: Participant flow.

Figure 1: Participant flow



Chronic disease and SARS-CoV-2 infection in the study population

Of the 6230 participants, 1145 reported a history of at least one chronic disease. Two hundred ninety-eight reported a history of more than one chronic disease.

Supplementary Table 1: Chronic disease in the study population

Chronic disease (n = 1145)	Number (%)
Hypertension	815 (13·1%)
Diabetes	314 (5·0%)
Chronic Obstructive Pulmonary Disease	39 (0·6%)
Coronary Heart Disease	35 (0·6%)
Cerebrovascular Disease	16 (0·3%)
Asthma	15 (0·2%)
Chronic Kidney Disease	10 (0·2%)
Chronic Liver Disease	5 (0·1%)
Cancer	4 (0·1%)

Supplementary Table 2: History of COVID-19 like symptoms and seropositivity vis-à-vis history of chronic disease

	..	History of COVID-19 like symptoms	
..	..	**Yes**	**No**
Reported history of chronic disease (n=1145)	**Seropositive**	78	417
..	**Seronegative**	63	587
Did not report any history of chronic disease (n=5085)	**Seropositive**	169	1751
..	**Seronegative**	164	3001

Of the 1154 participants who reported a history of chronic disease 78 (6.8%) reported a history of COVID-19 like symptoms within three months of the interview date and were seropositive for SARS-CoV-2 specific IgG (symptomatic infection). The proportion of symptomatic infection among participants who did not report any history of chronic disease was 3.3% (169/5085).

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	4, 5
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4, 5
		(*b*) Describe any methods used to examine subgroups and interactions	5
		(*c*) Explain how missing data were addressed	5
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	4, 5
		(*e*) Describe any sensitivity analyses	-
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure 1, and page 5
		(b) Give reasons for non-participation at each stage	Figure 1
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1, Figure 1
Outcome data	15*	Report numbers of outcome events or summary measures	Table 2

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2
		(*b*) Report category boundaries when continuous variables were categorized	Table 1, 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Table 2
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9, 10
Generalisability	21	Discuss the generalisability (external validity) of the study results	9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for exposed and unexposed groups.

BMJ Open

Seroprevalence of SARS-CoV-2 specific IgG antibodies in Kashmir, India, seven months after the first reported local COVID-19 case: results of a population-based seroprevalence survey from October-November, 2020

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-053791.R1
Article Type:	Original research
Date Submitted by the Author:	14-Jul-2021

Complete List of Authors:	Khan, S Muhammad; Government Medical College Srinagar, Community Medicine Qurieshi, Mariya; Government Medical College Srinagar, Community Medicine Haq, Inaamul; Government Medical College Srinagar, Community Medicine Majid, Sabhiya; Government Medical College Srinagar, Biochemistry Ahmad, Javid; Sher-i-Kashmir Institute of Medical Sciences, Community Medicine Ayub, Taha; Government Medical College Srinagar, Community Medicine Bhat, Ashfaq; SKIMS Medical College Srinagar, Community Medicine Fazili, Anjum; Sher-i-Kashmir Institute of Medical Sciences, Community Medicine Ganai, Abdul; Government Medical College Baramulla, Community Medicine Jan, Yasmeen; SKIMS Medical College Srinagar, Community Medicine Kaul, Rauf-ur-Rashid; Sher-i-Kashmir Institute of Medical Sciences, Community Medicine Khan, Zahid; Government Medical College Baramulla, Community Medicine Masoodi, Muneer; Government Medical College Anantnag, Community Medicine Mushtaq, Beenish; SKIMS Medical College Srinagar, Community Medicine Nazir, Fouzia; Government Medical College Anantnag, Community Medicine Nazir, Muzamil; Government Medical College Baramulla, Community Medicine Raja, Malik; Government Medical College Srinagar, Community Medicine Rasool, Mahbooba; Government Medical College Anantnag, Community Medicine Asma, Anjum; Government Medical College Srinagar, Community Medicine Ayoub, Shifana; Government Medical College Srinagar, Community Medicine Aziz, Munazza; Government of Jammu and Kashmir Directorate of Health Services Bhat, Arif; Government Medical College Srinagar, Biochemistry Chowdri, Iqra; Government Medical College Srinagar, Community

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Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Epidemiology
Keywords:	COVID-19, EPIDEMIOLOGY, Epidemiology < INFECTIOUS DISEASES

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Seroprevalence of SARS-CoV-2 specific IgG antibodies in Kashmir, India, seven months after the first reported local COVID-19 case: results of a population-based seroprevalence survey from October-November, 2020

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Word Count: 3353 (excluding abstract, tables, and references)

ABSTRACT

Objectives: We designed a population-based survey in Kashmir to estimate the seroprevalence of SARS-CoV-2 specific IgG antibodies in the general population aged 18 years and above.

Setting: The survey was conducted among 110 villages and urban wards across ten districts in Kashmir from 17 Oct 2020 to 04 Nov 2020.

Participants: Individuals aged 18 years and above were eligible to be included in the survey. Serum samples were tested for the presence of SARS-CoV-2 specific IgG antibodies using the Abbott SARS-CoV-2 IgG assay.

Primary and secondary outcome measures: We labeled assay results equal to or above the cut-off index value of 1·4 as positive for SARS-CoV-2 specific IgG antibodies. Seroprevalence estimates were adjusted for the sampling design and assay characteristics.

Results: Out of 6397 eligible individuals enumerated, 6315 (98.7%) agreed to participate. The final analysis was done on 6230 participants. Seroprevalence adjusted for the sampling design and assay characteristics was 36.7% (95% CI 34.3%-39.2%). Seroprevalence was higher among the older population. Among seropositive individuals, 10.2% (247/2415) reported a history of COVID-19 like symptoms. Out of 474 symptomatic individuals, 233 (49.2%) reported having been tested. We estimated an infection fatality rate of 0.034%.

Conclusions: During the first seven months of the COVID-19 epidemic in Kashmir valley, approximately 37% of individuals were infected. A large proportion of the population remains susceptible to the infection. The experience of a second wave of COVID-19 in April-June 2021, the appearance of virus variants, and the introduction of vaccination programs warrant robust surveillance of the epidemic.

ARTICLE SUMMARY

Strengths and limitations of this study

- The findings of our study are based on a representative sample of the population.
- The laboratory test used for the detection of SARS-CoV-2 specific IgG antibodies in serum samples provides valid results.
- We report seroprevalence estimates adjusted for sampling design and test performance.
- Even though we adjusted the weighted seroprevalence estimates for test performance using manufacturer-provided sensitivity and specificity (100% and 99·63% respectively), we did not quantify the test validity in-house.
- Because of lack of age- and gender-specific mortality data we could not estimate age- and gender-specific infection fatality rates.

INTRODUCTION

On 11 Feb 2020, the World Health Organization announced that the disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) will be named coronavirus disease (COVID-19).[1] In Kashmir, the first case of COVID-19 was confirmed in Srinagar city on 18 Mar 2020.[2] The government imposed the first phase of the lockdown in Kashmir on 24 March 2020. During this phase, inter-state travel remained suspended. People were barred from moving outside except in an emergency. Except

for essential services, all government and private offices were advised to work from home. Universal masking was made mandatory. The lockdown was extended till 31 May 2020 and later relaxed in a phased manner.

Mild or asymptomatic infections are common in Coronavirus disease 2019 (COVID-19) and are an important source of infection transmission.[3,4] Such cases are less likely to be detected by a surveillance system based on reverse-transcriptase polymerase chain reaction (RT-PCR) testing. The number of reported RT-PCR positive cases are an underestimate of the true number of infections in a population.

Seroprevalence surveys have been conducted in various countries at different stages of the current epidemic among various population groups.[5–14] Seroprevalence surveys provide a more accurate estimate of past infection, improve understanding of the infection transmission dynamics, and guide public health response.[15]

We designed this survey with the primary objective to estimate the seroprevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) specific IgG antibodies in the adult population of Kashmir valley.

METHODS

We designed a population-based cross-sectional study. The study covered all the ten districts of Kashmir, a valley in northern India. (Figure 1) We completed data collection in three weeks, from 17 Oct 2020 to 04 Nov 2020.

Ethics

We obtained written informed consent from all study participants. The study was approved by the Institutional Ethics Committee of Government Medical College Srinagar (reference number: 1004/ETH/GMC). We used anonymized participant data for analysis.

Sample size

Based on the results of a previous study conducted in July 2020, we speculated that, by October 2020, the prevalence would have increased to around 20%.[16] We calculated the minimum sample size based on an anticipated seroprevalence of 20%, an absolute precision of 2%, and a design effect of 2. We used OpenEpi to make sample size calculations.[17] We adjusted the sample size for a possible non-response of 10% to obtain a minimum size of 3376. We decided to select 3600 individuals from nine of the ten districts (except district Srinagar). To obtain precise estimates for district Srinagar, sample size estimation was made for the district separately. We used a design effect of 1.5, an anticipated seroprevalence of 20%, and absolute precision of 2% to obtain a sample size of 2302 for the district, which was further increased to 2400 to account for non-response. We thus targeted a total sample size of 6000 (3600 + 2400).

Participants

All adults ≥18 years of age were eligible to participate in the study. We selected eligible participants using a three-stage stratified cluster sampling technique. We listed all clusters in the valley using the Census 2011 data.[18] Within each of the ten districts in the valley, clusters were stratified into urban

and rural clusters. We selected clusters within each of the 20 strata by probability proportionate to size (PPS) sampling. Except for district Srinagar which we oversampled to obtain precise seroprevalence estimates for, ten clusters were randomly selected from each district. We selected 20 clusters from district Srinagar. We divided each selected cluster into four equal areas and chose a central location within each of the four areas as the starting point. Thereafter, we approached consecutive households to enroll at least ten eligible participants. We thus identified a total of 440 random locations within 110 clusters in ten districts. We invited all eligible adults in a household for participation.

Variables

The main outcome variable of interest was SARS-CoV-2 specific IgG antibodies. We obtained information from participants about their age, gender, history of COVID-19 like symptoms in the three months before the interview date, history of contact with a known COVID-19 patient, and history of COVID-19 testing.

Procedure

We informed eligible adults about the purpose and the procedure of the study. Study participation was voluntary. Participants were interviewed by health personnel specifically trained for the interview. Interview responses were recorded in an Epicollect5 form.[19] Once the interview was completed, a trained phlebotomist collected 3-5 mL of venous blood from the antecubital vein under aseptic precautions into a red-top collection tube containing a clot activator. The tube was left standing, undisturbed, for at least 30 minutes for clot formation. The sample was later transported to a central facility for centrifugation. Centrifuged samples were transported to a central laboratory for further processing and analysis. Serum samples were tested for the presence of SARS-CoV-2 specific IgG antibodies using the Abbott SARS-CoV-2 IgG assay. The assay uses chemiluminescence to detect IgG antibodies against the SARS-CoV-2 nucleocapsid protein. The reported sensitivity and specificity of the assay are 100% (95% CI 95.89-100.00)and 99·63% (99.05-99.90), respectively.[20] As recommended by the manufacturer, we labeled assay results equal to or above the cut-off index value of 1·4 as positive for SARS-CoV-2 specific IgG antibodies.

Statistical methods

We report unweighted seroprevalence estimates in percentages. We used the Agresti-Coull procedure to calculate a 95% confidence interval (CI) for seroprevalence estimates.[21] A weighted estimate of seroprevalence is provided. To calculate survey weights (inverse of sampling probability) we used the estimated population of the districts. We used the census 2011 data and growth rates from Sample Registration System to estimate the population of the districts in 2020.[18,22] Survey weights so obtained were further adjusted for non-response and age and sex structure (post-stratification weights). We further adjusted the weighted seroprevalence estimates for test performance to calculate "weighted seroprevalence adjusted for test performance". We did this using the formula:
$Weighted\ seroprevalence\ adjusted\ for\ test\ performance =$
$(Weighted\ seroprevalence + Test\ specificity - 1)/(Test\ sensitivity + Test\ specificity - 1)$.[23]

We used the manufacturer-provided sensitivity and specificity in the above formula.[20] We used the lower and upper bounds of the manufacturer-provided test performance to report sensitivity analyses.

We analyzed the difference in seroprevalence estimates across levels of a categorical variable using a Chi-square test adjusted for the sampling design.

We estimated the number of SARS-CoV-2 infections by multiplying the weighted seroprevalence adjusted for test performance with the estimated population of the valley. To estimate the number of infections per reported case, we divided the estimated number of SARS-CoV-2 infections by the reported number of COVID-19 cases two weeks before the survey date. We calculated the infection fatality rate by dividing the reported number of deaths by the number of estimated infections, assuming a three-week lag time from infection to death.[24]

We analyzed the data using Stata version 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LP.).

Patient and Public Involvement

No patients or members of the public were involved in this study.

RESULTS

We enumerated 6 397 individuals ≥18 years from 3 077 households and 110 clusters (34 urban clusters and 76 rural clusters) between 17 Oct 2020 and 04 Nov 2020. Out of the 6 397 eligible individuals, 6 315 (98·7%) agreed to participate and were enrolled. The final analysis was done on a sample of 6 230 participants. (Figure 2)

Of the 6 230 participants, 1 513 (24·3%) were between 18 and 30 years of age, 2 672 (42·9%) were aged 30-49 years, 1 643 (26·4%) were aged 50-69 years, and 402 (6·4%) were 70 years and older. (Table 1). There was equal representation from males and females, and 3 364 (54·0%) resided in a rural area. Of the 3 104 females, 56 (1·8%) reported pregnant at the time of the survey. Four hundred seventy-four (7·6%) reported COVID-19 like symptoms in the three months preceding the survey and 439 (7·0%) reported to have ever come in contact with a known COVID-19 case. One thousand ninety-two (17·5%) reported having been tested for COVID-19 using RT-PCR or a Rapid Antigen Test (RAT) previously, of whom 176 (16·2%) reported to have tested positive for the disease.

Table 1: Characteristics of study participants

	Frequency	Percent
Total	6230	
Age, years		
18-29	1513	24·3
30-49	2672	42·9
50-69	1643	26·4
≥70	402	6·5
Gender		
Male	3126	50·2
Female	3104	49·8
Residence		
Urban	2866	46·0
Rural	3364	54·0

Pregnant (n=3104)	56	1·8
Self-reported history of chronic disease	1145	18.4
History of COVID-19 like symptoms	474	7·6
History of contact with a known COVID-19 case	439	7·0
Ever tested for COVID-19 (RT-PCR)	1092	17·5
RT-PCR result (n=1088*)		
Positive	176	16·2
Negative	912	83·8

COVID-19: Coronavirus disease; RT-PCR: Reverse transcriptase-polymerase chain reaction
*RT-PCR result not known in four participants

We found an overall unweighted seroprevalence of 38·8% (95% CI 37·6 – 40·0). The seroprevalence ranged from 28·5% in district Kulgam to 43·1% in district Pulwama. (Figure 1 and online supplemental file 1) The overall weighted seroprevalence (adjusted for sampling design) was 36·9% (95% CI 34·5 – 39·4). The weighted seroprevalence adjusted for test performance was 36·7% (95% CI 34·3 – 39·2). (Table 2) Upon sensitivity analyses, the weighted seroprevalence adjusted for test performance ranged from 36·3% (95% CI 33·9 – 38·8) to 38·4% (95% CI 35·9 – 41·0). (Table 3)

Table 2: Seroprevalence of SARS-CoV-2 specific IgG antibodies by participant characteristics

	Number tested	Number seropositive	Unweighted seroprevalence, % (95% CI)	Weighted seroprevalence, % (95% CI)	Weighted seroprevalence adjusted for test performance, % (95% CI)	Design-based F, p-value
Total	6230	2415	38·8 (37·6-40·0)	36·9 (34·5-39·4)	36·7 (34·3-39·2)	
Age, years						
18-29	1513	538	35·6 (33·2-38·0)	33·7 (30·1-37·6)	33·5 (29·8-37·4)	6·42, 0·0006
30-49	2672	1000	37·4 (35·6-39·3)	36·3 (33·5-39·3)	36·1 (33·3-39·1)	
50-69	1643	691	42·1 (39·7-44·5)	42·5 (38·8-46·2)	42·3 (38·6-46·0)	
≥70	402	186	46·3 (41·5-51·2)	45·3 (37·8-53·0)	45·1 (37·6-52·8)	
Gender						
Male	3126	1166	37·3 (35·6-39·0)	36·1 (33·5-38·9)	35·9 (33·3-38·7)	0·94, 0·34
Female	3104	1249	40·2 (38·5-42·0)	37·8 (34·5-41·3)	37·6 (34·3-41·1)	
Residence						
Urban	2866	1180	41·2 (39·4-43·0)	40·2 (36·3-44·1)	40·0 (36·1-43·9)	3·43, 0·07
Rural	3364	1235	36·7 (35·1-38·4)	35·5 (32·5-38·7)	35·3 (32·2-38·5)	

Self-reported history of chronic disease						
Yes	1145	495	43·2 (40·4-46·1)	41·9 (37·4-46·6)	41·7 (37·2-46·4)	6.14, 0.02
No	5085	1920	37·8 (36·4-39·1)	36·2 (33·7-38·9)	36·0 (33·5-38·7)	
History of COVID-19 like symptoms						
Yes	474	247	52·1 (47·6-56·6)	47·4 (37·9-57·1)	47·2 (37·7-56·9)	5·53, 0·02
No	5756	2168	37·7 (36·4-38·9)	36·3 (33·9-38·8)	36·1 (33·7-38·6)	
History of contact with a known COVID-19 case						
Yes	439	219	49·9 (45·2-54·5)	45·2 (38·3-52·2)	45·0 (38·1-52·0)	7·13, 0·01
No	5791	2196	37·9 (36·7-39·2)	36·5 (34·1-39·0)	36·3 (33·9-38·8)	
Ever tested for COVID-19 (RT-PCR)						
Yes	1092	485	44·4 (41·5-47·4)	41·0 (35·4-46·9)	40·8 (35·2-46·7)	2·17, 0·14
No	5138	1930	37·6 (36·2-38·9)	36·2 (33·5-39·0)	36·0 (33·3-38·8)	
RT-PCR result (n=1088*)						
Positive	176	140	79·5 (73·0-84·9)	81·8 (74·8-87·1)	81·7 (74·7-87·1)	74·93, <0·0001
Negative	912	345	37·8 (34·7-41·0)	38·8 (33·3-44·7)	38·6 (33·1-44·5)	

COVID-19: Coronavirus disease; RT-PCR: Reverse transcriptase-polymerase chain reaction
*RT-PCR result not known in four participants

Table 3: Sensitivity analyses for seroprevalence at extremes of test performance

	Weighted seroprevalence, % (95% CI)	**Weighted seroprevalence adjusted for test performance, % (95% CI)** ***[Sensitivity 100·00%, Specificity 99·63%]***	**Weighted seroprevalence adjusted for test performance, % (95% CI)** ***[Sensitivity 95·89%, Specificity 99·90%]***	**Weighted seroprevalence adjusted for test performance, % (95% CI)** ***[Sensitivity 100·00%, Specificity 99·05%]***
Overall	36·9 (34·5-39·4)	36·7 (34·3-39·2)	38·4 (35·9-41.0)	36·3 (33·9-38·8)
Age, years				
18-29	33·7 (30·1-37·6)	33·5 (29·8-37·4)	35·1 (31·3-39·1)	33·1 (29·4-37·0)
30-49	36·3 (33·5-39·3)	36·1 (33·3-39·1)	37·8 (34·9-40·9)	35·7 (32·9-38·7)

50-69	42·5 938·8-46·2)	42·3 (38·6-46·0)	44·3 (40·4-48·1)	41·9 (38·2-45·7)
≥70	45·3 937·8-53·0)	45·1 (37·6-52·8)	47·2 (39·4-55·2)	44·8 (37·2-52·5)
Gender				
Male	36·1 (33·5-38·9)	35·9 (33·3-38·7)	37·6 (34·9-40·5)	35·5 (32·9-38·3)
Female	37·8 (34·5-41·30	37·6 (34·3-41·1)	39·4 (35·9-43.0)	37·2 (33·9-40·7)
Residence				
Urban	40·2 (36·3-44·1)	40·0 (36·1-43·9)	41·9 (37·8-45·9)	39·6 (35·7-43·6)
Rural	35·5 (32·5-38·7)	35·3 (32·2-38·5)	37·0 (33·8-40·3)	34·9 (31·9-38·1)
Self-reported history of chronic disease				
Yes	43·2 (40·4-46·1)	41·9 (37·4-46·6)	43·6 (38·9-48·5)	41·3 (36·8-46·1)
No	37·8 (36·4-39·1)	36·2 (33·7-38·9)	37·7 (35·1-40·5)	35·6 (33·1-38·3)
History of COVID-19 like symptoms				
Yes	52·1 (47·6-56·6)	47·4 (37·9-57·1)	49·4 (39·5-59·5)	46·9 (37·3-56·7)
No	37·7 (36·4-38·9)	36·3 (33·9-38·8)	37·8 (35·3-40·4)	35·7 (33·3-38·2)
History of contact with a known COVID-19 case				
Yes	49·9 (45·2-54·5)	45·2 (38·3-52·2)	47·1 (39·9-54·4)	44·7 (37·7-51·7)
No	37·9 (36·7-39·2)	36·5 (34·1-39·0)	38·0 (35·5-40·6)	35·9 (33·5-38·4)
Ever tested for COVID-19 (RT-PCR)				
Yes	44·4 (41·5-47·4)	41·0 (35·4-46·9)	42·7 (36·9-48·9)	40·4 (34·8-46·4)
No	37·6 (36·2-38·9)	36·2 (33·5-39·0)	37·7 (34·9-40·6)	35·6 (32·9-38·4)
RT-PCR result (n=1088*)				
Positive	79·5 (73·0-84·9)	81·8 (74·8-87·1)	85·3 (78·0-90·8)	81·6 (74·6-87·0)
Negative	37·8 (34·7-41·0)	38·8 (33·3-44·7)	40·4 (34·7-46·6)	38·2 (32·7-44·2)

Seroprevalence was lowest among participants aged 18-29 years [33·5% (95% CI 29·8 – 37·4)] and was higher in older age groups. Seroprevalence was highest in those aged 70 years and above [45·1% (95% CI 37·6 – 52·8)]. Seroprevalence was not significantly different among males and females (p=0·34). The seroprevalence among urban residents was 40·0% (95% CI 36·1 – 43·9), slightly but not significantly, higher than rural residents [35·3% (95% CI 32·2 – 38·5), p=0·07]. (Table 2)

One in five participants (1145/6230, 18·4%) self-reported a history of at least one chronic disease (Table 1). Hypertension (815/6230, 13.1%) and diabetes mellitus (314/6230, 5.0%) were the most commonly reported chronic diseases (online supplemental file 2). Seroprevalence was significantly higher in participants who self-reported history of chronic disease (41·7%, 95% CI 37·2 – 46·4) as compared to those who did not report a history of chronic disease (36·0%, 95% CI 33·5 - 38·7) (Table 2).

Among participants who reported a history of COVID-19 like symptoms, seroprevalence was 47·2% (95% CI 37·7 – 56·9) compared with 36·1% (95% CI 33·7 – 38·6) among participants who did not report such

symptoms. Seroprevalence was higher among those who reported contact with a known COVID-19 case [45·0% (95% CI 38·1 – 52·0)] than participants who did not report any history of such contact [36·3% (95% CI 33·9 – 38·8)]. (Table 2)

Seroprevalence was not significantly related to being tested for COVID-19 (RT-PCR). However, those who reported a positive RT-PCR COVID-19 test had significantly higher seroprevalence (81·7%, 95% CI 74·7 – 87·1) as compared to those who reported a negative RT-PCR COVID-19 test (38·6%, 95% CI 33·1 – 44·5). (Table 2)

Among 2 415 seropositive individuals, only 247 (10·2%) reported a history of COVID-19 like symptoms. Only 20.1% (485/2415) of the seropositive individuals were tested for COVID-19 (RT-PCR). Among 474 who reported a history of COVID-19 like symptoms, 233 (49·2%) were tested for COVID-19 (RT-PCR). Among 4897 individuals who did not report a history of COVID-19 like symptoms and were never tested for COVID-19 (RT-PCR), 1825 (37.3%) were seropositive. (Figure 3)

Among 36 participants who reported a positive RT-PCR COVID-19 test but were seronegative, the duration between COVID-19 RT-PCR test and serological testing ranged from 9 days to 101 days. In only four of these 36 participants, the duration between the COVID-19 RT-PCR test and the serological test was 14 days or less. Of the remaining 32 participants, 21 did not report a history of CVOID-19 like symptoms, nine did not report a history of contact with a known COVID-19 case, and eight reported neither a history of COVID-19 like symptoms nor a history of contact with a known COVID-19 case.

We estimated that there were 1 673 484 (95% CI 1 564 047 – 1 787 482) cumulative number of infections among adults aged ≥18 years in the valley by 03 Oct 2020, two weeks before the start of the survey. If we assume that the seroprevalence was similar to the overall seroprevalence in the population not included in our study (<18 years of age) then the estimated cumulative number of infections in the valley by 03 Oct 2020 was 2 791 933 (95% CI 2 609 354 – 2 982 119). Considering that the cumulative number of reported COVID-19 cases was 47 071 by 03 Oct 2020 (Figure 4), we estimate the number of infections per reported case as 59·3 (95% CI 55·4 – 63·4). The number of reported COVID-19 deaths after a three-week lag period (on 24 Oct 2020) was 955. Thus, we estimated the infection fatality rate as 0.034% (95% CI 0.032 – 0.037).

DISCUSSION

The results of our study indicate that by the first week of October 2020, nearly seven months after the appearance of the first laboratory-confirmed COVID-19 case on 18 March 2020, close to 37% of the valley's population aged ≥18 years had been infected. Our results suggest that the cumulative number of SARS-CoV-2 infections by the first week of October 2020 was nearly 2·8 million with an estimated infection fatality rate of 0.034%. Seroprevalence did not differ by gender but was higher in older age groups.

The findings of our study are based on a representative sample of the population. The laboratory test used for the detection of SARS-CoV-2 specific IgG antibodies in serum samples provides valid results.[20,25] We report seroprevalence estimates adjusted for sampling design and test performance.

The overall adjusted seroprevalence of around 37% indicates that a large proportion of the valley's population has been infected with the virus. Easing of lockdown, being fed up with the restrictions, and non-adherence to prevention norms are the possible reasons. Even though a large proportion of the

population has been infected, the transmission of infection is expected to continue till most of the susceptible population becomes immune. Herd immunity in the context of COVID-19 is a matter of debate as reports of a second infection continue to pour in.[26] The emergence of several Variants of Concern and the introduction of COVID-19 vaccination will also influence population immunity. Several factors potentially influence the seroprevalence rates. These include population density, social and demographic structure of the population, governmental policies and the extent of their implementation, immunity level of the population, time since the start of infection transmission, adherence to infection prevention guidelines, quality of contact tracing and quarantine, and possibly the geography and environment of an area.

Comparison with previous reports suggests that the seroprevalence has increased almost ten-fold since July 2020.[16,27] The second of the three nationwide seroprevalence surveys in India conducted in August-September 2020 reports an overall seroprevalence of 6.6% ranging from 5.2% in rural areas to 16.9% in urban slums.[28] A nationwide survey conducted in December 2020-January 2021 reported an overall seroprevalence of 24.1% ranging from 4.9% - 44.4% across districts.[29] Kashmir is thus not a low-infection area. Being an oft-visited tourist area, Kashmir is at an increased risk of infection transmission. Adherence to COVID appropriate behavior (use of face masks in public, frequent handwashing, physical and social distancing) has been poor. With the introduction of the COVID-19 vaccination program in January 2021 and the emergence of a 'second wave' in Kashmir in April 2021, the seroprevalence estimates are expected to increase in the future.

The seroprevalence of SARS-CoV-2 IgG antibodies was higher in older age groups. During the early period of the pandemic, people were adherent to social distancing and other non-pharmaceutical interventions because of a fear of the disease and administrative restrictions. With time, administrative restrictions were relaxed, fear of the disease attenuated, and people became sort of fed up with the social restrictions. This not only led to an increase in the number of reported COVID-19 cases but also provided the population, including older age groups, an opportunity to contract the infection. That older people have an increased risk of symptomatic and more severe disease is now well known.[30,31] However, age-based differential susceptibility to SARS-CoV-2 infection antibody response and the reasons thereof are still a grey area and need further understanding. Existing literature might suggest that the young who are more mobile and socially active have a higher risk of infection.[6,7] However, this should not imply that the elderly have a decreased susceptibility to SARS-CoV-2 infection or a decreased antibody response.[32] On the contrary, several studies suggest that the seroprevalence of SARS-CoV-2 specific IgG antibodies is higher in the older age groups and particularly so in more dense population groups.[4,5,8–11,13] Furthermore, SARS-CoV-2 antibody levels have been reported to be higher in older people.[12]

The seroprevalence of SARS-CoV-2 specific IgG antibodies did not differ significantly by gender, though the figure was slightly higher for females. These findings are consistent with the available literature.[6,13] Difference in seroprevalence by gender has been suggested by some studies and females have been reported to have lower antibody levels.[5,7,9,11,12,14,33]

Urban areas are more densely populated as compared to rural areas which accelerate the transmission of infections in the population. The seroprevalence of SARS-CoV-2 specific IgG antibodies is thus expected to be higher in urban areas especially during the early phases of an epidemic. As the epidemic progresses the seroprevalence gap between urban and rural areas will wane off. We estimated an

adjusted seroprevalence of 40·0% (95% CI 36·1 – 43·9) in urban areas as compared to 35·3% (95% CI 32·2 – 38·5) in rural areas (p=0·07).

People with a chronic disease experience more severe COVID-19 symptoms and are more likely to die when compared to people with no chronic disease.[34] We found a higher proportion of symptomatic infection among participants with a self-reported history of chronic disease (78/1145, 6·8%) as compared to participants with no chronic disease (169/5085, 3·3%) (online supplemental file 3). Little is, however, known about the risk of infection in chronic disease patients. We found a significantly higher seroprevalence among participants with a self-reported history of chronic disease (Table 2). This finding needs further research for corroboration and possible explanations.

People with a self-reported history of COVID-19 related symptoms, contact with a known COVID-19 case, or a positive COVID-19 RT-PCR had a higher seroprevalence as compared to their complement. Among seropositive individuals, only 10·2% reported being symptomatic. The percentage of asymptomatic infections, thus, was 90%. However, only 49% of individuals with a history of COVID-19 like symptoms were tested using RT-PCR. We also estimated that only one out of almost 59 infections gets reported. This reflects the necessity of improving the efficiency of RT-PCR testing so that more symptomatic individuals receive the test. Not all individuals with a known RT-PCR positive result showed the presence of IgG antibodies. Around 20% of RT-PCR positive individuals were seronegative and in a large majority of them (32 out of 36) the duration since RT-PCR positivity was more than two weeks. This may be attributed to a poor B cell response or a false negative antibody test.[35] Around 38% of RT-PCR negative individuals were seropositive suggesting a false-negative RT-PCR or infection acquisition at a date later than the RT-PCR test.

We estimated an infection fatality rate of 0.034% (95% CI 0.032 – 0.037). The infection fatality rate in SARS-CoV-2 infection has been reported to range from as low as 0.00% to 1.63%.[36] Our estimates of the infection fatality rate are low as compared to estimates from several Indian studies.[5,28,37] Under-reporting of COVID-19 deaths because of non-uniform definition for a 'COVID-19 death' may falsely lower the infection fatality rates.[38]The infection fatality rate is, however, known to be lower in developing nations.[30,39] In developed nations like the United States and many European countries, a higher infection fatality rate has been reported.[30,40]

Limitations

One important limitation of our study is that even though we adjusted the weighted seroprevalence estimates for test performance using manufacturer-provided sensitivity and specificity (100% and 99·63% respectively), we did not quantify the test validity in-house. Another limitation of our study estimates is that we excluded people <18 years of age. The results of our study may not thus be generalizable to this group of the population.

Our estimated seroprevalence was much higher than we anticipated at the designing stage. This has impacted the precision of our estimates to some extent. However, we believe we still have been able to estimate the seroprevalence with reasonable precision.

Lack of reliable death counts is another potential limitation. This may have led to an underestimation of the infection fatality rate. We did not perform any adjustment for death counts. Further, because of lack

of age- and gender-specific mortality data we could not estimate age- and gender-specific infection fatality rates.

CONCLUSIONS

We conclude that nearly 37% of individuals aged 18 years and above were infected with SARS-CoV-2 in Kashmir by October 2020. The infection fatality rate in the valley is around 0.034%. A majority of cases go unreported. For every reported case there are 59 unreported infections in the population. Since almost half of the symptomatic individuals go unreported, testing of symptomatic individuals and effective contact tracing needs to continue. Given the emergence of mutant Variants of Concern, increasing the population immunity through augmented and sustained vaccination is needed. We further recommend that adherence to COVID-19 prevention measures should be ensured at least till a large proportion of the population gets vaccinated.

ACKNOWLEDGMENTS

We thank Prof. Samia Rashid for her support. We acknowledge the help from Divisional COVID-19 Control Room Kashmir, especially from Dr. Rauf Hussain Rather. We thank the following coordinating center teams and the laboratory support team for their support and coordination:

Coordinating center teams: Dr. Sabira Aalia Dkhar, Dr. Kouser Sideeq, Dr. Uruj Qureshi, Dr. Mehraj u Din Sofi, Dr. Yasmin Kango, Dr. Suhail Manzoor Shah, Dr. Bashir Ahmad Khan, Dr. Nabeel Ishtiyaq, Dr. Tajamul Hussain Khan, Dr. Lahoot Hassan Khan, Dr. Muzaffer Ahmad, Dr. Ramesh Kumar Malla, Dr. Ruqaya Rasool Kapta, Dr. Mohammad Yousuf Naikoo, Dr. Khalid Abbass, Dr. Zarar Ahmad Bhat, Dr. Sumair Rashid, Dr. Zubair Ahmad, Dr. Shalender Singh Bali, Dr. Abdul Rouf, Dr. Abdul Hamid, Dr. Khalid Bashir, Dr. Qazi Najeeb Ahmad, Dr. Syed Irtiza, Dr. P Sharik Shaheen Khaki, Dr. Sheikh Mohammad Shafi, Dr. Mohammad Yousuf Zagoo, Dr. Mukhtar Ahmad Shah, Dr. Imtiyaz-ur-Rehman Bhat, Dr. Showkat Parray, Dr. Fazil Ali Kochak, Dr. Muzaffar Ahmad Zargar, Dr. Abdul Rashid Shah, Dr. Shagufta Saleem, Dr. Abdul Gani, Dr. Nighat Nasreen, Dr. Asima Nazeer, Dr. Zahoor Ahmad Lone.

Laboratory support team: Ishtiyaq Ahmad Handoo, Rahiba Makhdoomi, Iyman Wani, Romana Rasool, Asiya Rehman, Insha Rehman, Zahoor Ahmad, Gulzar Ahmad Wani, Javid Ahmad Bhat, M Saleem Malik, Naveed Manzoor, Mubeena.

COMPETING INTERESTS

We declare no competing interests, financial or otherwise.

FUNDING

The National Health Mission Jammu & Kashmir funded the study. The funding agency was not involved in study designing, implementation, analysis, or interpretation of the study findings.

CONTRIBUTORS

S Muhammad Salim Khan: Conceptualization, Funding acquisition, Methodology, Writing-review & editing.

Mariya Amin Qurieshi, Inaamul Haq, Sabhiya Majid: Formal analysis, Data curation, Methodology, Project administration, Writing-original draft, Writing-review & editing.

Javid Ahmad, Taha Ayub, Anjum Bashir, Ashfaq Ahmad Bhat, Abdul Majeed Ganai, Yasmeen Jan, Rauf-ur-Rashid Kaul, Zahid Ali Khan, Muneer Ahmad Masoodi, Beenish Mushtaq, Fouzia Nazir, Muzamil Nazir, Malik Waseem Raja, Mahbooba Rasool: Project administration, Supervision, Investigation, Writing-review & editing.

Anjum Asma, Munazza Aziz, Shifana Ayoub, Arif Akbar Bhat, Iqra Nisar Chowdri, Shaista Ismail, Misbah Ferooz Kawoosa, Mehvish Afzal Khan, Mosin Saleem Khan, Rafiya Kousar, Ab Aziz Lone, Shahroz Nabi, Mohammad Obaid, Tanzeela Bashir Qazi, Iram Sabah, Ishtiyaq Ahmad Sumji: Supervision, Investigation, Writing-review & editing.

S Muhammad Salim Khan, Mariya Amin Qurieshi, Inaamul Haq, and Sabhiya Majid have verified the underlying data.

DATA SHARING

Anonymized data collected for the study, including individual participant data and a data dictionary defining each field in the set, will be made available to interested researchers on request by Inaamul Haq (haqinaam@yahoo.co.in) for two years from publication. The study protocol, statistical analysis plan, and informed consent forms are also available from Inaamul Haq.

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Figure 1 legend:

Figure 1: Location of the ten districts and seroprevalence (%) by district. Figures in parentheses indicate a 95% Confidence Interval for seroprevalence.

Figure 2 legend:

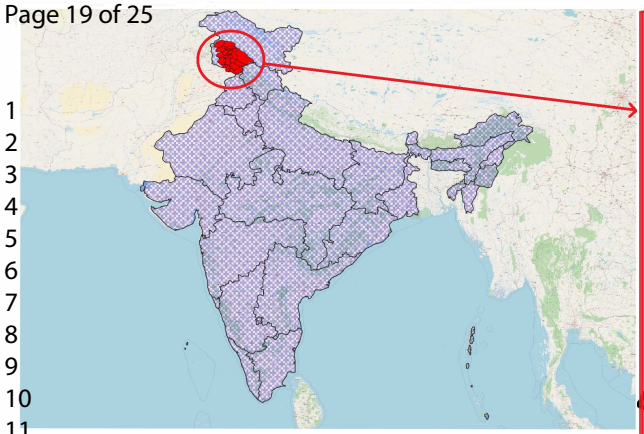
Figure 2: Participant flow.

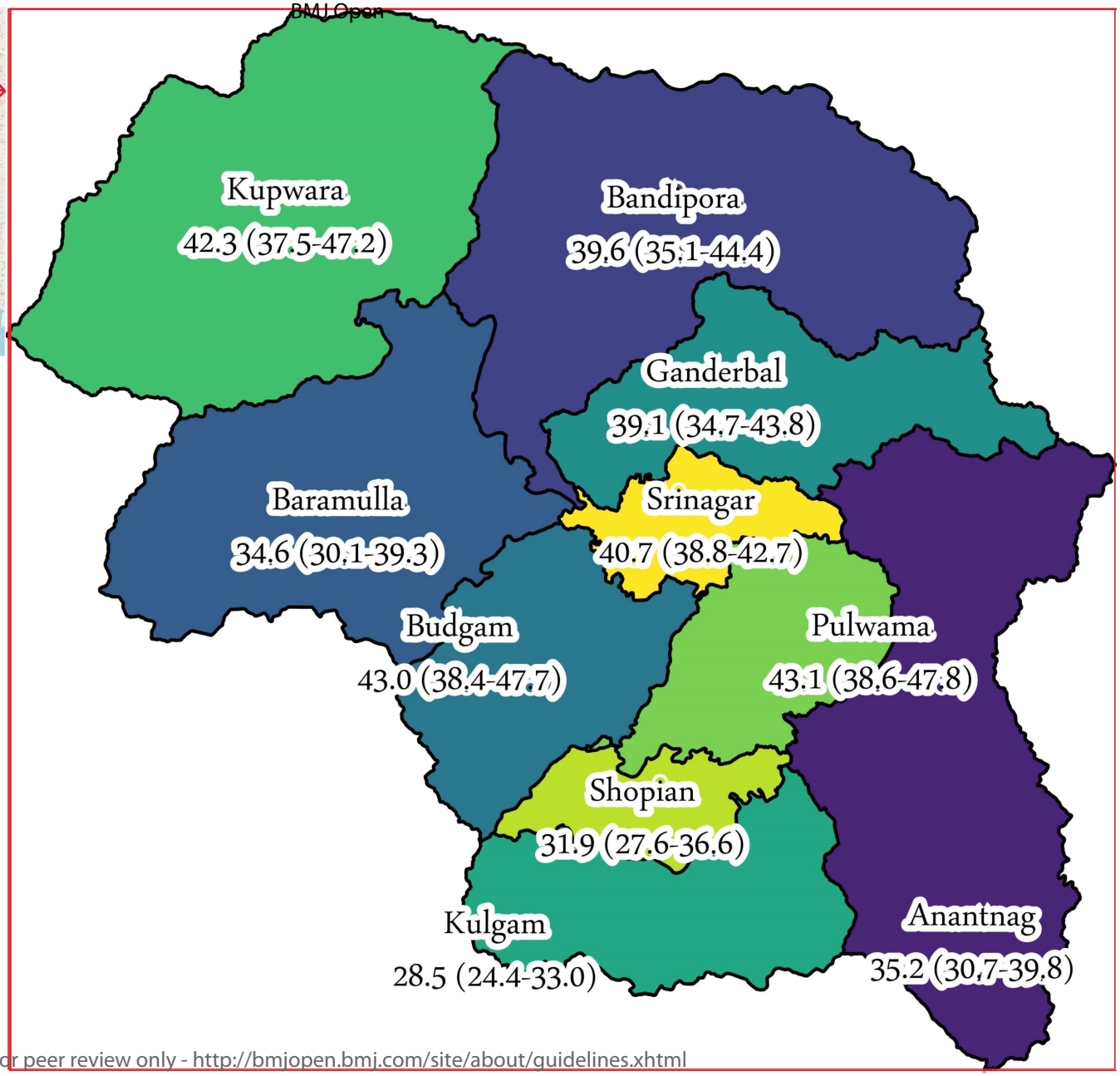
Figure 3 legend:

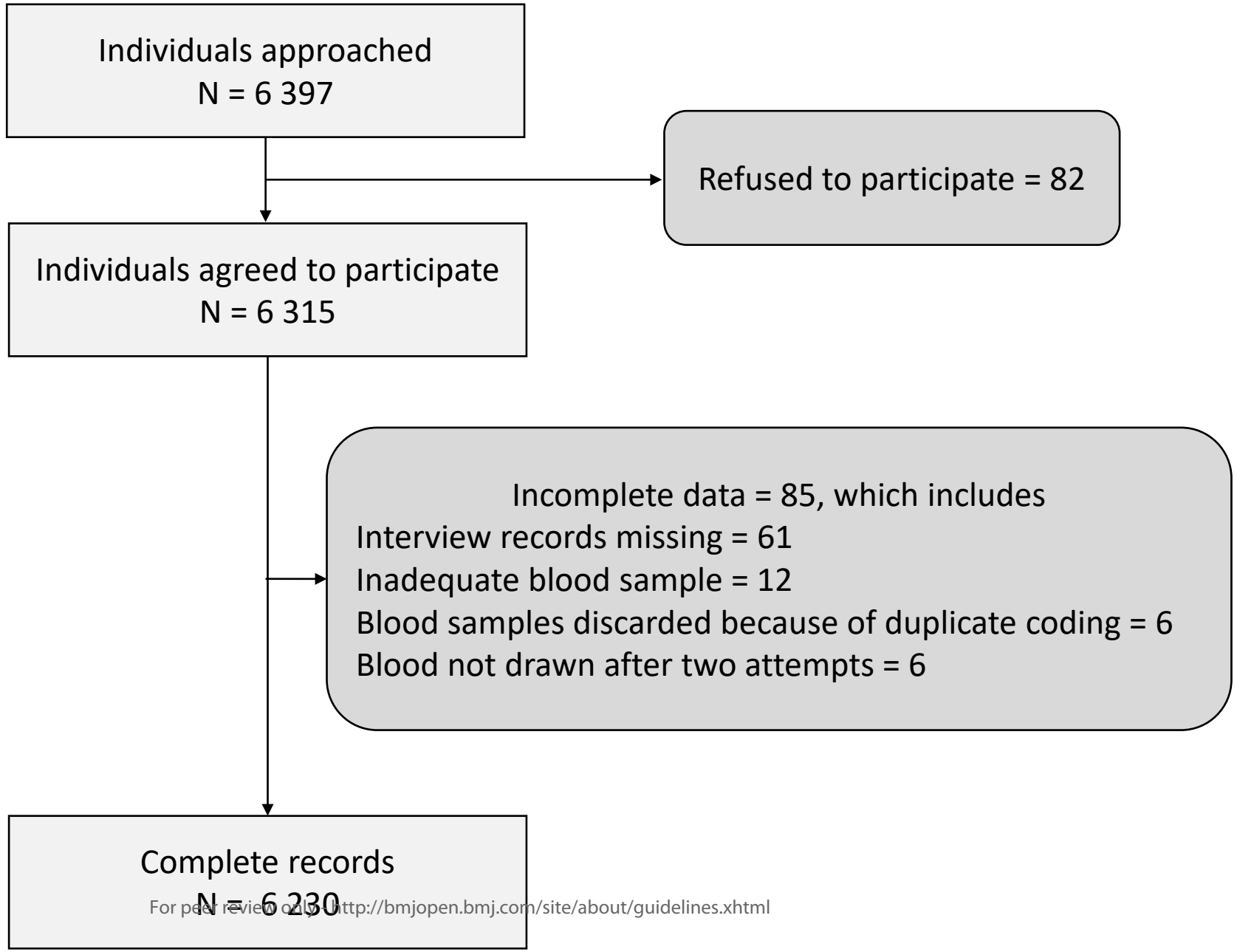
Figure 3: A. Seropositivity by the history of COVID-19 like symptoms, RT-PCR testing, and test result. B. History of COVID-19 like symptoms by seropositivity, RT-PCR testing, and test result.

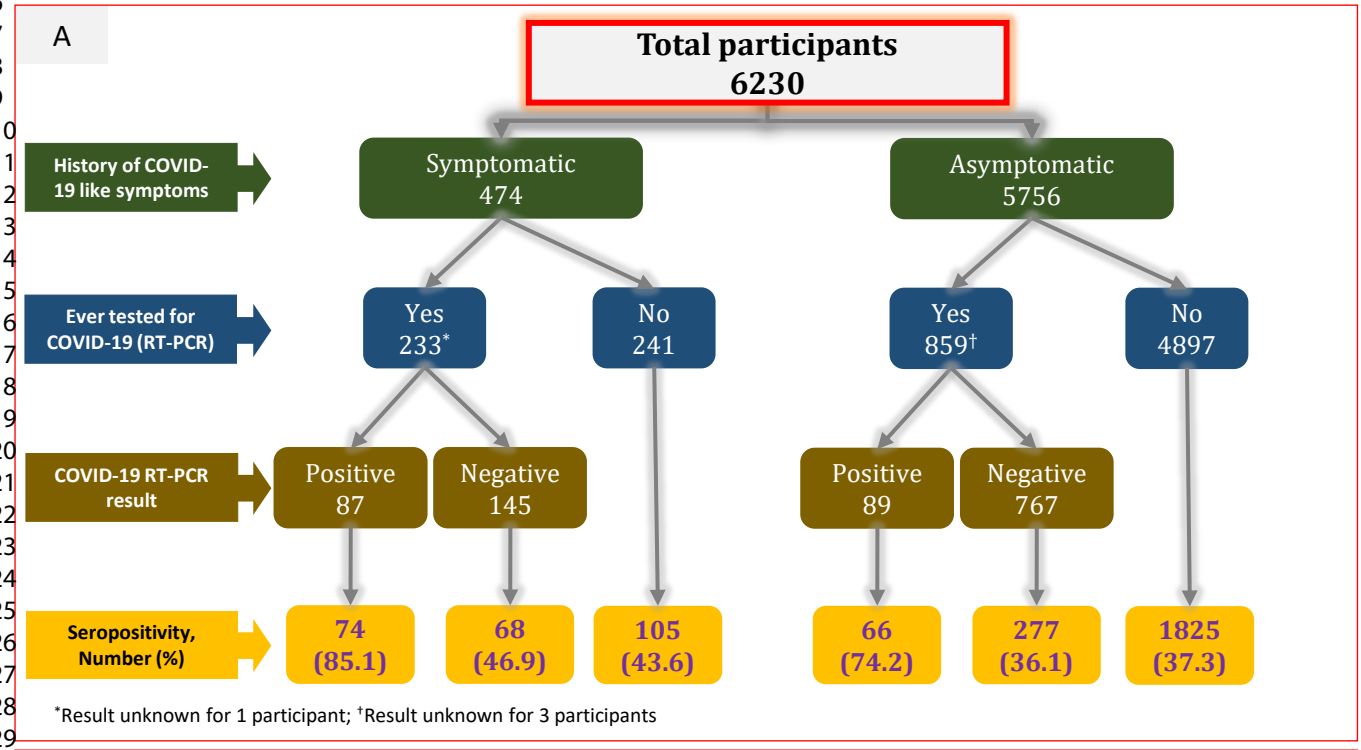
Figure 4 legend:

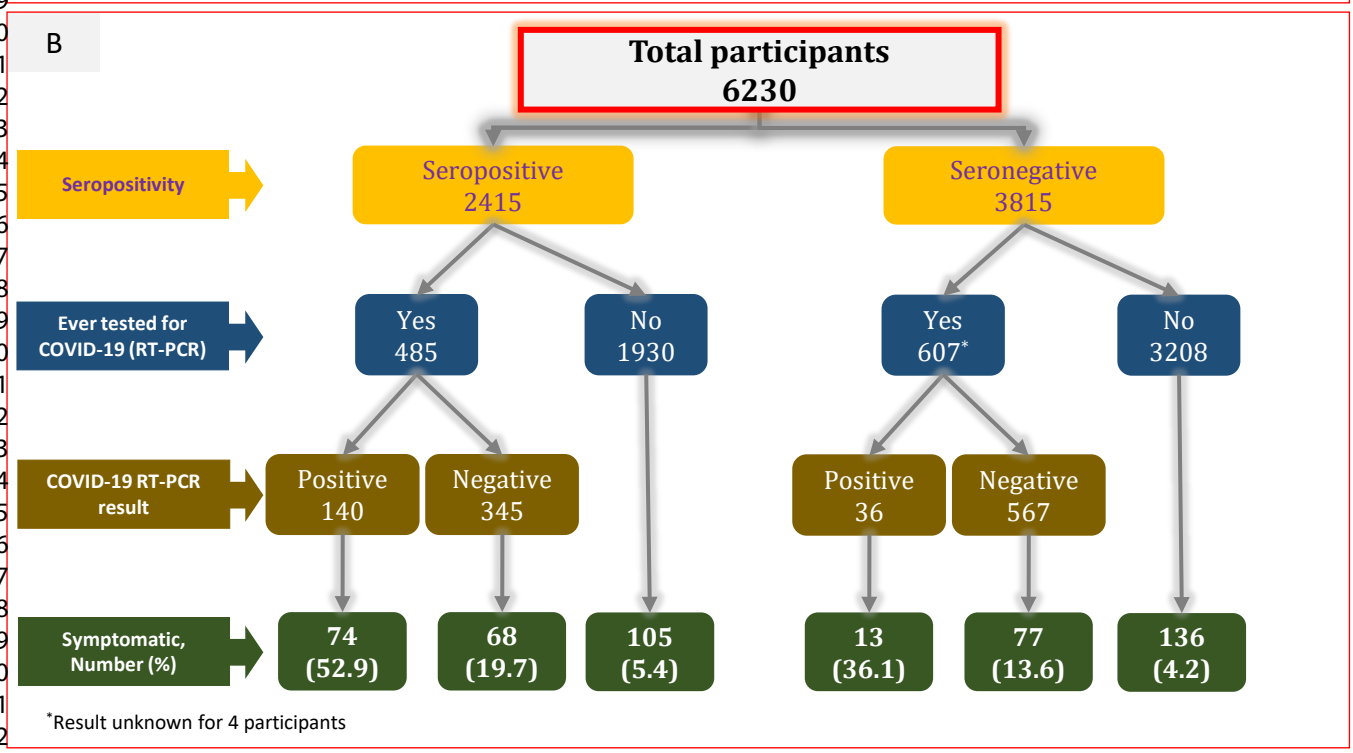
Figure 4: A. Daily cases and deaths in Kashmir since the start of the COVID-19 pandemic; B. Cumulative number of cases and deaths in Kashmir.

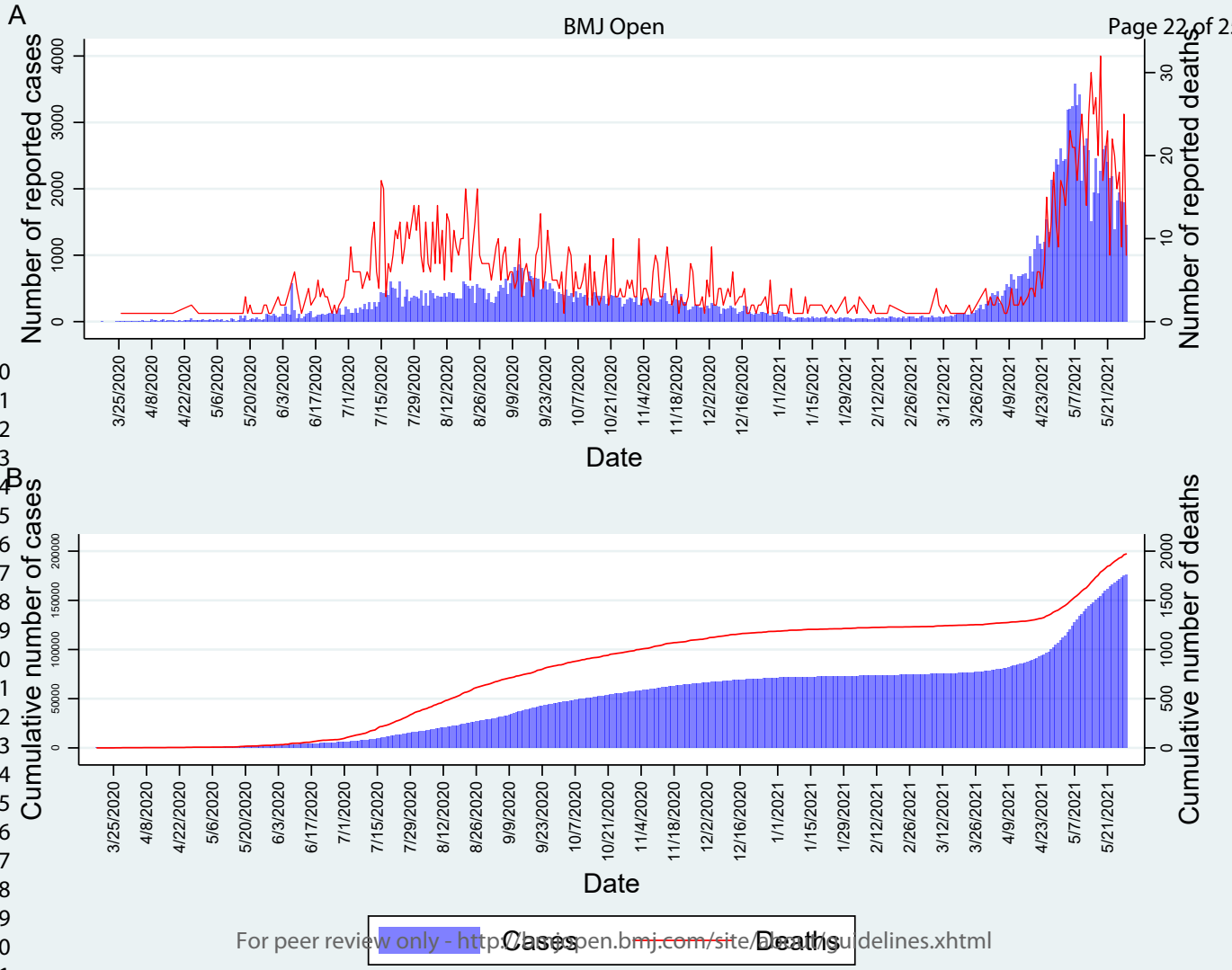







A
Total participants
6230
History of COVID-19 like symptoms
Symptomatic
474
Asymptomatic
5756
Ever tested for COVID-19 (RT-PCR)
Yes
233*
No
241
Yes
859†
No
4897
COVID-19 RT-PCR result
Positive
87
Negative
145
Positive
89
Negative
767
Seropositivity, Number (%)
74
(85.1)
68
(46.9)
105
(43.6)
66
(74.2)
277
(36.1)
1825
(37.3)
*Result unknown for 1 participant; †Result unknown for 3 participants


B
Total participants
6230
Seropositivity
Seropositive
2415
Seronegative
3815
Ever tested for COVID-19 (RT-PCR)
Yes
485
No
1930
Yes
607*
No
3208
COVID-19 RT-PCR result
Positive
140
Negative
345
Positive
36
Negative
567
Symptomatic, Number (%)
74
(52.9)
68
(19.7)
105
(5.4)
13
(36.1)
77
(13.6)
136
(4.2)
*Result unknown for 4 participants



Supplemental Table 1: Participant characteristics by district

District	Total	Age (years)				Gender		Residence	
		<30	30-49	50-69	70+	Female	Male	Rural	Urban
Anantnag	421	84	197	113	27	214	207	295	126
Budgam	442	113	190	105	34	263	179	354	88
Bandipora	424	106	174	114	30	227	197	341	83
Baramulla	405	113	176	98	18	214	191	325	80
Ganderbal	442	92	210	123	17	233	209	346	96
Kulgam	428	102	194	113	19	257	171	346	82
Kupwara	400	81	171	105	43	215	185	360	40
Pulwama	443	102	176	126	39	218	225	396	47
Shopiyan	407	119	152	90	46	211	196	368	39
Srinagar	2418	601	1032	656	129	1052	1366	233	2185
Total	6230	1513	2672	1643	402	3104	3126	3364	2866

Supplemental Table 2: Seroprevalence (unadjusted) by district and participant characteristics

District	Overall	Age (years)				Gender		Residence	
		<30	30-49	50-69	70+	Male	Female	Urban	Rural
Anantnag	35.2 (30.7-39.8)	29.8 (21-40.4)	34.5 (28.2-41.4)	38.9 (30.4-48.2)	40.7 (24.2-59.7)	36.2 (30-43)	34.1 (28.1-40.7)	42.9 (34.5-51.6)	31.9 (26.8-37.4)
Budgam	43 (38.4-47.7)	44.2 (35.4-53.5)	37.9 (31.3-45)	48.6 (39.2-58.1)	50 (33.8-66.2)	41.9 (34.9-49.3)	43.7 (37.8-49.8)	38.6 (29.1-49.2)	44.1 (39-49.3)
Bandipora	39.6 (35.1-44.4)	37.7 (29-47.3)	42 (34.8-49.4)	40.4 (31.8-49.6)	30 (16.4-48.3)	37.6 (31.1-44.5)	41.4 (35.2-47.9)	55.4 (44.6-65.7)	35.8 (30.9-41)
Baramulla	34.6 (30.1-39.3)	27.4 (20-36.4)	32.4 (25.9-39.6)	44.9 (35.4-54.8)	44.4 (24-67)	39.3 (32.6-46.4)	30.4 (24.6-36.9)	36.3 (26.5-47.3)	34.2 (29.2-39.5)
Ganderbal	39.1 (34.7-43.8)	34.8 (25.8-45)	40.5 (34-47.3)	39.8 (31.6-48.7)	41.2 (21-64.8)	39.2 (32.8-46)	39.1 (33-45.5)	42.7 (33.2-52.8)	38.2 (33.2-43.4)
Kulgam	28.5 (24.4-33)	27.5 (19.7-36.9)	26.8 (21-33.5)	31 (23.1-40.1)	36.8 (18.7-59.7)	25.1 (19.2-32.2)	30.7 (25.4-36.6)	37.8 (28-48.7)	26.3 (21.9-31.2)
Kupwara	42.3 (37.5-47.2)	33.3 (24-44.2)	39.8 (32.7-47.3)	50.5 (41-59.9)	48.8 (34.4-63.4)	41.6 (34.7-48.9)	42.8 (36.3-49.5)	50 (35-65)	41.4 (36.4-46.6)
Pulwama	43.1 (38.6-47.8)	35.3 (26.7-45)	42.6 (35.5-50)	45.2 (36.8-54)	59 (43.2-73.1)	39.6 (33.4-46.1)	46.8 (40.3-53.4)	40.4 (27.5-54.9)	43.4 (38.6-48.4)
Shopiyan	31.9 (27.6-36.6)	28.6 (21.2-37.3)	29.6 (22.9-37.3)	41.1 (31.4-51.5)	30.4 (18.9-45.1)	31.1 (25-37.9)	32.7 (26.7-39.3)	38.5 (24.7-54.4)	31.3 (26.7-36.2)
Srinagar	40.7 (38.8-42.7)	39.1 (35.3-43.1)	39.2 (36.3-42.3)	41.9 (38.2-45.7)	53.5 (44.9-61.9)	37.7 (35.2-40.3)	44.6 (41.6-47.6)	40.8 (38.7-42.9)	39.9 (33.8-46.3)

Chronic disease and SARS-CoV-2 infection in the study population

Of the 6230 participants, 1145 reported a history of at least one chronic disease. Two hundred ninety-eight reported a history of more than one chronic disease.

Supplementary Table 3: Chronic disease in the study population

Chronic disease (n = 1145)	Number (%)
Hypertension	815 (13·1%)
Diabetes	314 (5·0%)
Chronic Obstructive Pulmonary Disease	39 (0·6%)
Coronary Heart Disease	35 (0·6%)
Cerebrovascular Disease	16 (0·3%)
Asthma	15 (0·2%)
Chronic Kidney Disease	10 (0·2%)
Chronic Liver Disease	5 (0·1%)
Cancer	4 (0·1%)

Supplementary Table 4: History of COVID-19 like symptoms and seropositivity vis-à-vis history of chronic disease

		History of COVID-19 like symptoms	
		Yes	**No**
Reported history of chronic disease (n=1145)	**Seropositive**	78	417
	Seronegative	63	587
Did not report any history of chronic disease (n=5085)	**Seropositive**	169	1751
	Seronegative	164	3001

Of the 1154 participants who reported a history of chronic disease 78 (6.8%) reported a history of COVID-19 like symptoms within three months of the interview date and were seropositive for SARS-CoV-2 specific IgG (symptomatic infection). The proportion of symptomatic infection among participants who did not report any history of chronic disease was 3.3% (169/5085).

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3 and Figure 1
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	3, 4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	4, 5
Study size	10	Explain how the study size was arrived at	3
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4, 5
		(*b*) Describe any methods used to examine subgroups and interactions	5
		(*c*) Explain how missing data were addressed	5
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	4, 5
		(*e*) Describe any sensitivity analyses	6, Table 3
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure 2, and page 5
		(b) Give reasons for non-participation at each stage	Figure 2
		(c) Consider use of a flow diagram	Figure 2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1, Figure 2
Outcome data	15*	Report numbers of outcome events or summary measures	Table 2

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2
		(*b*) Report category boundaries when continuous variables were categorized	Table 1, 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Table 2, Table 3
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11, 12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9, 10, 11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for exposed and unexposed groups.

BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-053791.R2
Article Type:	Original research
Date Submitted by the Author:	14-Aug-2021
Complete List of Authors:	Khan, S Muhammad; Government Medical College Srinagar, Community Medicine Qurieshi, Mariya; Government Medical College Srinagar, Community Medicine Haq, Inaamul; Government Medical College Srinagar, Community Medicine Majid, Sabhiya; Government Medical College Srinagar, Biochemistry Ahmad, Javid; Sher-i-Kashmir Institute of Medical Sciences, Community Medicine Ayub, Taha; Government Medical College Srinagar, Community Medicine Bhat, Ashfaq; SKIMS Medical College Srinagar, Community Medicine Fazili, Anjum; Sher-i-Kashmir Institute of Medical Sciences, Community Medicine Ganai, Abdul; Government Medical College Baramulla, Community Medicine Jan, Yasmeen; SKIMS Medical College Srinagar, Community Medicine Kaul, Rauf-ur-Rashid; Sher-i-Kashmir Institute of Medical Sciences, Community Medicine Khan, Zahid; Government Medical College Baramulla, Community Medicine Masoodi, Muneer; Government Medical College Anantnag, Community Medicine Mushtaq, Beenish; SKIMS Medical College Srinagar, Community Medicine Nazir, Fouzia; Government Medical College Anantnag, Community Medicine Nazir, Muzamil; Government Medical College Baramulla, Community Medicine Raja, Malik; Government Medical College Srinagar, Community Medicine Rasool, Mahbooba; Government Medical College Anantnag, Community Medicine Asma, Anjum; Government Medical College Srinagar, Community Medicine Ayoub, Shifana; Government Medical College Srinagar, Community Medicine Aziz, Munazza; Government of Jammu and Kashmir Directorate of Health Services Bhat, Arif; Government Medical College Srinagar, Biochemistry Chowdri, Iqra; Government Medical College Srinagar, Community

	Medicine Ismail, Shaista; Government Medical College Srinagar, Community Medicine Kawoosa, Misbah; Government Medical College Srinagar, Community Medicine Khan, Mehvish; Government Medical College Srinagar, Community Medicine Khan, Mosin; Government Medical College Srinagar, Biochemistry Kousar, Rafiya; Government Medical College Srinagar, Community Medicine Lone, Ab; Government Medical College Srinagar, Community Medicine Nabi, Shahroz; Government Medical College Srinagar, Community Medicine Obaid, Mohammad; Government Medical College Srinagar, Biochemistry Qazi, Tanzeela; Government Medical College Srinagar, Community Medicine Sabah, Iram; Government Medical College Srinagar, Community Medicine Sumji, Ishtiyaq; Government Medical College Srinagar, Community Medicine
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Epidemiology
Keywords:	COVID-19, EPIDEMIOLOGY, Epidemiology < INFECTIOUS DISEASES

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Seroprevalence of SARS-CoV-2 specific IgG antibodies in Kashmir, India, seven months after the first reported local COVID-19 case: results of a population-based seroprevalence survey from October-November, 2020

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Word Count: 3353 (excluding abstract, tables, and references)

ABSTRACT

Objectives: We designed a population-based survey in Kashmir to estimate the seroprevalence of SARS-CoV-2 specific IgG antibodies in the general population aged 18 years and above.

Setting: The survey was conducted among 110 villages and urban wards across ten districts in Kashmir from 17 Oct 2020 to 04 Nov 2020.

Participants: Individuals aged 18 years and above were eligible to be included in the survey. Serum samples were tested for the presence of SARS-CoV-2 specific IgG antibodies using the Abbott SARS-CoV-2 IgG assay.

Primary and secondary outcome measures: We labeled assay results equal to or above the cut-off index value of 1·4 as positive for SARS-CoV-2 specific IgG antibodies. Seroprevalence estimates were adjusted for the sampling design and assay characteristics.

Results: Out of 6397 eligible individuals enumerated, 6315 (98.7%) agreed to participate. The final analysis was done on 6230 participants. Seroprevalence adjusted for the sampling design and assay characteristics was 36.7% (95% CI 34.3%-39.2%). Seroprevalence was higher among the older population. Among seropositive individuals, 10.2% (247/2415) reported a history of COVID-19 like symptoms. Out of 474 symptomatic individuals, 233 (49.2%) reported having been tested. We estimated an infection fatality rate of 0.034%.

Conclusions: During the first seven months of the COVID-19 epidemic in Kashmir valley, approximately 37% of individuals were infected. A large proportion of the population remains susceptible to the infection. The experience of a second wave of COVID-19 in April-June 2021, the appearance of virus variants, and the introduction of vaccination programs warrant robust surveillance of the epidemic.

ARTICLE SUMMARY

Strengths and limitations of this study

- The findings of our study are based on a representative sample of the population.
- The laboratory test used for the detection of SARS-CoV-2 specific IgG antibodies in serum samples provides valid results.
- We report seroprevalence estimates adjusted for sampling design and test performance.
- Even though we adjusted the weighted seroprevalence estimates for test performance using manufacturer-provided sensitivity and specificity (100% and 99·63% respectively), we did not quantify the test validity in-house.
- Because of lack of age- and gender-specific mortality data we could not estimate age- and gender-specific infection fatality rates.

INTRODUCTION

On 11 Feb 2020, the World Health Organization announced that the disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) will be named coronavirus disease (COVID-19).[1] In Kashmir, the first case of COVID-19 was confirmed in Srinagar city on 18 Mar 2020.[2] The government imposed the first phase of the lockdown in Kashmir on 24 March 2020. During this phase, inter-state travel remained suspended. People were barred from moving outside except in an emergency. Except

for essential services, all government and private offices were advised to work from home. Universal masking was made mandatory. The lockdown was extended till 31 May 2020 and later relaxed in a phased manner.

Mild or asymptomatic infections are common in Coronavirus disease 2019 (COVID-19) and are an important source of infection transmission.[3,4] Such cases are less likely to be detected by a surveillance system based on reverse-transcriptase polymerase chain reaction (RT-PCR) testing. The number of reported RT-PCR positive cases are an underestimate of the true number of infections in a population.

Seroprevalence surveys have been conducted in various countries at different stages of the current epidemic among various population groups.[5–14] Seroprevalence surveys provide a more accurate estimate of past infection, improve understanding of the infection transmission dynamics, and guide public health response.[15]

We designed this survey with the primary objective to estimate the seroprevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) specific IgG antibodies in the adult population of Kashmir valley.

METHODS

We designed a population-based cross-sectional study. The study covered all the ten districts of Kashmir, a valley in northern India. (Figure 1) We completed data collection in three weeks, from 17 Oct 2020 to 04 Nov 2020.

Ethics

We obtained written informed consent from all study participants. The study was approved by the Institutional Ethics Committee of Government Medical College Srinagar (reference number: 1004/ETH/GMC). We used anonymized participant data for analysis.

Sample size

Based on the results of a previous study conducted in July 2020, we speculated that, by October 2020, the prevalence would have increased to around 20%.[16] We calculated the minimum sample size based on an anticipated seroprevalence of 20%, an absolute precision of 2%, and a design effect of 2. We used OpenEpi to make sample size calculations.[17] We adjusted the sample size for a possible non-response of 10% to obtain a minimum size of 3376. We decided to select 3600 individuals from nine of the ten districts (except district Srinagar). To obtain precise estimates for district Srinagar, sample size estimation was made for the district separately. We used a design effect of 1.5, an anticipated seroprevalence of 20%, and absolute precision of 2% to obtain a sample size of 2302 for the district, which was further increased to 2400 to account for non-response. We thus targeted a total sample size of 6000 (3600 + 2400).

Participants

All adults ≥18 years of age were eligible to participate in the study. We selected eligible participants using a three-stage stratified cluster sampling technique. We listed all clusters in the valley using the Census 2011 data.[18] Within each of the ten districts in the valley, clusters were stratified into urban

and rural clusters. We selected clusters within each of the 20 strata by probability proportionate to size (PPS) sampling. Except for district Srinagar which we oversampled to obtain precise seroprevalence estimates for, ten clusters were randomly selected from each district. We selected 20 clusters from district Srinagar. We divided each selected cluster into four equal areas and chose a central location within each of the four areas as the starting point. Thereafter, we approached consecutive households to enroll at least ten eligible participants. We thus identified a total of 440 random locations within 110 clusters in ten districts. We invited all eligible adults in a household for participation.

Variables

The main outcome variable of interest was SARS-CoV-2 specific IgG antibodies. We obtained information from participants about their age, gender, history of COVID-19 like symptoms in the three months before the interview date, history of contact with a known COVID-19 patient, and history of COVID-19 testing.

Procedure

We informed eligible adults about the purpose and the procedure of the study. Study participation was voluntary. Participants were interviewed by health personnel specifically trained for the interview. Interview responses were recorded in an Epicollect5 form.[19] Once the interview was completed, a trained phlebotomist collected 3-5 mL of venous blood from the antecubital vein under aseptic precautions into a red-top collection tube containing a clot activator. The tube was left standing, undisturbed, for at least 30 minutes for clot formation. The sample was later transported to a central facility for centrifugation. Centrifuged samples were transported to a central laboratory for further processing and analysis. Serum samples were tested for the presence of SARS-CoV-2 specific IgG antibodies using the Abbott SARS-CoV-2 IgG assay. The assay uses chemiluminescence to detect IgG antibodies against the SARS-CoV-2 nucleocapsid protein. The reported sensitivity and specificity of the assay are 100% (95% CI 95.89-100.00)and 99·63% (99.05-99.90), respectively.[20] As recommended by the manufacturer, we labeled assay results equal to or above the cut-off index value of 1·4 as positive for SARS-CoV-2 specific IgG antibodies.

Statistical methods

We report unweighted seroprevalence estimates in percentages. We used the Agresti-Coull procedure to calculate a 95% confidence interval (CI) for seroprevalence estimates.[21] A weighted estimate of seroprevalence is provided. To calculate survey weights (inverse of sampling probability) we used the estimated population of the districts. We used the census 2011 data and growth rates from Sample Registration System to estimate the population of the districts in 2020.[18,22] Survey weights so obtained were further adjusted for non-response and age and sex structure (post-stratification weights). We further adjusted the weighted seroprevalence estimates for test performance to calculate "weighted seroprevalence adjusted for test performance". We did this using the formula:
$Weighted\ seroprevalence\ adjusted\ for\ test\ performance =$
$(Weighted\ seroprevalence + Test\ specificity - 1)/(Test\ sensitivity + Test\ specificity - 1)$.[23]

We used the manufacturer-provided sensitivity and specificity in the above formula.[20] We used the lower and upper bounds of the manufacturer-provided test performance to report sensitivity analyses.

We analyzed the difference in seroprevalence estimates across levels of a categorical variable using a Chi-square test adjusted for the sampling design.

We estimated the number of SARS-CoV-2 infections by multiplying the weighted seroprevalence adjusted for test performance with the estimated population of the valley. To estimate the number of infections per reported case, we divided the estimated number of SARS-CoV-2 infections by the reported number of COVID-19 cases two weeks before the survey date. We calculated the infection fatality rate by dividing the reported number of deaths by the number of estimated infections, assuming a three-week lag time from infection to death.[24]

We analyzed the data using Stata version 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LP.).

Patient and Public Involvement

No patients or members of the public were involved in this study.

RESULTS

We enumerated 6 397 individuals ≥18 years from 3 077 households and 110 clusters (34 urban clusters and 76 rural clusters) between 17 Oct 2020 and 04 Nov 2020. Out of the 6 397 eligible individuals, 6 315 (98·7%) agreed to participate and were enrolled. The final analysis was done on a sample of 6 230 participants. (Figure 2)

Of the 6 230 participants, 1 513 (24·3%) were between 18 and 30 years of age, 2 672 (42·9%) were aged 30-49 years, 1 643 (26·4%) were aged 50-69 years, and 402 (6·4%) were 70 years and older. (Table 1). There was equal representation from males and females, and 3 364 (54·0%) resided in a rural area. Of the 3 104 females, 56 (1·8%) reported pregnant at the time of the survey. Four hundred seventy-four (7·6%) reported COVID-19 like symptoms in the three months preceding the survey and 439 (7·0%) reported to have ever come in contact with a known COVID-19 case. One thousand ninety-two (17·5%) reported having been tested for COVID-19 using RT-PCR or a Rapid Antigen Test (RAT) previously, of whom 176 (16·2%) reported to have tested positive for the disease.

Table 1: Characteristics of study participants

	Frequency	Percent
Total	6230	
Age, years		
18-29	1513	24·3
30-49	2672	42·9
50-69	1643	26·4
≥70	402	6·5
Gender		
Male	3126	50·2
Female	3104	49·8
Residence		
Urban	2866	46·0
Rural	3364	54·0

Pregnant (n=3104)	56	1·8
Self-reported history of chronic disease	1145	18.4
History of COVID-19 like symptoms	474	7·6
History of contact with a known COVID-19 case	439	7·0
Ever tested for COVID-19 (RT-PCR)	1092	17·5
RT-PCR result (n=1088*)		
Positive	176	16·2
Negative	912	83·8

COVID-19: Coronavirus disease; RT-PCR: Reverse transcriptase-polymerase chain reaction
*RT-PCR result not known in four participants

We found an overall unweighted seroprevalence of 38·8% (95% CI 37·6 – 40·0). The seroprevalence ranged from 28·5% in district Kulgam to 43·1% in district Pulwama. (Figure 1 and online supplemental file 1) The overall weighted seroprevalence (adjusted for sampling design) was 36·9% (95% CI 34·5 – 39·4). The weighted seroprevalence adjusted for test performance was 36·7% (95% CI 34·3 – 39·2). (Table 2) Upon sensitivity analyses, the weighted seroprevalence adjusted for test performance ranged from 36·3% (95% CI 33·9 – 38·8) to 38·4% (95% CI 35·9 – 41·0). (Table 3)

Table 2: Seroprevalence of SARS-CoV-2 specific IgG antibodies by participant characteristics

	Number tested	Number seropositive	Unweighted seroprevalence, % (95% CI)	Weighted seroprevalence, % (95% CI)	Weighted seroprevalence adjusted for test performance, % (95% CI)	Design-based F, p-value
Total	6230	2415	38·8 (37·6-40·0)	36·9 (34·5-39·4)	36·7 (34·3-39·2)	
Age, years						
18-29	1513	538	35·6 (33·2-38·0)	33·7 (30·1-37·6)	33·5 (29·8-37·4)	6·42, 0·0006
30-49	2672	1000	37·4 (35·6-39·3)	36·3 (33·5-39·3)	36·1 (33·3-39·1)	
50-69	1643	691	42·1 (39·7-44·5)	42·5 (38·8-46·2)	42·3 (38·6-46·0)	
≥70	402	186	46·3 (41·5-51·2)	45·3 (37·8-53·0)	45·1 (37·6-52·8)	
Gender						
Male	3126	1166	37·3 (35·6-39·0)	36·1 (33·5-38·9)	35·9 (33·3-38·7)	0·94, 0·34
Female	3104	1249	40·2 (38·5-42·0)	37·8 (34·5-41·3)	37·6 (34·3-41·1)	
Residence						
Urban	2866	1180	41·2 (39·4-43·0)	40·2 (36·3-44·1)	40·0 (36·1-43·9)	3·43, 0·07
Rural	3364	1235	36·7 (35·1-38·4)	35·5 (32·5-38·7)	35·3 (32·2-38·5)	

Self-reported history of chronic disease						
Yes	1145	495	43·2 (40·4-46·1)	41·9 (37·4-46·6)	41·7 (37·2-46·4)	6.14, 0.02
No	5085	1920	37·8 (36·4-39·1)	36·2 (33·7-38·9)	36·0 (33·5-38·7)	
History of COVID-19 like symptoms						
Yes	474	247	52·1 (47·6-56·6)	47·4 (37·9-57·1)	47·2 (37·7-56·9)	5·53, 0·02
No	5756	2168	37·7 (36·4-38·9)	36·3 (33·9-38·8)	36·1 (33·7-38·6)	
History of contact with a known COVID-19 case						
Yes	439	219	49·9 (45·2-54·5)	45·2 (38·3-52·2)	45·0 (38·1-52·0)	7·13, 0·01
No	5791	2196	37·9 (36·7-39·2)	36·5 (34·1-39·0)	36·3 (33·9-38·8)	
Ever tested for COVID-19 (RT-PCR)						
Yes	1092	485	44·4 (41·5-47·4)	41·0 (35·4-46·9)	40·8 (35·2-46·7)	2·17, 0·14
No	5138	1930	37·6 (36·2-38·9)	36·2 (33·5-39·0)	36·0 (33·3-38·8)	
RT-PCR result (n=1088*)						
Positive	176	140	79·5 (73·0-84·9)	81·8 (74·8-87·1)	81·7 (74·7-87·1)	74·93, <0·0001
Negative	912	345	37·8 (34·7-41·0)	38·8 (33·3-44·7)	38·6 (33·1-44·5)	

COVID-19: Coronavirus disease; RT-PCR: Reverse transcriptase-polymerase chain reaction
*RT-PCR result not known in four participants

Table 3: Sensitivity analyses for seroprevalence at extremes of test performance

	Weighted seroprevalence, % (95% CI)	**Weighted seroprevalence adjusted for test performance, % (95% CI)** ***[Sensitivity 100·00%, Specificity 99·63%]***	**Weighted seroprevalence adjusted for test performance, % (95% CI)** ***[Sensitivity 95·89%, Specificity 99·90%]***	**Weighted seroprevalence adjusted for test performance, % (95% CI)** ***[Sensitivity 100·00%, Specificity 99·05%]***
Overall	36·9 (34·5-39·4)	36·7 (34·3-39·2)	38·4 (35·9-41.0)	36·3 (33·9-38·8)
Age, years				
18-29	33·7 (30·1-37·6)	33·5 (29·8-37·4)	35·1 (31·3-39·1)	33·1 (29·4-37·0)
30-49	36·3 (33·5-39·3)	36·1 (33·3-39·1)	37·8 (34·9-40·9)	35·7 (32·9-38·7)

50-69	42·5 938·8-46·2)	42·3 (38·6-46·0)	44·3 (40·4-48·1)	41·9 (38·2-45·7)
≥70	45·3 937·8-53·0)	45·1 (37·6-52·8)	47·2 (39·4-55·2)	44·8 (37·2-52·5)
Gender				
Male	36·1 (33·5-38·9)	35·9 (33·3-38·7)	37·6 (34·9-40·5)	35·5 (32·9-38·3)
Female	37·8 (34·5-41·30	37·6 (34·3-41·1)	39·4 (35·9-43.0)	37·2 (33·9-40·7)
Residence				
Urban	40·2 (36·3-44·1)	40·0 (36·1-43·9)	41·9 (37·8-45·9)	39·6 (35·7-43·6)
Rural	35·5 (32·5-38·7)	35·3 (32·2-38·5)	37·0 (33·8-40·3)	34·9 (31·9-38·1)
Self-reported history of chronic disease				
Yes	43·2 (40·4-46·1)	41·9 (37·4-46·6)	43·6 (38·9-48·5)	41·3 (36·8-46·1)
No	37·8 (36·4-39·1)	36·2 (33·7-38·9)	37·7 (35·1-40·5)	35·6 (33·1-38·3)
History of COVID-19 like symptoms				
Yes	52·1 (47·6-56·6)	47·4 (37·9-57·1)	49·4 (39·5-59·5)	46·9 (37·3-56·7)
No	37·7 (36·4-38·9)	36·3 (33·9-38·8)	37·8 (35·3-40·4)	35·7 (33·3-38·2)
History of contact with a known COVID-19 case				
Yes	49·9 (45·2-54·5)	45·2 (38·3-52·2)	47·1 (39·9-54·4)	44·7 (37·7-51·7)
No	37·9 (36·7-39·2)	36·5 (34·1-39·0)	38·0 (35·5-40·6)	35·9 (33·5-38·4)
Ever tested for COVID-19 (RT-PCR)				
Yes	44·4 (41·5-47·4)	41·0 (35·4-46·9)	42·7 (36·9-48·9)	40·4 (34·8-46·4)
No	37·6 (36·2-38·9)	36·2 (33·5-39·0)	37·7 (34·9-40·6)	35·6 (32·9-38·4)
RT-PCR result (n=1088*)				
Positive	79·5 (73·0-84·9)	81·8 (74·8-87·1)	85·3 (78·0-90·8)	81·6 (74·6-87·0)
Negative	37·8 (34·7-41·0)	38·8 (33·3-44·7)	40·4 (34·7-46·6)	38·2 (32·7-44·2)

Seroprevalence was lowest among participants aged 18-29 years [33·5% (95% CI 29·8 – 37·4)] and was higher in older age groups. Seroprevalence was highest in those aged 70 years and above [45·1% (95% CI 37·6 – 52·8)]. Seroprevalence was not significantly different among males and females (p=0·34). The seroprevalence among urban residents was 40·0% (95% CI 36·1 – 43·9), slightly but not significantly, higher than rural residents [35·3% (95% CI 32·2 – 38·5), p=0·07]. (Table 2)

One in five participants (1145/6230, 18·4%) self-reported a history of at least one chronic disease (Table 1). Hypertension (815/6230, 13.1%) and diabetes mellitus (314/6230, 5.0%) were the most commonly reported chronic diseases (online supplemental file 2). Seroprevalence was significantly higher in participants who self-reported history of chronic disease (41·7%, 95% CI 37·2 – 46·4) as compared to those who did not report a history of chronic disease (36·0%, 95% CI 33·5 - 38·7) (Table 2).

Among participants who reported a history of COVID-19 like symptoms, seroprevalence was 47·2% (95% CI 37·7 – 56·9) compared with 36·1% (95% CI 33·7 – 38·6) among participants who did not report such

symptoms. Seroprevalence was higher among those who reported contact with a known COVID-19 case [45·0% (95% CI 38·1 – 52·0)] than participants who did not report any history of such contact [36·3% (95% CI 33·9 – 38·8)]. (Table 2)

Seroprevalence was not significantly related to being tested for COVID-19 (RT-PCR). However, those who reported a positive RT-PCR COVID-19 test had significantly higher seroprevalence (81·7%, 95% CI 74·7 – 87·1) as compared to those who reported a negative RT-PCR COVID-19 test (38·6%, 95% CI 33·1 – 44·5). (Table 2)

Among 2 415 seropositive individuals, only 247 (10·2%) reported a history of COVID-19 like symptoms. Only 20.1% (485/2415) of the seropositive individuals were tested for COVID-19 (RT-PCR). Among 474 who reported a history of COVID-19 like symptoms, 233 (49·2%) were tested for COVID-19 (RT-PCR). Among 4897 individuals who did not report a history of COVID-19 like symptoms and were never tested for COVID-19 (RT-PCR), 1825 (37.3%) were seropositive. (Figure 3)

Among 36 participants who reported a positive RT-PCR COVID-19 test but were seronegative, the duration between COVID-19 RT-PCR test and serological testing ranged from 9 days to 101 days. In only four of these 36 participants, the duration between the COVID-19 RT-PCR test and the serological test was 14 days or less. Of the remaining 32 participants, 21 did not report a history of CVOID-19 like symptoms, nine did not report a history of contact with a known COVID-19 case, and eight reported neither a history of COVID-19 like symptoms nor a history of contact with a known COVID-19 case.

We estimated that there were 1 673 484 (95% CI 1 564 047 – 1 787 482) cumulative number of infections among adults aged ≥18 years in the valley by 03 Oct 2020, two weeks before the start of the survey. If we assume that the seroprevalence was similar to the overall seroprevalence in the population not included in our study (<18 years of age) then the estimated cumulative number of infections in the valley by 03 Oct 2020 was 2 791 933 (95% CI 2 609 354 – 2 982 119). Considering that the cumulative number of reported COVID-19 cases was 47 071 by 03 Oct 2020 (Figure 4), we estimate the number of infections per reported case as 59·3 (95% CI 55·4 – 63·4). The number of reported COVID-19 deaths after a three-week lag period (on 24 Oct 2020) was 955. Thus, we estimated the infection fatality rate as 0.034% (95% CI 0.032 – 0.037).

Figure 5 depicts the relationship between the estimated number of SARS-CoV-2 infected persons, reported COVID-19 cases, and reported COVID-19 deaths. Of the total estimated SARS-CoV-2 infected persons, only 1.69% were reported. Of the total reported COVID-19 cases, 2.03% died.**DISCUSSION**

The results of our study indicate that by the first week of October 2020, nearly seven months after the appearance of the first laboratory-confirmed COVID-19 case on 18 March 2020, close to 37% of the valley's population aged ≥18 years had been infected. Our results suggest that the cumulative number of SARS-CoV-2 infections by the first week of October 2020 was nearly 2·8 million with an estimated infection fatality rate of 0.034%. Seroprevalence did not differ by gender but was higher in older age groups.

The findings of our study are based on a representative sample of the population. The laboratory test used for the detection of SARS-CoV-2 specific IgG antibodies in serum samples provides valid results.[20,25] We report seroprevalence estimates adjusted for sampling design and test performance.

The overall adjusted seroprevalence of around 37% indicates that a large proportion of the valley's population has been infected with the virus. Easing of lockdown, being fed up with the restrictions, and non-adherence to prevention norms are the possible reasons. Even though a large proportion of the population has been infected, the transmission of infection is expected to continue till most of the susceptible population becomes immune. Herd immunity in the context of COVID-19 is a matter of debate as reports of a second infection continue to pour in.[26] The emergence of several Variants of Concern and the introduction of COVID-19 vaccination will also influence population immunity. Several factors potentially influence the seroprevalence rates. These include population density, social and demographic structure of the population, governmental policies and the extent of their implementation, immunity level of the population, time since the start of infection transmission, adherence to infection prevention guidelines, quality of contact tracing and quarantine, and possibly the geography and environment of an area.

Comparison with previous reports suggests that the seroprevalence has increased almost ten-fold since July 2020.[16,27] The second of the three nationwide seroprevalence surveys in India conducted in August-September 2020 reports an overall seroprevalence of 6.6% ranging from 5.2% in rural areas to 16.9% in urban slums.[28] A nationwide survey conducted in December 2020-January 2021 reported an overall seroprevalence of 24.1% ranging from 4.9% - 44.4% across districts.[29] Kashmir is thus not a low-infection area. Being an oft-visited tourist area, Kashmir is at an increased risk of infection transmission. Adherence to COVID appropriate behavior (use of face masks in public, frequent handwashing, physical and social distancing) has been poor. With the introduction of the COVID-19 vaccination program in January 2021 and the emergence of a 'second wave' in Kashmir in April 2021, the seroprevalence estimates are expected to increase in the future.

The seroprevalence of SARS-CoV-2 IgG antibodies was higher in older age groups. During the early period of the pandemic, people were adherent to social distancing and other non-pharmaceutical interventions because of a fear of the disease and administrative restrictions. With time, administrative restrictions were relaxed, fear of the disease attenuated, and people became sort of fed up with the social restrictions. This not only led to an increase in the number of reported COVID-19 cases but also provided the population, including older age groups, an opportunity to contract the infection. That older people have an increased risk of symptomatic and more severe disease is now well known.[30,31] However, age-based differential susceptibility to SARS-CoV-2 infection antibody response and the reasons thereof are still a grey area and need further understanding. Existing literature might suggest that the young who are more mobile and socially active have a higher risk of infection.[6,7] However, this should not imply that the elderly have a decreased susceptibility to SARS-CoV-2 infection or a decreased antibody response.[32] On the contrary, several studies suggest that the seroprevalence of SARS-CoV-2 specific IgG antibodies is higher in the older age groups and particularly so in more dense population groups.[4,5,8–11,13] Furthermore, SARS-CoV-2 antibody levels have been reported to be higher in older people.[12]

The seroprevalence of SARS-CoV-2 specific IgG antibodies did not differ significantly by gender, though the figure was slightly higher for females. These findings are consistent with the available literature.[6,13] Difference in seroprevalence by gender has been suggested by some studies and females have been reported to have lower antibody levels.[5,7,9,11,12,14,33]

Urban areas are more densely populated as compared to rural areas which accelerate the transmission of infections in the population. The seroprevalence of SARS-CoV-2 specific IgG antibodies is thus expected to be higher in urban areas especially during the early phases of an epidemic. As the epidemic progresses the seroprevalence gap between urban and rural areas will wane off. We estimated an adjusted seroprevalence of 40·0% (95% CI 36·1 – 43·9) in urban areas as compared to 35·3% (95% CI 32·2 – 38·5) in rural areas (p=0·07).

People with a chronic disease experience more severe COVID-19 symptoms and are more likely to die when compared to people with no chronic disease.[34] We found a higher proportion of symptomatic infection among participants with a self-reported history of chronic disease (78/1145, 6·8%) as compared to participants with no chronic disease (169/5085, 3·3%) (online supplemental file 3). Little is, however, known about the risk of infection in chronic disease patients. We found a significantly higher seroprevalence among participants with a self-reported history of chronic disease (Table 2). This finding needs further research for corroboration and possible explanations.

People with a self-reported history of COVID-19 related symptoms, contact with a known COVID-19 case, or a positive COVID-19 RT-PCR had a higher seroprevalence as compared to their complement. Among seropositive individuals, only 10·2% reported being symptomatic. The percentage of asymptomatic infections, thus, was 90%. However, only 49% of individuals with a history of COVID-19 like symptoms were tested using RT-PCR. We also estimated that only one out of almost 59 infections gets reported. This reflects the necessity of improving the efficiency of RT-PCR testing so that more symptomatic individuals receive the test. Not all individuals with a known RT-PCR positive result showed the presence of IgG antibodies. Around 20% of RT-PCR positive individuals were seronegative and in a large majority of them (32 out of 36) the duration since RT-PCR positivity was more than two weeks. This may be attributed to a poor B cell response or a false negative antibody test.[35] Around 38% of RT-PCR negative individuals were seropositive suggesting a false-negative RT-PCR or infection acquisition at a date later than the RT-PCR test.

We estimated an infection fatality rate of 0.034% (95% CI 0.032 – 0.037). The infection fatality rate in SARS-CoV-2 infection has been reported to range from as low as 0.00% to 1.63%.[36] Our estimates of the infection fatality rate are low as compared to estimates from several Indian studies.[5,28,37] Under-reporting of COVID-19 deaths because of non-uniform definition for a 'COVID-19 death' may falsely lower the infection fatality rates.[38] Many other factors can influence the infection fatality rate in SARS-CoV-2 infection – the quality of available health facilities, the age structure of the population, and COVID-19 epidemic intensity.[39,40] Developing countries usually have a younger population as compared to the developed countries and Kashmir is not an exception. However, because of the possibility of under-reporting of COVID-19 deaths, the true infection fatality rate in Kashmir may be higher than our estimates. The infection fatality rate is, however, known to be lower in developing nations.[30,41] In developed nations like the United States and many European countries, a higher infection fatality rate has been reported.[30,42]

Limitations

One important limitation of our study is that even though we adjusted the weighted seroprevalence estimates for test performance using manufacturer-provided sensitivity and specificity (100% and 99·63% respectively), we did not quantify the test validity in-house. Another limitation of our study

estimates is that we excluded people <18 years of age. The results of our study may not thus be generalizable to this group of the population.

Our estimated seroprevalence was much higher than we anticipated at the designing stage. This has impacted the precision of our estimates to some extent. However, we believe we still have been able to estimate the seroprevalence with reasonable precision.

Lack of reliable death counts is another potential limitation. This may have led to an underestimation of the infection fatality rate. We did not perform any adjustment for death counts. Further, because of lack of age- and gender-specific mortality data we could not estimate age- and gender-specific infection fatality rates.

CONCLUSIONS

We conclude that nearly 37% of individuals aged 18 years and above were infected with SARS-CoV-2 in Kashmir by October 2020. The infection fatality rate in the valley is around 0.034%. A majority of cases go unreported. For every reported case there are 59 unreported infections in the population. Since almost half of the symptomatic individuals go unreported, testing of symptomatic individuals and effective contact tracing needs to continue. Given the emergence of mutant Variants of Concern, increasing the population immunity through augmented and sustained vaccination is needed. We further recommend that adherence to COVID-19 prevention measures should be ensured at least till a large proportion of the population gets vaccinated.

ACKNOWLEDGMENTS

We thank Prof. Samia Rashid for her support. We acknowledge the help from Divisional COVID-19 Control Room Kashmir, especially from Dr. Rauf Hussain Rather. We thank the following coordinating center teams and the laboratory support team for their support and coordination:

Coordinating center teams: Dr. Sabira Aalia Dkhar, Dr. Kouser Sideeq, Dr. Uruj Qureshi, Dr. Mehraj u Din Sofi, Dr. Yasmin Kango, Dr. Suhail Manzoor Shah, Dr. Bashir Ahmad Khan, Dr. Nabeel Ishtiyaq, Dr. Tajamul Hussain Khan, Dr. Lahoot Hassan Khan, Dr. Muzaffer Ahmad, Dr. Ramesh Kumar Malla, Dr. Ruqaya Rasool Kapta, Dr. Mohammad Yousuf Naikoo, Dr. Khalid Abbass, Dr. Zarar Ahmad Bhat, Dr. Sumair Rashid, Dr. Zubair Ahmad, Dr. Shalender Singh Bali, Dr. Abdul Rouf, Dr. Abdul Hamid, Dr. Khalid Bashir, Dr. Qazi Najeeb Ahmad, Dr. Syed Irtiza, Dr. P Sharik Shaheen Khaki, Dr. Sheikh Mohammad Shafi, Dr. Mohammad Yousuf Zagoo, Dr. Mukhtar Ahmad Shah, Dr. Imtiyaz-ur-Rehman Bhat, Dr. Showkat Parray, Dr. Fazil Ali Kochak, Dr. Muzaffar Ahmad Zargar, Dr. Abdul Rashid Shah, Dr. Shagufta Saleem, Dr. Abdul Gani, Dr. Nighat Nasreen, Dr. Asima Nazeer, Dr. Zahoor Ahmad Lone.

Laboratory support team: Ishtiyaq Ahmad Handoo, Rahiba Makhdoomi, Iyman Wani, Romana Rasool, Asiya Rehman, Insha Rehman, Zahoor Ahmad, Gulzar Ahmad Wani, Javid Ahmad Bhat, M Saleem Malik, Naveed Manzoor, Mubeena.

COMPETING INTERESTS

We declare no competing interests, financial or otherwise.

FUNDING

The National Health Mission Jammu & Kashmir funded the study. The funding agency was not involved in study designing, implementation, analysis, or interpretation of the study findings.

CONTRIBUTORS

S Muhammad Salim Khan: Conceptualization, Funding acquisition, Methodology, Writing-review & editing.

Mariya Amin Qurieshi, Inaamul Haq, Sabhiya Majid: Formal analysis, Data curation, Methodology, Project administration, Writing-original draft, Writing-review & editing.

Javid Ahmad, Taha Ayub, Anjum Bashir, Ashfaq Ahmad Bhat, Abdul Majeed Ganai, Yasmeen Jan, Rauf-ur-Rashid Kaul, Zahid Ali Khan, Muneer Ahmad Masoodi, Beenish Mushtaq, Fouzia Nazir, Muzamil Nazir, Malik Waseem Raja, Mahbooba Rasool: Project administration, Supervision, Investigation, Writing-review & editing.

Anjum Asma, Munazza Aziz, Shifana Ayoub, Arif Akbar Bhat, Iqra Nisar Chowdri, Shaista Ismail, Misbah Ferooz Kawoosa, Mehvish Afzal Khan, Mosin Saleem Khan, Rafiya Kousar, Ab Aziz Lone, Shahroz Nabi, Mohammad Obaid, Tanzeela Bashir Qazi, Iram Sabah, Ishtiyaq Ahmad Sumji: Supervision, Investigation, Writing-review & editing.

S Muhammad Salim Khan, Mariya Amin Qurieshi, Inaamul Haq, and Sabhiya Majid have verified the underlying data.

DATA SHARING

Anonymized data collected for the study, including individual participant data and a data dictionary defining each field in the set, will be made available to interested researchers on request by Inaamul Haq (haqinaam@yahoo.co.in) for two years from publication. The study protocol, statistical analysis plan, and informed consent forms are also available from Inaamul Haq.

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Figure 1 legend:

Figure 1: Location of the ten districts and seroprevalence (%) by district. Figures in parentheses indicate a 95% Confidence Interval for seroprevalence.

Figure 2 legend:

Figure 2: Participant flow.

Figure 3 legend:

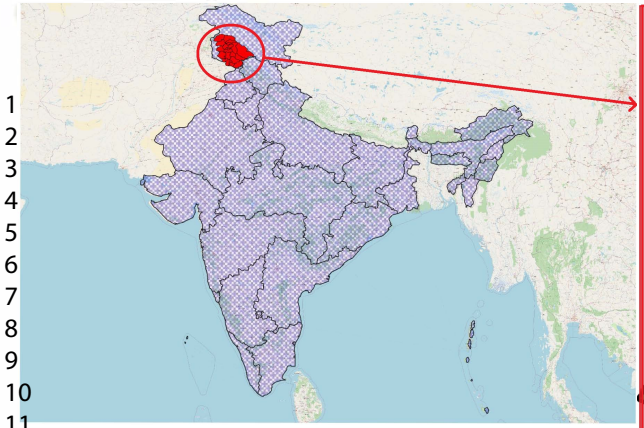
Figure 3: A. Seropositivity by the history of COVID-19 like symptoms, RT-PCR testing, and test result. B. History of COVID-19 like symptoms by seropositivity, RT-PCR testing, and test result.

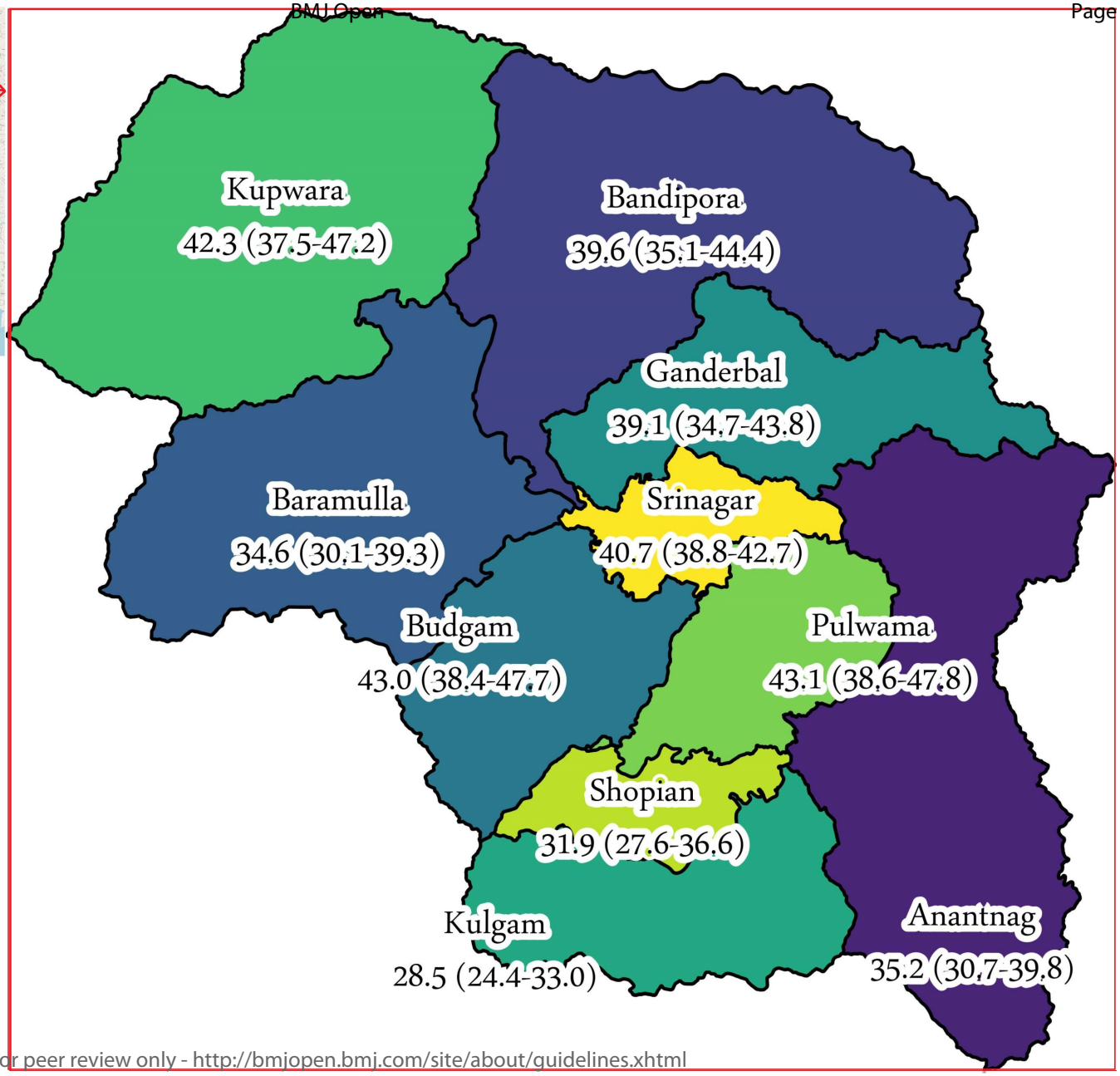
Figure 4 legend:

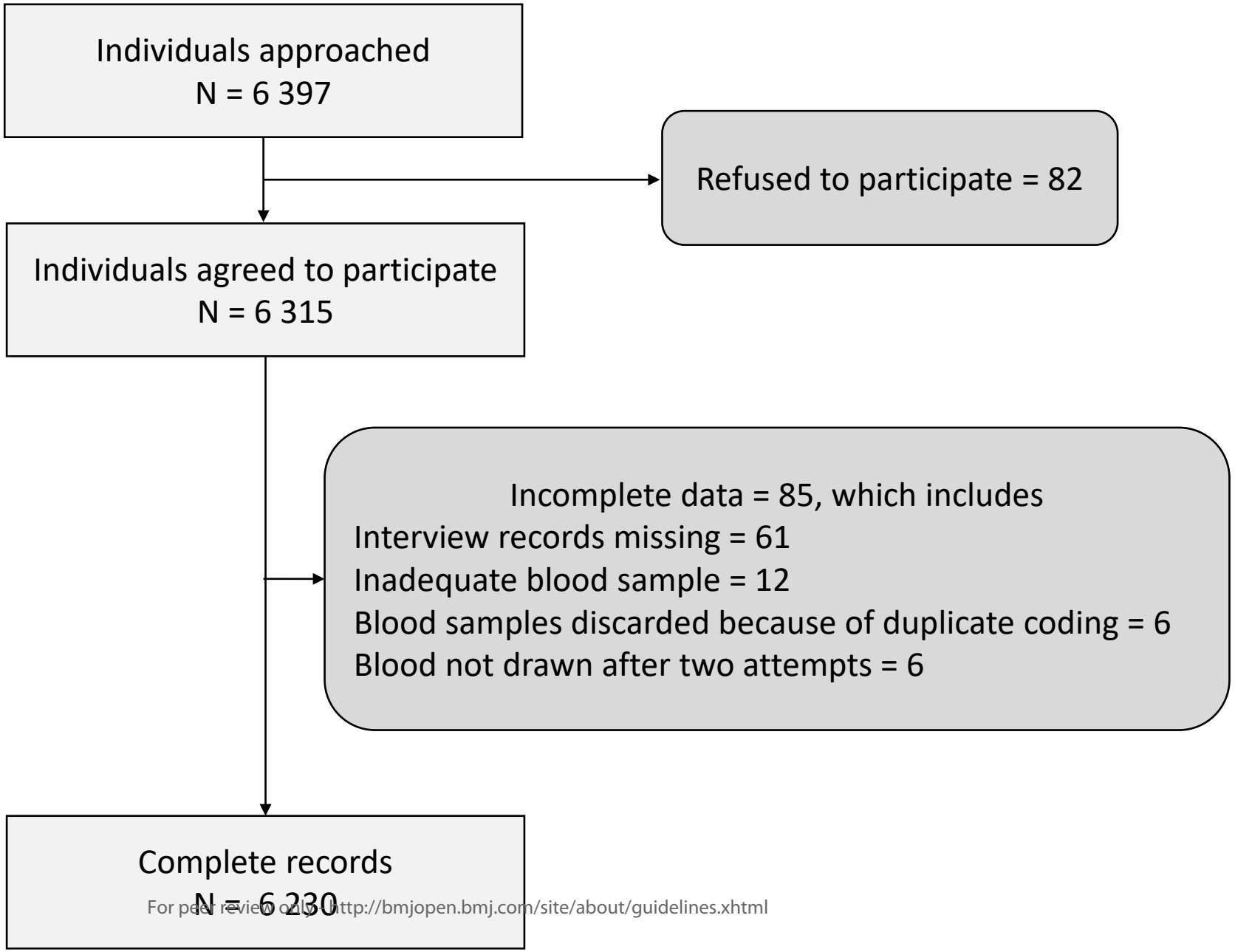
Figure 4: A. Daily cases and deaths in Kashmir since the start of the COVID-19 pandemic; B. Cumulative number of cases and deaths in Kashmir.

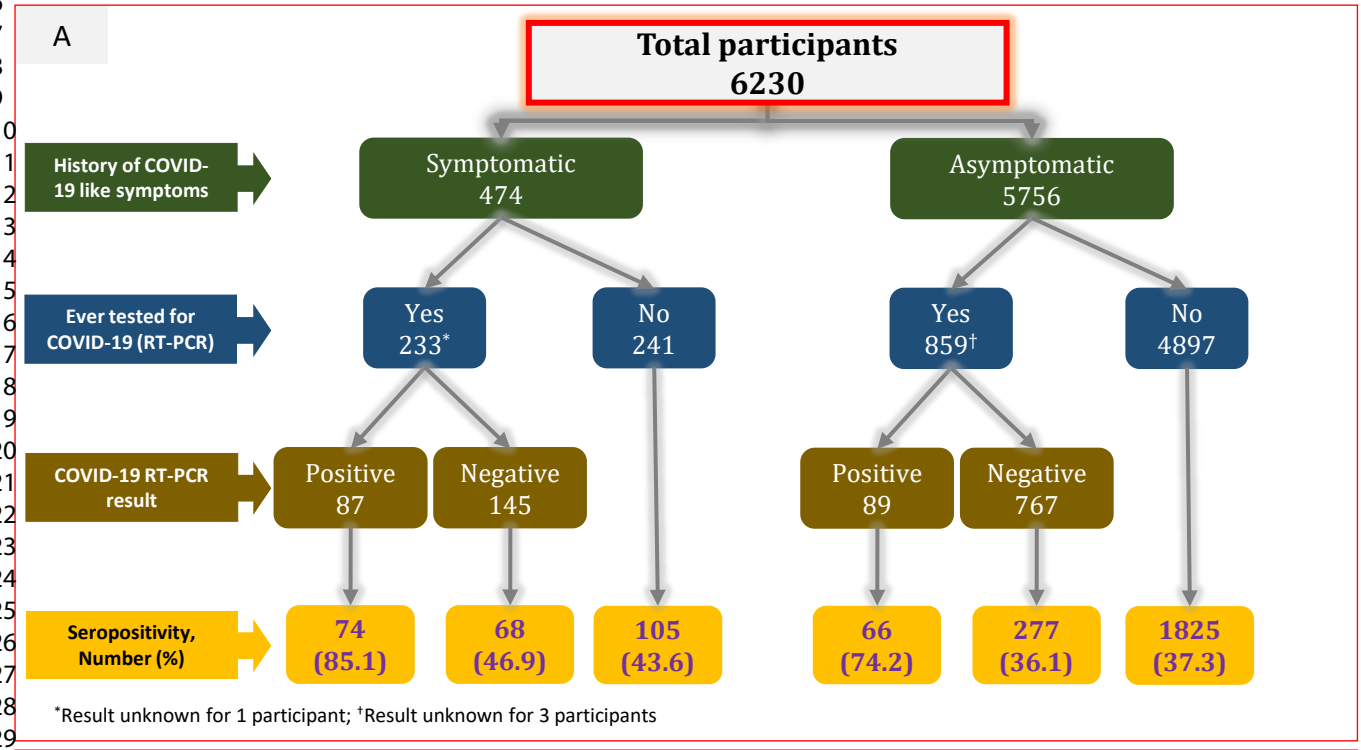
Figure 5 legend:

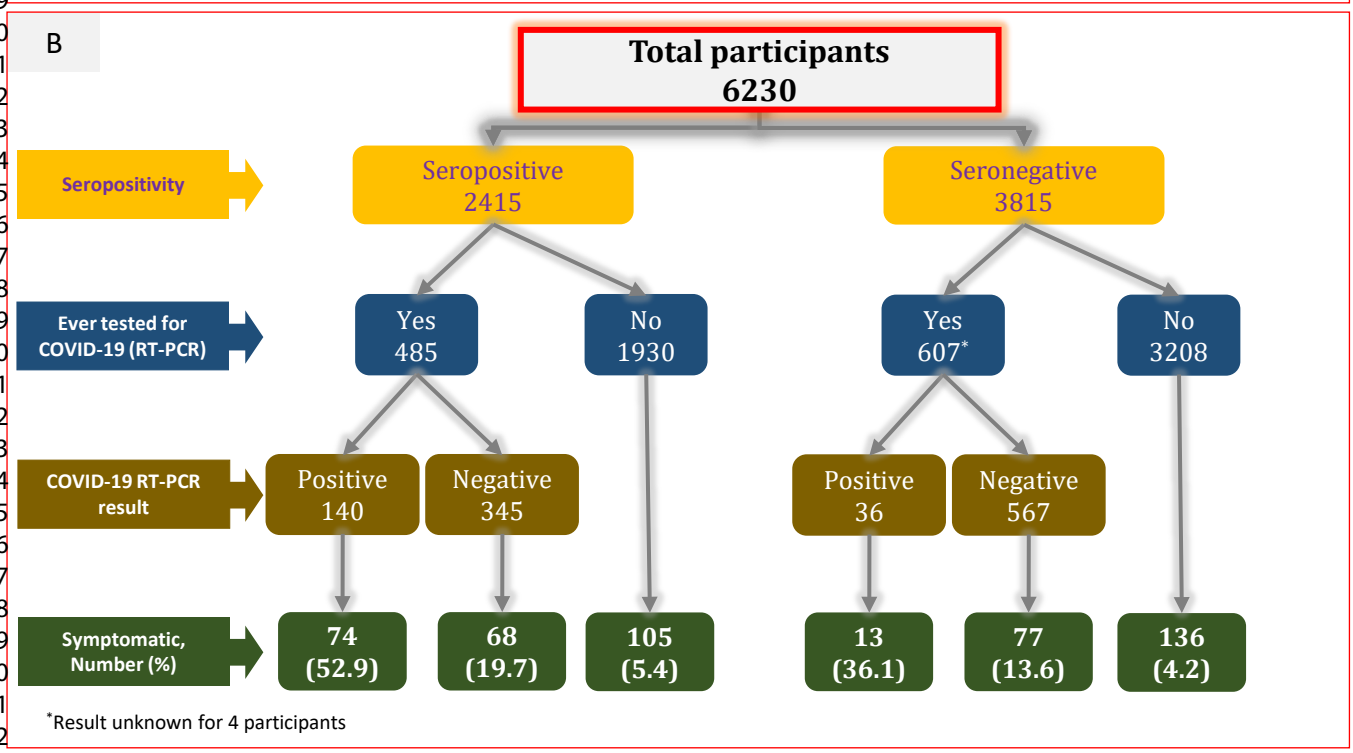
Figure 5: Cumulative estimated SARS-CoV-2 infections, reported cases, and deaths in Kashmir, October-November 2020. The bars represent the number of persons at each step. The percentages above the bars represent the percentage out of the total population. The percentages within the triangles represent percentages out of the previous step who proceed to the next step.

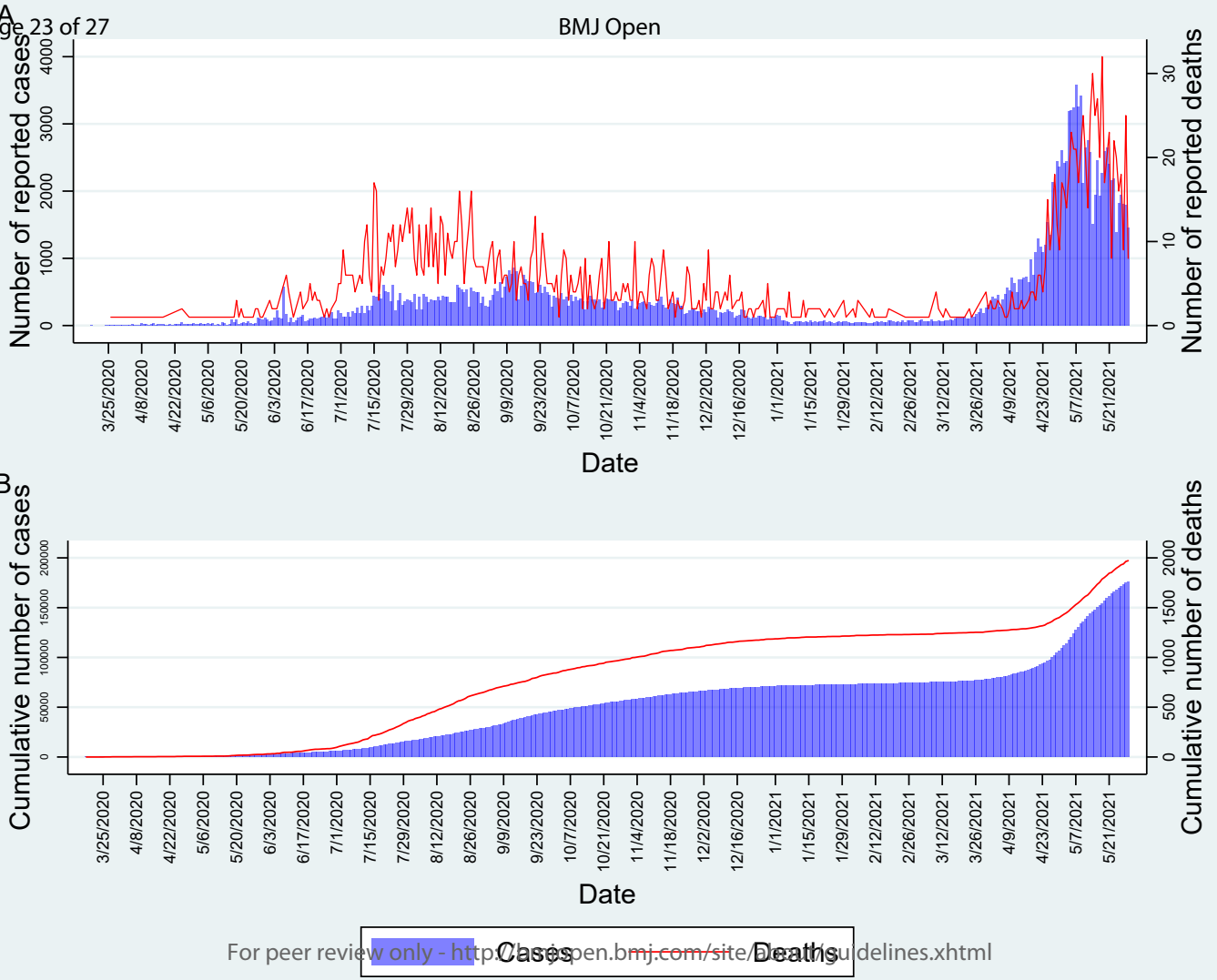


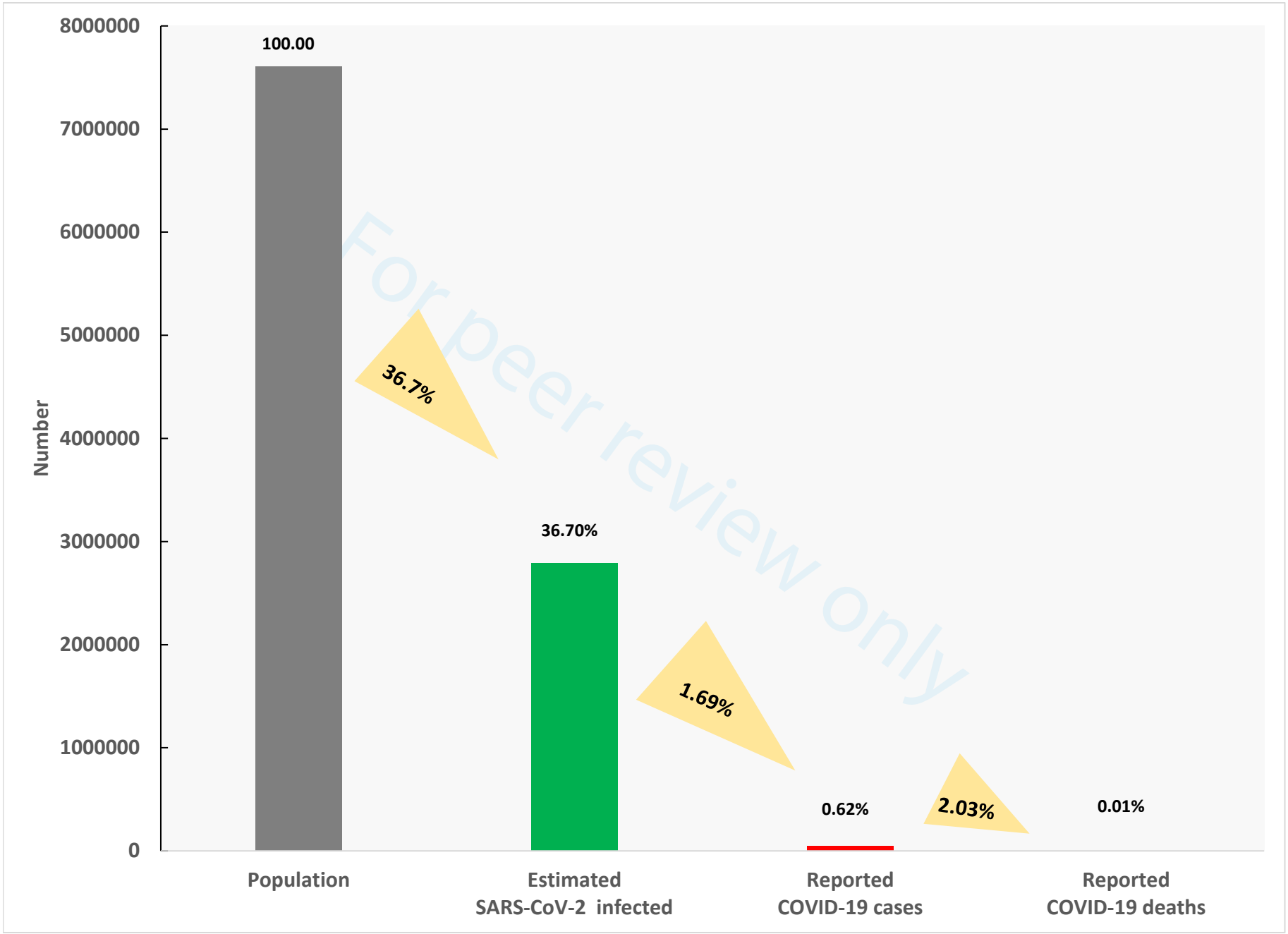

Kupwara
42.3 (37.5-47.2)
Bandipora
39.6 (35.1-44.4)
Ganderbal
39.1 (34.7-43.8)
Baramulla
34.6 (30.1-39.3)
Srinagar
40.7 (38.8-42.7)
Budgam
43.0 (38.4-47.7)
Pulwama
43.1 (38.6-47.8)
Shopian
31.9 (27.6-36.6)
Kulgam
28.5 (24.4-33.0)
Anantnag
35.2 (30.7-39.8)




A
Total participants
6230
History of COVID-19 like symptoms
Symptomatic
474
Asymptomatic
5756
Ever tested for COVID-19 (RT-PCR)
Yes
233*
No
241
Yes
859†
No
4897
COVID-19 RT-PCR result
Positive
87
Negative
145
Positive
89
Negative
767
Seropositivity, Number (%)
74
(85.1)
68
(46.9)
105
(43.6)
66
(74.2)
277
(36.1)
1825
(37.3)
*Result unknown for 1 participant; †Result unknown for 3 participants


B
Total participants
6230
Seropositivity
Seropositive
2415
Seronegative
3815
Ever tested for COVID-19 (RT-PCR)
Yes
485
No
1930
Yes
607*
No
3208
COVID-19 RT-PCR result
Positive
140
Negative
345
Positive
36
Negative
567
Symptomatic, Number (%)
74
(52.9)
68
(19.7)
105
(5.4)
13
(36.1)
77
(13.6)
136
(4.2)
*Result unknown for 4 participants





Supplemental Table 1: Participant characteristics by district

District	Total	Age (years)				Gender		Residence	
		<30	30-49	50-69	70+	Female	Male	Rural	Urban
Anantnag	421	84	197	113	27	214	207	295	126
Budgam	442	113	190	105	34	263	179	354	88
Bandipora	424	106	174	114	30	227	197	341	83
Baramulla	405	113	176	98	18	214	191	325	80
Ganderbal	442	92	210	123	17	233	209	346	96
Kulgam	428	102	194	113	19	257	171	346	82
Kupwara	400	81	171	105	43	215	185	360	40
Pulwama	443	102	176	126	39	218	225	396	47
Shopiyan	407	119	152	90	46	211	196	368	39
Srinagar	2418	601	1032	656	129	1052	1366	233	2185
Total	6230	1513	2672	1643	402	3104	3126	3364	2866

Supplemental Table 2: Seroprevalence (unadjusted) by district and participant characteristics

District	Overall	Age (years)				Gender		Residence	
		<30	30-49	50-69	70+	Male	Female	Urban	Rural
Anantnag	35.2 (30.7-39.8)	29.8 (21-40.4)	34.5 (28.2-41.4)	38.9 (30.4-48.2)	40.7 (24.2-59.7)	36.2 (30-43)	34.1 (28.1-40.7)	42.9 (34.5-51.6)	31.9 (26.8-37.4)
Budgam	43 (38.4-47.7)	44.2 (35.4-53.5)	37.9 (31.3-45)	48.6 (39.2-58.1)	50 (33.8-66.2)	41.9 (34.9-49.3)	43.7 (37.8-49.8)	38.6 (29.1-49.2)	44.1 (39-49.3)
Bandipora	39.6 (35.1-44.4)	37.7 (29-47.3)	42 (34.8-49.4)	40.4 (31.8-49.6)	30 (16.4-48.3)	37.6 (31.1-44.5)	41.4 (35.2-47.9)	55.4 (44.6-65.7)	35.8 (30.9-41)
Baramulla	34.6 (30.1-39.3)	27.4 (20-36.4)	32.4 (25.9-39.6)	44.9 (35.4-54.8)	44.4 (24-67)	39.3 (32.6-46.4)	30.4 (24.6-36.9)	36.3 (26.5-47.3)	34.2 (29.2-39.5)
Ganderbal	39.1 (34.7-43.8)	34.8 (25.8-45)	40.5 (34-47.3)	39.8 (31.6-48.7)	41.2 (21-64.8)	39.2 (32.8-46)	39.1 (33-45.5)	42.7 (33.2-52.8)	38.2 (33.2-43.4)
Kulgam	28.5 (24.4-33)	27.5 (19.7-36.9)	26.8 (21-33.5)	31 (23.1-40.1)	36.8 (18.7-59.7)	25.1 (19.2-32.2)	30.7 (25.4-36.6)	37.8 (28-48.7)	26.3 (21.9-31.2)
Kupwara	42.3 (37.5-47.2)	33.3 (24-44.2)	39.8 (32.7-47.3)	50.5 (41-59.9)	48.8 (34.4-63.4)	41.6 (34.7-48.9)	42.8 (36.3-49.5)	50 (35-65)	41.4 (36.4-46.6)
Pulwama	43.1 (38.6-47.8)	35.3 (26.7-45)	42.6 (35.5-50)	45.2 (36.8-54)	59 (43.2-73.1)	39.6 (33.4-46.1)	46.8 (40.3-53.4)	40.4 (27.5-54.9)	43.4 (38.6-48.4)
Shopiyan	31.9 (27.6-36.6)	28.6 (21.2-37.3)	29.6 (22.9-37.3)	41.1 (31.4-51.5)	30.4 (18.9-45.1)	31.1 (25-37.9)	32.7 (26.7-39.3)	38.5 (24.7-54.4)	31.3 (26.7-36.2)
Srinagar	40.7 (38.8-42.7)	39.1 (35.3-43.1)	39.2 (36.3-42.3)	41.9 (38.2-45.7)	53.5 (44.9-61.9)	37.7 (35.2-40.3)	44.6 (41.6-47.6)	40.8 (38.7-42.9)	39.9 (33.8-46.3)

Chronic disease and SARS-CoV-2 infection in the study population

Of the 6230 participants, 1145 reported a history of at least one chronic disease. Two hundred ninety-eight reported a history of more than one chronic disease.

Supplementary Table 3: Chronic disease in the study population

Chronic disease (n = 1145)	Number (%)
Hypertension	815 (13·1%)
Diabetes	314 (5·0%)
Chronic Obstructive Pulmonary Disease	39 (0·6%)
Coronary Heart Disease	35 (0·6%)
Cerebrovascular Disease	16 (0·3%)
Asthma	15 (0·2%)
Chronic Kidney Disease	10 (0·2%)
Chronic Liver Disease	5 (0·1%)
Cancer	4 (0·1%)

Supplementary Table 4: History of COVID-19 like symptoms and seropositivity vis-à-vis history of chronic disease

		History of COVID-19 like symptoms	
		Yes	**No**
Reported history of chronic disease (n=1145)	**Seropositive**	78	417
	Seronegative	63	587
Did not report any history of chronic disease (n=5085)	**Seropositive**	169	1751
	Seronegative	164	3001

Of the 1154 participants who reported a history of chronic disease 78 (6.8%) reported a history of COVID-19 like symptoms within three months of the interview date and were seropositive for SARS-CoV-2 specific IgG (symptomatic infection). The proportion of symptomatic infection among participants who did not report any history of chronic disease was 3.3% (169/5085).

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3 and Figure 1
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	3, 4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	4, 5
Study size	10	Explain how the study size was arrived at	3
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4, 5
		(*b*) Describe any methods used to examine subgroups and interactions	5
		(*c*) Explain how missing data were addressed	5
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	4, 5
		(*e*) Describe any sensitivity analyses	6, Table 3
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure 2, and page 5
		(b) Give reasons for non-participation at each stage	Figure 2
		(c) Consider use of a flow diagram	Figure 2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1, Figure 2
Outcome data	15*	Report numbers of outcome events or summary measures	Table 2

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2
		(*b*) Report category boundaries when continuous variables were categorized	Table 1, 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Table 2, Table 3
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11, 12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9, 10, 11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for exposed and unexposed groups.

BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-053791.R3
Article Type:	Original research
Date Submitted by the Author:	29-Aug-2021
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Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Epidemiology
Keywords:	COVID-19, EPIDEMIOLOGY, Epidemiology < INFECTIOUS DISEASES

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Seroprevalence of SARS-CoV-2 specific IgG antibodies in Kashmir, India, seven months after the first reported local COVID-19 case: results of a population-based seroprevalence survey from October-November, 2020

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Word Count: 3727 (excluding abstract, tables, and references)

ABSTRACT

Objectives: We designed a population-based survey in Kashmir to estimate the seroprevalence of SARS-CoV-2 specific IgG antibodies in the general population aged 18 years and above.

Setting: The survey was conducted among 110 villages and urban wards across ten districts in Kashmir from 17 Oct 2020 to 04 Nov 2020.

Participants: Individuals aged 18 years and above were eligible to be included in the survey. Serum samples were tested for the presence of SARS-CoV-2 specific IgG antibodies using the Abbott SARS-CoV-2 IgG assay.

Primary and secondary outcome measures: We labeled assay results equal to or above the cut-off index value of 1·4 as positive for SARS-CoV-2 specific IgG antibodies. Seroprevalence estimates were adjusted for the sampling design and assay characteristics.

Results: Out of 6397 eligible individuals enumerated, 6315 (98.7%) agreed to participate. The final analysis was done on 6230 participants. Seroprevalence adjusted for the sampling design and assay characteristics was 36.7% (95% CI 34.3%-39.2%). Seroprevalence was higher among the older population. Among seropositive individuals, 10.2% (247/2415) reported a history of COVID-19 like symptoms. Out of 474 symptomatic individuals, 233 (49.2%) reported having been tested. We estimated an infection fatality rate of 0.034%.

Conclusions: During the first seven months of the COVID-19 epidemic in Kashmir valley, approximately 37% of individuals were infected. The reported number of COVID-19 cases was only a small fraction of the estimated number of infections. A more efficient surveillance system with strengthened reporting of COVID-19 cases and deaths is warranted.

ARTICLE SUMMARY

Strengths and limitations of this study

- The findings of our study are based on a representative sample of the population.
- The laboratory test used for the detection of SARS-CoV-2 specific IgG antibodies in serum samples provides valid results.
- We report seroprevalence estimates adjusted for sampling design and test performance.
- Even though we adjusted the weighted seroprevalence estimates for test performance using manufacturer-provided sensitivity and specificity (100% and 99·63%, respectively), we did not quantify the test validity in-house.
- Because of lack of age- and gender-specific mortality data, we could not estimate age- and gender-specific infection fatality rates.

INTRODUCTION

On 11 Feb 2020, the World Health Organization announced that the disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) would be named coronavirus disease (COVID-19).[1] In Kashmir, the first case of COVID-19 was confirmed in Srinagar city on 18 Mar 2020.[2] The government imposed the first phase of the lockdown in Kashmir on 24 March 2020. During this phase, inter-state travel remained suspended. People were barred from moving outside except in an

emergency. Except for essential services, all government and private offices were advised to work from home. Universal masking was made mandatory. The lockdown was extended till 31 May 2020 and later relaxed in a phased manner.

Mild or asymptomatic infections are common in Coronavirus disease 2019 (COVID-19) and are an important source of infection transmission.[3,4] Such cases are less likely to be detected by a surveillance system based on reverse-transcriptase polymerase chain reaction (RT-PCR) testing. Therefore, the number of reported RT-PCR positive cases are an underestimate of the true number of infections in a population.

Seroprevalence surveys have been conducted in various countries at different stages of the current epidemic among various population groups.[5–14] Seroprevalence surveys provide a more accurate estimate of past infection, improve understanding of the infection transmission dynamics, and guide public health response.[15]

We designed this survey with the primary objective to estimate the seroprevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) specific IgG antibodies in the adult population of Kashmir valley.

METHODS

We designed a population-based cross-sectional study. The study covered all the ten districts of Kashmir, a valley in northern India. (Figure 1) We completed data collection in three weeks, from 17 Oct 2020 to 04 Nov 2020.

Ethics

We obtained written informed consent from all study participants. The study was approved by the Institutional Ethics Committee of Government Medical College Srinagar (reference number: 1004/ETH/GMC). We used anonymized participant data for analysis.

Sample size

Based on the results of a previous study conducted in July 2020, we speculated that, by October 2020, the prevalence would have increased to around 20%.[16] We calculated the minimum sample size based on an anticipated seroprevalence of 20%, an absolute precision of 2%, and a design effect of 2. We used OpenEpi to make sample size calculations.[17] We adjusted the sample size for a possible non-response of 10% to obtain a minimum size of 3376. We decided to select 3600 individuals from nine of the ten districts (except district Srinagar). To obtain precise estimates for district Srinagar, sample size estimation was made for the district separately. We used a design effect of 1.5, an anticipated seroprevalence of 20%, and absolute precision of 2% to obtain a sample size of 2302 for the district, further increasing to 2400 to account for non-response. We thus targeted a total sample size of 6000 (3600 + 2400).

Participants

All adults ≥18 years of age were eligible to participate in the study. We selected eligible participants using a three-stage stratified cluster sampling technique. We listed all clusters in the valley using the Census 2011 data.[18] Within each of the ten districts in the valley, clusters were stratified into urban

and rural clusters. We selected clusters within each of the 20 strata by probability proportionate to size (PPS) sampling. Except for district Srinagar which we oversampled to obtain precise seroprevalence estimates for, ten clusters were randomly selected from each district. We selected 20 clusters from district Srinagar. We divided each selected cluster into four equal areas and chose a central location within each of the four areas as the starting point. Thereafter, we approached consecutive households to enroll at least ten eligible participants. We thus identified a total of 440 random locations within 110 clusters in ten districts. We invited all eligible adults in a household for participation.

Variables

The primary outcome variable of interest was SARS-CoV-2 specific IgG antibodies. In addition, we obtained information from participants about their age, gender, history of COVID-19 like symptoms in the three months before the interview date, history of contact with a known COVID-19 patient, and history of COVID-19 testing.

Procedure

We informed eligible adults about the purpose and the procedure of the study. Study participation was voluntary. Participants were interviewed by health personnel specifically trained for the interview. Interview responses were recorded in an Epicollect5 form.[19] Once the interview was completed, a trained phlebotomist collected 3-5 mL of venous blood from the antecubital vein under aseptic precautions into a red-top collection tube containing a clot activator. The tube was left standing, undisturbed, for at least 30 minutes for clot formation. The sample was later transported to a central facility for centrifugation. Centrifuged samples were transported to a central laboratory for further processing and analysis. Serum samples were tested for the presence of SARS-CoV-2 specific IgG antibodies using the Abbott SARS-CoV-2 IgG assay. The assay uses chemiluminescence to detect IgG antibodies against the SARS-CoV-2 nucleocapsid protein. The reported sensitivity and specificity of the assay are 100% (95% CI 95.89-100.00)and 99·63% (99.05-99.90), respectively.[20] As recommended by the manufacturer, we labeled assay results equal to or above the cut-off index value of 1·4 as positive for SARS-CoV-2 specific IgG antibodies.

Statistical methods

We report unweighted seroprevalence estimates in percentages. We used the Agresti-Coull procedure to calculate a 95% confidence interval (CI) for seroprevalence estimates.[21] A weighted estimate of seroprevalence is provided. To calculate survey weights (inverse of sampling probability), we used the estimated population of the districts. We used the census 2011 data and growth rates from Sample Registration System to estimate the population of the districts in 2020.[18,22] Survey weights obtained were further adjusted for non-response and age and sex structure (post-stratification weights). We further adjusted the weighted seroprevalence estimates for test performance to calculate "weighted seroprevalence adjusted for test performance". We did this using the formula:

$$Weighted\ seroprevalence\ adjusted\ for\ test\ performance = (Weighted\ seroprevalence + Test\ specificity - 1)/(Test\ sensitivity + Test\ specificity - 1).$$[23]

We used the manufacturer-provided sensitivity and specificity in the above formula.[20] We used the extremes of the manufacturer-provided 95% CI of the test sensitivity and specificity (upper limit of

sensitivity, lower limit of specificity; and lower limit of sensitivity, upper limit of specificity) to report sensitivity analyses.

We analyzed the difference in seroprevalence estimates across levels of a categorical variable using a Chi-square test adjusted for the sampling design.

We estimated the number of SARS-CoV-2 infections by multiplying the weighted seroprevalence adjusted for test performance with the estimated population of the valley. To estimate the number of infections per reported case, we divided the estimated number of SARS-CoV-2 infections by the reported number of COVID-19 cases two weeks before the survey date. We calculated the infection fatality rate by dividing the reported number of deaths by the number of estimated infections, assuming a three-week lag time from infection to death.[24]

We analyzed the data using Stata version 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LP.).

Patient and Public Involvement

No patients or members of the public were involved in this study.

RESULTS

We enumerated 6 397 individuals ≥18 years from 3 077 households and 110 clusters (34 urban clusters and 76 rural clusters) between 17 Oct 2020 and 04 Nov 2020. Of the 6 397 eligible individuals, 6 315 (98·7%) agreed to participate and were enrolled. The final analysis was done on a sample of 6 230 participants. (Figure 2)

Of the 6 230 participants, 1 513 (24·3%) were between 18 and 30 years of age, 2 672 (42·9%) were aged 30-49 years, 1 643 (26·4%) were aged 50-69 years, and 402 (6·4%) were 70 years and older. (Table 1). There was equal representation from males and females, and 3 364 (54·0%) resided in a rural area. Of the 3 104 females, 56 (1·8%) reported being pregnant at the time of the survey. Four hundred seventy-four (7·6%) reported COVID-19 like symptoms in the three months preceding the survey, and 439 (7·0%) reported to have ever come in contact with a known COVID-19 case. One thousand ninety-two (17·5%) reported having been tested for COVID-19 using RT-PCR or a Rapid Antigen Test (RAT) previously, of whom 176 (16·2%) reported to have tested positive for the disease.

Table 1: Characteristics of study participants

	Frequency	Percent
Total	6230	
Age, years		
18-29	1513	24·3
30-49	2672	42·9
50-69	1643	26·4
≥70	402	6·5
Gender		
Male	3126	50·2
Female	3104	49·8

Residence		
Urban	2866	46·0
Rural	3364	54·0
Pregnant (n=3104)	56	1·8
Self-reported history of chronic disease	1145	18.4
History of COVID-19 like symptoms	474	7·6
History of contact with a known COVID-19 case	439	7·0
Ever tested for COVID-19 (RT-PCR)	1092	17·5
RT-PCR result (n=1088*)		
Positive	176	16·2
Negative	912	83·8

COVID-19: Coronavirus disease; RT-PCR: Reverse transcriptase-polymerase chain reaction
*RT-PCR result not known in four participants

We found an overall unweighted seroprevalence of 38·8% (95% CI 37·6 – 40·0). The seroprevalence ranged from 28·5% in district Kulgam to 43·1% in district Pulwama. (Figure 1 and online supplemental file 1) The overall weighted seroprevalence (adjusted for sampling design) was 36·9% (95% CI 34·5 – 39·4). The weighted seroprevalence adjusted for test performance was 36·7% (95% CI 34·3 – 39·2). (Table 2) Upon sensitivity analyses, the weighted seroprevalence adjusted for test performance ranged from 36·3% (95% CI 33·9 – 38·8) to 38·4% (95% CI 35·9 – 41·0). (Table 3)

Table 2: Seroprevalence of SARS-CoV-2 specific IgG antibodies by participant characteristics

	Number tested	Number seropositive	Unweighted seroprevalence, % (95% CI)	Weighted seroprevalence, % (95% CI)	Weighted seroprevalence adjusted for test performance, % (95% CI)	Design-based F, p-value
Total	6230	2415	38·8 (37·6-40·0)	36·9 (34·5-39·4)	36·7 (34·3-39·2)	
Age, years						
18-29	1513	538	35·6 (33·2-38·0)	33·7 (30·1-37·6)	33·5 (29·8-37·4)	6·42, 0·0006
30-49	2672	1000	37·4 (35·6-39·3)	36·3 (33·5-39·3)	36·1 (33·3-39·1)	
50-69	1643	691	42·1 (39·7-44·5)	42·5 (38·8-46·2)	42·3 (38·6-46·0)	
≥70	402	186	46·3 (41·5-51·2)	45·3 (37·8-53·0)	45·1 (37·6-52·8)	
Gender						
Male	3126	1166	37·3 (35·6-39·0)	36·1 (33·5-38·9)	35·9 (33·3-38·7)	0·94, 0·34
Female	3104	1249	40·2 (38·5-42·0)	37·8 (34·5-41·3)	37·6 (34·3-41·1)	
Residence						
Urban	2866	1180	41·2 (39·4-43·0)	40·2 (36·3-44·1)	40·0 (36·1-43·9)	3·43, 0·07

Rural	3364	1235	36·7 (35·1-38·4)	35·5 (32·5-38·7)	35·3 (32·2-38·5)	
Self-reported history of chronic disease						
Yes	1145	495	43·2 (40·4-46·1)	41·9 (37·4-46·6)	41·7 (37·2-46·4)	6.14, 0.02
No	5085	1920	37·8 (36·4-39·1)	36·2 (33·7-38·9)	36·0 (33·5-38·7)	
History of COVID-19 like symptoms						
Yes	474	247	52·1 (47·6-56·6)	47·4 (37·9-57·1)	47·2 (37·7-56·9)	5·53, 0·02
No	5756	2168	37·7 (36·4-38·9)	36·3 (33·9-38·8)	36·1 (33·7-38·6)	
History of contact with a known COVID-19 case						
Yes	439	219	49·9 (45·2-54·5)	45·2 (38·3-52·2)	45·0 (38·1-52·0)	7·13, 0·01
No	5791	2196	37·9 (36·7-39·2)	36·5 (34·1-39·0)	36·3 (33·9-38·8)	
Ever tested for COVID-19 (RT-PCR)						
Yes	1092	485	44·4 (41·5-47·4)	41·0 (35·4-46·9)	40·8 (35·2-46·7)	2·17, 0·14
No	5138	1930	37·6 (36·2-38·9)	36·2 (33·5-39·0)	36·0 (33·3-38·8)	
RT-PCR result (n=1088*)						
Positive	176	140	79·5 (73·0-84·9)	81·8 (74·8-87·1)	81·7 (74·7-87·1)	74·93, <0·0001
Negative	912	345	37·8 (34·7-41·0)	38·8 (33·3-44·7)	38·6 (33·1-44·5)	

COVID-19: Coronavirus disease; RT-PCR: Reverse transcriptase-polymerase chain reaction
*RT-PCR result not known in four participants

Table 3: Sensitivity analyses for seroprevalence at extremes of test performance

	Weighted seroprevalence, % (95% CI)	**Weighted seroprevalence adjusted for test performance, % (95% CI)** ***[Sensitivity 100·00%, Specificity 99·63%]***	**Weighted seroprevalence adjusted for test performance, % (95% CI)** ***[Sensitivity 95·89%, Specificity 99·90%]***	**Weighted seroprevalence adjusted for test performance, % (95% CI)** ***[Sensitivity 100·00%, Specificity 99·05%]***
Overall	36·9 (34·5-39·4)	36·7 (34·3-39·2)	38·4 (35·9-41.0)	36·3 (33·9-38·8)
Age, years				
18-29	33·7 (30·1-37·6)	33·5 (29·8-37·4)	35·1 (31·3-39·1)	33·1 (29·4-37·0)

30-49	36·3 (33·5-39·3)	36·1 (33·3-39·1)	37·8 (34·9-40·9)	35·7 (32·9-38·7)
50-69	42·5 938·8-46·2)	42·3 (38·6-46·0)	44·3 (40·4-48·1)	41·9 (38·2-45·7)
≥70	45·3 937·8-53·0)	45·1 (37·6-52·8)	47·2 (39·4-55·2)	44·8 (37·2-52·5)
Gender				
Male	36·1 (33·5-38·9)	35·9 (33·3-38·7)	37·6 (34·9-40·5)	35·5 (32·9-38·3)
Female	37·8 (34·5-41·30	37·6 (34·3-41·1)	39·4 (35·9-43.0)	37·2 (33·9-40·7)
Residence				
Urban	40·2 (36·3-44·1)	40·0 (36·1-43·9)	41·9 (37·8-45·9)	39·6 (35·7-43·6)
Rural	35·5 (32·5-38·7)	35·3 (32·2-38·5)	37·0 (33·8-40·3)	34·9 (31·9-38·1)
Self-reported history of chronic disease				
Yes	43·2 (40·4-46·1)	41·9 (37·4-46·6)	43·6 (38·9-48·5)	41·3 (36·8-46·1)
No	37·8 (36·4-39·1)	36·2 (33·7-38·9)	37·7 (35·1-40·5)	35·6 (33·1-38·3)
History of COVID-19 like symptoms				
Yes	52·1 (47·6-56·6)	47·4 (37·9-57·1)	49·4 (39·5-59·5)	46·9 (37·3-56·7)
No	37·7 (36·4-38·9)	36·3 (33·9-38·8)	37·8 (35·3-40·4)	35·7 (33·3-38·2)
History of contact with a known COVID-19 case				
Yes	49·9 (45·2-54·5)	45·2 (38·3-52·2)	47·1 (39·9-54·4)	44·7 (37·7-51·7)
No	37·9 (36·7-39·2)	36·5 (34·1-39·0)	38·0 (35·5-40·6)	35·9 (33·5-38·4)
Ever tested for COVID-19 (RT-PCR)				
Yes	44·4 (41·5-47·4)	41·0 (35·4-46·9)	42·7 (36·9-48·9)	40·4 (34·8-46·4)
No	37·6 (36·2-38·9)	36·2 (33·5-39·0)	37·7 (34·9-40·6)	35·6 (32·9-38·4)
RT-PCR result (n=1088*)				
Positive	79·5 (73·0-84·9)	81·8 (74·8-87·1)	85·3 (78·0-90·8)	81·6 (74·6-87·0)
Negative	37·8 (34·7-41·0)	38·8 (33·3-44·7)	40·4 (34·7-46·6)	38·2 (32·7-44·2)

Seroprevalence was lowest among participants aged 18-29 years [33·5% (95% CI 29·8 – 37·4)] and was higher in older age groups. Seroprevalence was highest in those aged 70 years and above [45·1% (95% CI 37·6 – 52·8)]. Seroprevalence was not significantly different among males and females (p=0·34). The seroprevalence among urban residents was 40·0% (95% CI 36·1 – 43·9), slightly but not significantly, higher than rural residents [35·3% (95% CI 32·2 – 38·5), p=0·07]. (Table 2)

One in five participants (1145/6230, 18·4%) self-reported history of at least one chronic disease (Table 1). Hypertension (815/6230, 13.1%) and diabetes mellitus (314/6230, 5.0%) were the most commonly reported chronic diseases (online supplemental file 2). Seroprevalence was significantly higher in participants who self-reported history of chronic disease (41·7%, 95% CI 37·2 – 46·4) as compared to those who did not report a history of chronic disease (36·0%, 95% CI 33·5 - 38·7) (Table 2).

Among participants who reported a history of COVID-19 like symptoms, seroprevalence was 47·2% (95% CI 37·7 – 56·9) compared with 36·1% (95% CI 33·7 – 38·6) among participants who did not report such symptoms. Seroprevalence was higher among those who reported contact with a known COVID-19 case [45·0% (95% CI 38·1 – 52·0)] than participants who did not report any history of such contact [36·3% (95% CI 33·9 – 38·8)]. (Table 2)

Seroprevalence was not significantly related to being tested for COVID-19 (RT-PCR). However, those who reported a positive RT-PCR COVID-19 test had significantly higher seroprevalence (81·7%, 95% CI 74·7 – 87·1) as compared to those who reported a negative RT-PCR COVID-19 test (38·6%, 95% CI 33·1 – 44·5). (Table 2)

Among 2 415 seropositive individuals, only 247 (10·2%) reported a history of COVID-19 like symptoms. Only 20.1% (485/2415) of the seropositive individuals were tested for COVID-19 (RT-PCR). Among 474 who reported a history of COVID-19 like symptoms, 233 (49·2%) were tested for COVID-19 (RT-PCR). Among 4897 individuals who did not report a history of COVID-19 like symptoms and were never tested for COVID-19 (RT-PCR), 1825 (37.3%) were seropositive. (Figure 3)

Among 36 participants who reported a positive RT-PCR COVID-19 test but were seronegative, the duration between COVID-19 RT-PCR test and serological testing ranged from 9 days to 101 days. In only four of these 36 participants, the duration between the COVID-19 RT-PCR test and the serological test was 14 days or less. Of the remaining 32 participants, 21 did not report a history of CVOID-19 like symptoms, nine did not report a history of contact with a known COVID-19 case, and eight reported neither a history of COVID-19 like symptoms nor a history of contact with a known COVID-19 case.

We estimated that there were 1 673 484 (95% CI 1 564 047 – 1 787 482) cumulative number of infections among adults aged ≥18 years in the valley by 03 Oct 2020, two weeks before the start of the survey. If we assume that the seroprevalence was similar to the overall seroprevalence in the population not included in our study (<18 years of age) then the estimated cumulative number of infections in the valley by 03 Oct 2020 was 2 791 933 (95% CI 2 609 354 – 2 982 119). Considering that the cumulative number of reported COVID-19 cases was 47 071 by 03 Oct 2020 (Figure 4), we estimate the number of infections per reported case as 59·3 (95% CI 55·4 – 63·4). The number of reported COVID-19 deaths after a three-week lag period (on 24 Oct 2020) was 955. Thus, we estimated the infection fatality rate as 0.034% (95% CI 0.032 – 0.037).

Figure 5 depicts the relationship between the estimated number of SARS-CoV-2 infected persons, reported COVID-19 cases, and reported COVID-19 deaths. Of the total estimated SARS-CoV-2 infected persons, only 1.69% were reported. Of the total reported COVID-19 cases, 2.03% died.

DISCUSSION

We report the results of a seroprevalence survey conducted in Kashmir from October-November 2020, seven months after the appearance of the first local COVID-19 case. The COVID-19 pandemic is rapidly evolving worldwide. In Kashmir, several important events happened since we completed our survey. From 16 Jan 2021, COVID-19 vaccination was introduced in a phased manner. Healthcare workers were given preference during the first phase. From 01 Mar 2021, the vaccine was made available for people ≥60 years of age and those with chronic diseases in the age group of 45-59 years. However, especially during the early phases of the COVID-19 vaccination campaign, many people were hesitant to receive

the vaccine doses. During the same time, SARS-CoV-2 Variants of Concern began to emerge and circulate. The daily number of COVID-19 cases started to rise again. The 'second wave' in April 2021 was more explosive than the 'first wave' at the beginning of the pandemic. The fear of the disease had diminished, and COVID appropriate behaviour was no more a norm. The government and the people were caught unawares. There were several reports of a possible 'second infection' and reports of cases among previously vaccinated individuals. Given these developments, the current seroprevalence in Kashmir will be higher than what we report in this study.

The results of our study indicate that by the first week of October 2020, nearly seven months after the appearance of the first laboratory-confirmed COVID-19 case on 18 March 2020, close to 37% of the valley's population aged ≥18 years had been infected. Our results suggest that the cumulative number of SARS-CoV-2 infections by the first week of October 2020 was nearly 2·8 million, with an estimated infection fatality rate of 0.034%. Seroprevalence did not differ by gender but was higher in older age groups.

The findings of our study are based on a representative sample of the population. The laboratory test used for the detection of SARS-CoV-2 specific IgG antibodies in serum samples provides valid results.[20,25] We report seroprevalence estimates adjusted for sampling design and test performance.

The overall adjusted seroprevalence of around 37% indicates that, by October 2020, a large proportion of the valley's population had been infected with the virus. Easing of lockdown, being fed up with the restrictions, and non-adherence to prevention norms are the possible reasons. Using several assumptions about the test sensitivity and specificity to calculate adjusted seroprevalence estimates yielded small differences.

Several factors potentially influence the seroprevalence rates. These include population density, social and demographic structure of the population, governmental policies and the extent of their implementation, immunity level of the population, time since the start of infection transmission, adherence to infection prevention guidelines, quality of contact tracing and quarantine, and possibly the geography and environment of an area. The emergence of several Variants of Concern and the introduction of COVID-19 vaccination will also influence population immunity. Herd immunity in the context of COVID-19 is a matter of debate as reports of a second infection continue to pour in.[26]

Comparison with previous reports suggests that, by October 2020, the seroprevalence had increased almost ten-fold since July 2020.[16,27] The second of the three nationwide seroprevalence surveys in India conducted in August-September 2020 reports an overall seroprevalence of 6.6%, ranging from 5.2% in rural areas to 16.9% in urban slums.[28] A nationwide survey conducted in December 2020-January 2021 reported an overall seroprevalence of 24.1% ranging from 4.9% - 44.4% across districts.[29] Kashmir is thus not a low-infection area. Being an oft-visited tourist area, Kashmir is at an increased risk of infection transmission. Adherence to COVID appropriate behavior (use of face masks in public, frequent handwashing, physical and social distancing) has been poor. The experience of a 'second wave' of COVID-19 in April-June 2021, the appearance of virus variants, and the introduction of vaccination programs warrant robust surveillance of the epidemic.

The seroprevalence of SARS-CoV-2 IgG antibodies was higher in older age groups. During the early period of the pandemic, people were adherent to social distancing and other non-pharmaceutical interventions because of a fear of the disease and administrative restrictions. With time, administrative

restrictions were relaxed, fear of the disease attenuated, and people became fed up with the social restrictions. This led to an increase in the number of reported COVID-19 cases and provided the population, including older age groups, an opportunity to contract the infection. That older people have an increased risk of symptomatic and more severe disease is now well known.[30,31] However, age-based differential susceptibility to SARS-CoV-2 infection antibody response and the reasons thereof are still a grey area and need further understanding. Existing literature might suggest that the more mobile and socially active young have a higher risk of infection.[6,7] However, this should not imply that the elderly have a decreased susceptibility to SARS-CoV-2 infection or a decreased antibody response.[32] On the contrary, several studies suggest that the seroprevalence of SARS-CoV-2 specific IgG antibodies is higher in the older age groups and particularly so in more dense population groups.[4,5,8–11,13] Furthermore, SARS-CoV-2 antibody levels have been reported to be higher in older people.[12]

The seroprevalence of SARS-CoV-2 specific IgG antibodies did not differ significantly by gender, though the figure was slightly higher for females. These findings are consistent with the available literature.[6,13] Difference in seroprevalence by gender has been suggested by some studies, and females have been reported to have lower antibody levels.[5,7,9,11,12,14,33]

Urban areas are more densely populated than rural areas, accelerating the transmission of infections in the population. The seroprevalence of SARS-CoV-2 specific IgG antibodies is thus expected to be higher in urban areas, especially during the early phases of an epidemic. However, as the epidemic progresses, the seroprevalence gap between urban and rural areas will wane off. We estimated an adjusted seroprevalence of 40·0% (95% CI 36·1 – 43·9) in urban areas as compared to 35·3% (95% CI 32·2 – 38·5) in rural areas (p=0·07).

People with a chronic disease experience more severe COVID-19 symptoms and are more likely to die when compared to people with no chronic disease.[34] We found a higher proportion of symptomatic infection among participants with a self-reported history of chronic disease (78/1145, 6·8%) as compared to participants with no chronic disease (169/5085, 3·3%) (online supplemental file 3). Little is, however, known about the risk of infection in chronic disease patients. We found a significantly higher seroprevalence among participants with a self-reported history of chronic disease (Table 2). This finding needs further research for corroboration and possible explanations.

People with a self-reported history of COVID-19 related symptoms, contact with a known COVID-19 case, or a positive COVID-19 RT-PCR had a higher seroprevalence as compared to their complement. Among seropositive individuals, only 10·2% reported being symptomatic. The percentage of asymptomatic infections, thus, was 90%. However, only 49% of individuals with a history of COVID-19 like symptoms were tested using RT-PCR. We also estimated that only one out of almost 59 infections gets reported. This reflects the necessity of improving the efficiency of RT-PCR testing so that more symptomatic individuals receive the test. Not all individuals with a known RT-PCR positive result showed the presence of IgG antibodies. Around 20% of RT-PCR positive individuals were seronegative, and in a large majority of them (32 out of 36), the duration since RT-PCR positivity was more than two weeks. This may be attributed to a poor B cell response or a false negative antibody test.[35] Around 38% of RT-PCR negative individuals were seropositive, suggesting a false-negative RT-PCR or infection acquisition at a date later than the RT-PCR test.

We estimated an infection fatality rate of 0.034% (95% CI 0.032 – 0.037). The infection fatality rate in SARS-CoV-2 infection has been reported to range from as low as 0.00% to 1.63%.[36] Our estimates of

the infection fatality rate are low as compared to estimates from several Indian studies.[5,28,37] Under-reporting COVID-19 deaths because of the non-uniform definition for a 'COVID-19 death' may falsely lower the infection fatality rates.[38] Many other factors can influence the infection fatality rate in SARS-CoV-2 infection – the quality of available health facilities, the age structure of the population, and COVID-19 epidemic intensity.[39,40] Developing countries usually have a younger population as compared to the developed countries, and Kashmir is not an exception. However, because of the possibility of under-reporting of COVID-19 deaths, the true infection fatality rate in Kashmir may be higher than our estimates. The infection fatality rate is, however, known to be lower in developing nations.[30,41] In developed nations like the United States and many European countries, a higher infection fatality rate has been reported.[30,42]

Limitations

One important limitation of our study is that even though we adjusted the weighted seroprevalence estimates for test performance using manufacturer-provided sensitivity and specificity (100% and 99·63% respectively), we did not quantify the test validity in-house. Another limitation of our study estimates is that we excluded people <18 years of age. The results of our study may not thus be generalizable to this group of the population.

Our estimated seroprevalence was much higher than we anticipated at the designing stage. This has impacted the precision of our estimates to some extent. However, we believe we still have been able to estimate the seroprevalence with reasonable precision.

Lack of reliable death counts is another potential limitation. This may have led to an underestimation of the infection fatality rate. We did not perform any adjustment for death counts. Further, because of lack of age- and gender-specific mortality data, we could not estimate age- and gender-specific infection fatality rates.

CONCLUSIONS

We conclude that nearly 37% of individuals aged 18 years and above were infected with SARS-CoV-2 in Kashmir by October 2020. The infection fatality rate in the valley is around 0.034%. A majority of cases go unreported. For every reported case, there are 59 unreported infections in the population. Since almost half of the symptomatic individuals go unreported, testing of symptomatic individuals and effective contact tracing needs to continue. Given the emergence of mutant Variants of Concern, increasing the population immunity through augmented and sustained vaccination is necessary. We further recommend that adherence to COVID-19 prevention measures should be ensured until a large proportion of the population gets vaccinated.

ACKNOWLEDGMENTS

We thank Prof. Samia Rashid for her support. We acknowledge the help from Divisional COVID-19 Control Room Kashmir, especially from Dr. Rauf Hussain Rather. We thank the following coordinating center teams and the laboratory support team for their support and coordination:

Coordinating center teams: Dr. Sabira Aalia Dkhar, Dr. Kouser Sideeq, Dr. Uruj Qureshi, Dr. Mehraj u Din Sofi, Dr. Yasmin Kango, Dr. Suhail Manzoor Shah, Dr. Bashir Ahmad Khan, Dr. Nabeel Ishtiyaq, Dr. Tajamul Hussain Khan, Dr. Lahoot Hassan Khan, Dr. Muzaffer Ahmad, Dr. Ramesh Kumar Malla, Dr.

Ruqaya Rasool Kapta, Dr. Mohammad Yousuf Naikoo, Dr. Khalid Abbass, Dr. Zarar Ahmad Bhat, Dr. Sumair Rashid, Dr. Zubair Ahmad, Dr. Shalender Singh Bali, Dr. Abdul Rouf, Dr. Abdul Hamid, Dr. Khalid Bashir, Dr. Qazi Najeeb Ahmad, Dr. Syed Irtiza, Dr. P Sharik Shaheen Khaki, Dr. Sheikh Mohammad Shafi, Dr. Mohammad Yousuf Zagoo, Dr. Mukhtar Ahmad Shah, Dr. Imtiyaz-ur-Rehman Bhat, Dr. Showkat Parray, Dr. Fazil Ali Kochak, Dr. Muzaffar Ahmad Zargar, Dr. Abdul Rashid Shah, Dr. Shagufta Saleem, Dr. Abdul Gani, Dr. Nighat Nasreen, Dr. Asima Nazeer, Dr. Zahoor Ahmad Lone.

Laboratory support team: Ishtiyaq Ahmad Handoo, Rahiba Makhdoomi, Iyman Wani, Romana Rasool, Asiya Rehman, Insha Rehman, Zahoor Ahmad, Gulzar Ahmad Wani, Javid Ahmad Bhat, M Saleem Malik, Naveed Manzoor, Mubeena.

COMPETING INTERESTS

We declare no competing interests, financial or otherwise.

FUNDING

The National Health Mission Jammu & Kashmir funded the study. The funding agency was not involved in study designing, implementation, analysis, or interpretation of the study findings.

CONTRIBUTORS

S Muhammad Salim Khan: Conceptualization, Funding acquisition, Methodology, Writing-review & editing.

Mariya Amin Qurieshi, Inaamul Haq, Sabhiya Majid: Formal analysis, Data curation, Methodology, Project administration, Writing-original draft, Writing-review & editing.

Javid Ahmad, Taha Ayub, Anjum Bashir, Ashfaq Ahmad Bhat, Abdul Majeed Ganai, Yasmeen Jan, Rauf-ur-Rashid Kaul, Zahid Ali Khan, Muneer Ahmad Masoodi, Beenish Mushtaq, Fouzia Nazir, Muzamil Nazir, Malik Waseem Raja, Mahbooba Rasool: Project administration, Supervision, Investigation, Writing-review & editing.

Anjum Asma, Munazza Aziz, Shifana Ayoub, Arif Akbar Bhat, Iqra Nisar Chowdri, Shaista Ismail, Misbah Ferooz Kawoosa, Mehvish Afzal Khan, Mosin Saleem Khan, Rafiya Kousar, Ab Aziz Lone, Shahroz Nabi, Mohammad Obaid, Tanzeela Bashir Qazi, Iram Sabah, Ishtiyaq Ahmad Sumji: Supervision, Investigation, Writing-review & editing.

S Muhammad Salim Khan, Mariya Amin Qurieshi, Inaamul Haq, and Sabhiya Majid have verified the underlying data.

DATA SHARING

Anonymized data collected for the study, including individual participant data and a data dictionary defining each field in the set, will be made available to interested researchers on request by Inaamul Haq (haqinaam@yahoo.co.in) for two years from publication. The study protocol, statistical analysis plan, and informed consent forms are also available from Inaamul Haq.

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Figure 1 legend:

Figure 1: Location of the ten districts and seroprevalence (%) by district. Figures in parentheses indicate a 95% Confidence Interval for seroprevalence.

Figure 2 legend:

Figure 2: Participant flow.

Figure 3 legend:

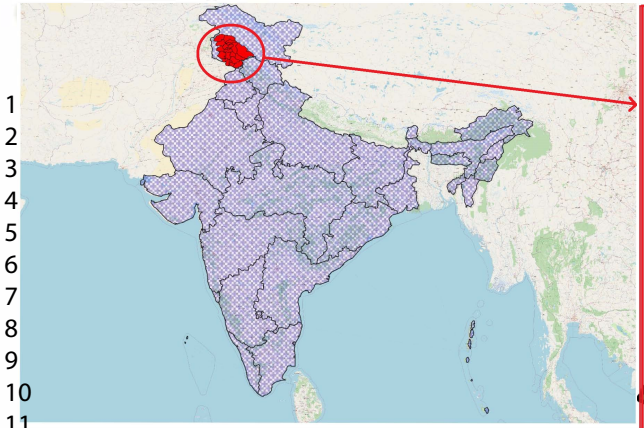
Figure 3: A. Seropositivity by the history of COVID-19 like symptoms, RT-PCR testing, and test result. B. History of COVID-19 like symptoms by seropositivity, RT-PCR testing, and test result.

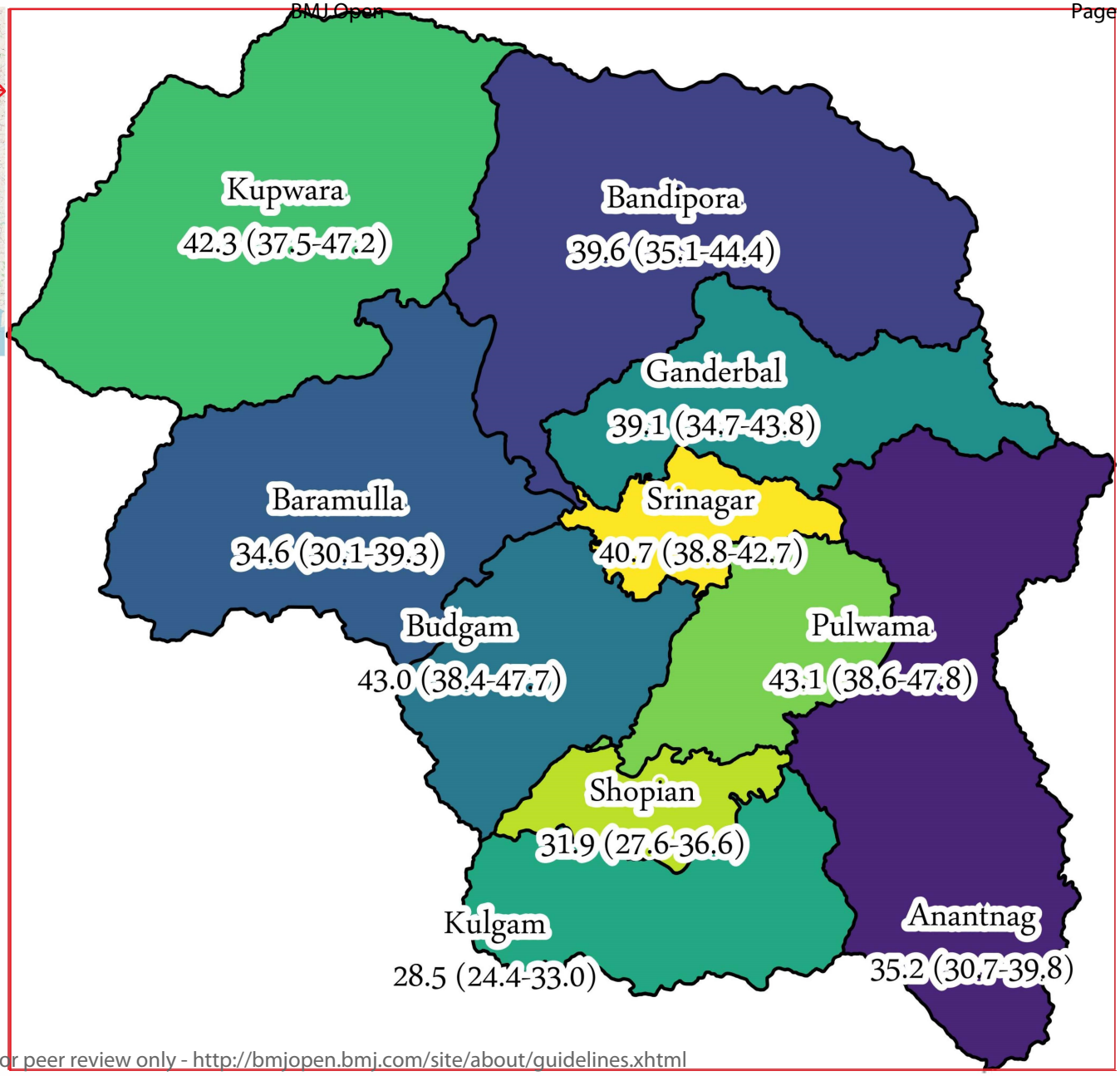
Figure 4 legend:

Figure 4: A. Daily cases and deaths in Kashmir since the start of the COVID-19 pandemic; B. Cumulative number of cases and deaths in Kashmir.

Figure 5 legend:

Figure 5: Cumulative estimated SARS-CoV-2 infections, reported cases, and deaths in Kashmir, October-November 2020. The bars represent the number of persons at each step. The percentages above the bars represent the percentage out of the total population. The percentages within the triangles represent percentages out of the previous step who proceed to the next step. (Adapted from Holtgrave DR, Barranco MA, Tesoriero JM, Blog DS, Rosenberg ES. Assessing racial and ethnic disparities using a COVID-19 outcomes continuum for New York State. Ann Epidemiol. 2020 Aug;48:9-14. doi: 10.1016/j.annepidem.2020.06.010. Copyright 2020 Elsevier Inc.).

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Bandipora
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Ganderbal
39.1 (34.7-43.8)
Baramulla
34.6 (30.1-39.3)
Srinagar
40.7 (38.8-42.7)
Budgam
43.0 (38.4-47.7)
Pulwama
43.1 (38.6-47.8)
Shopian
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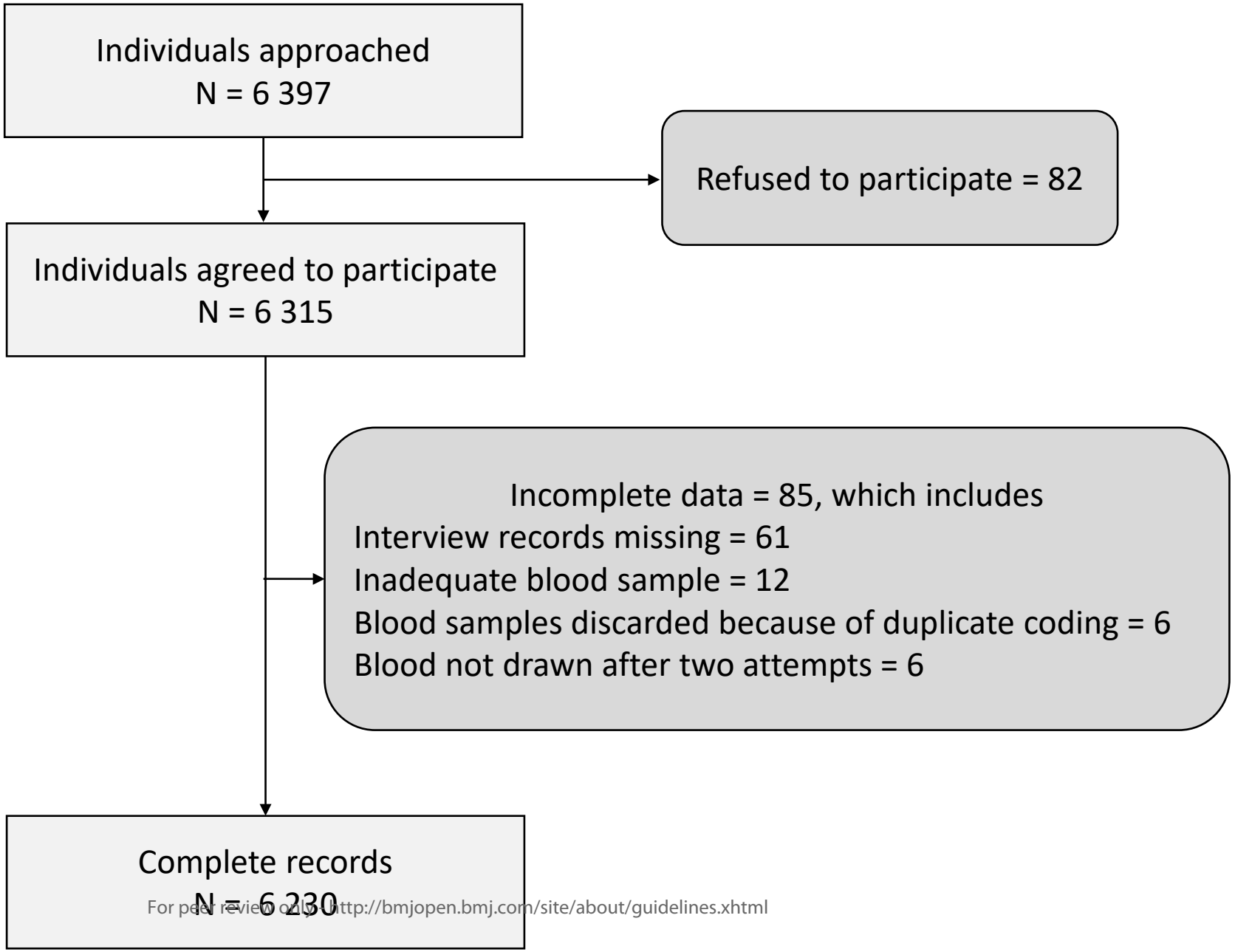
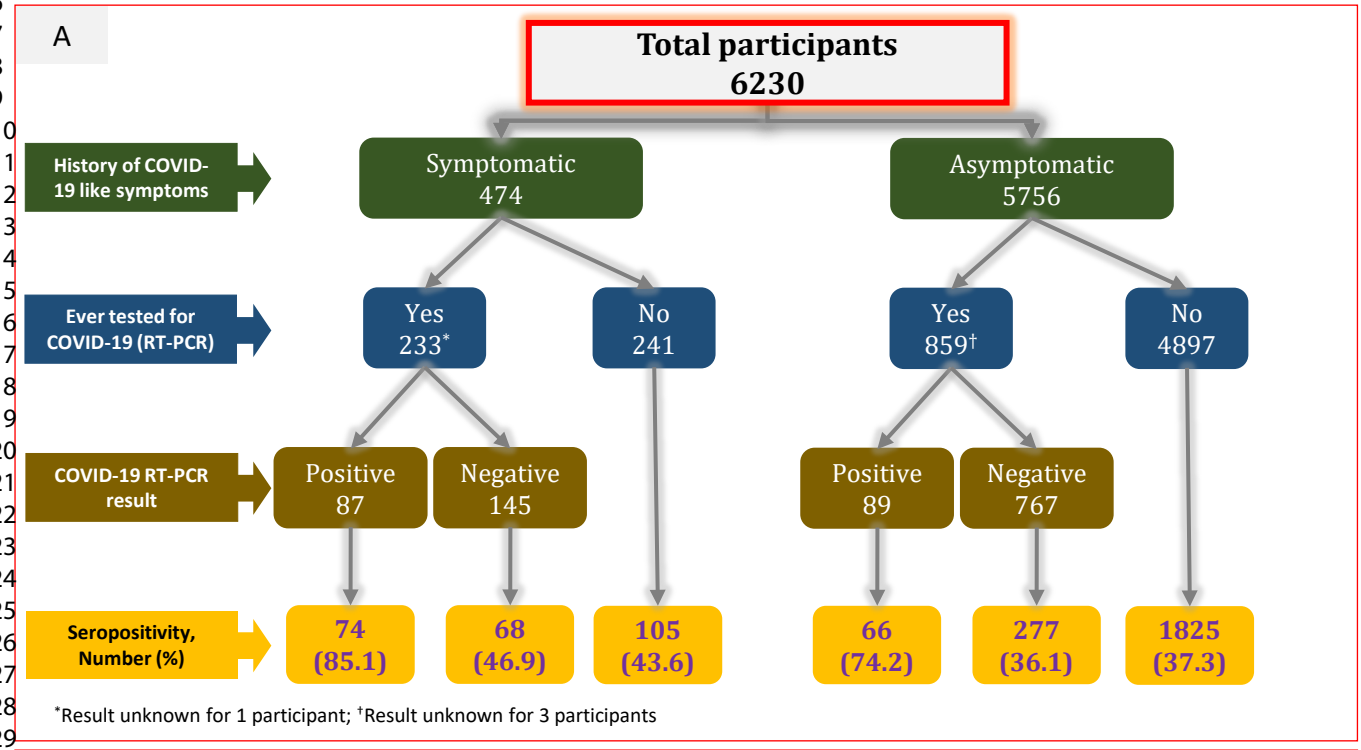
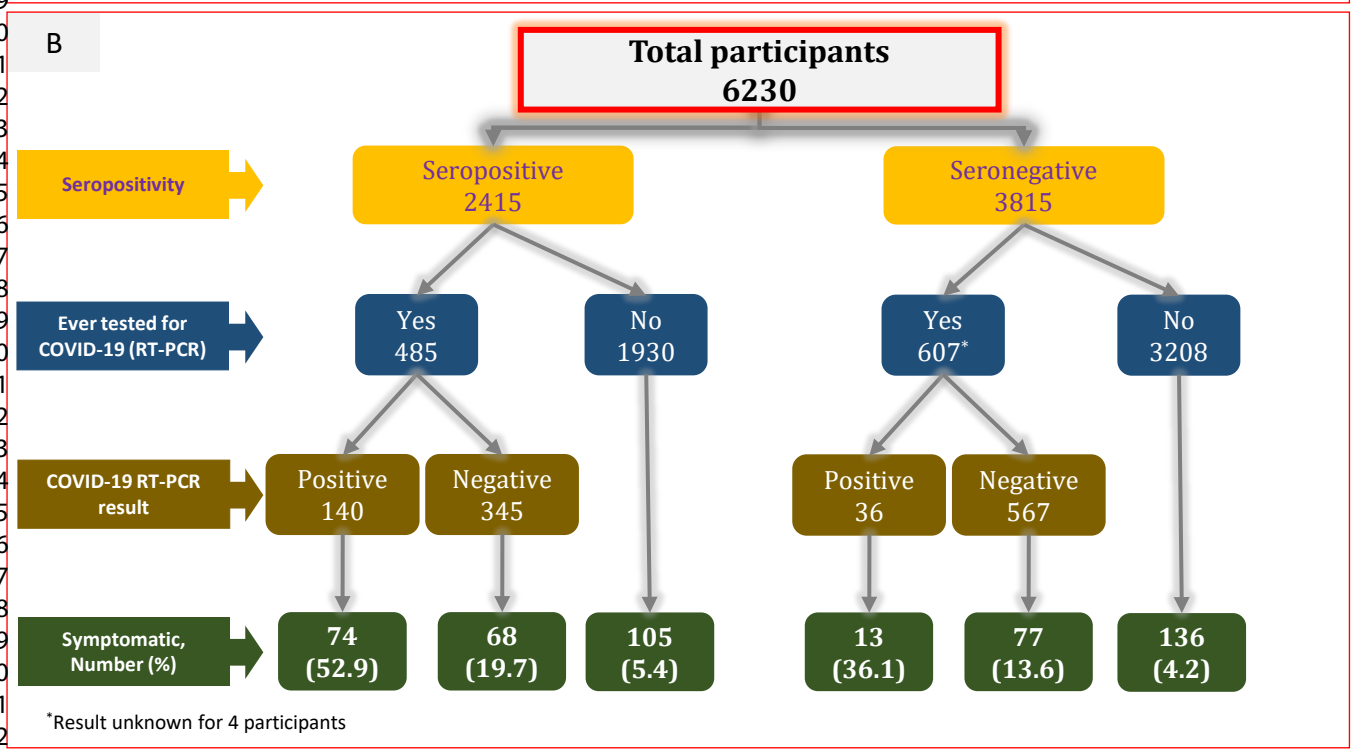
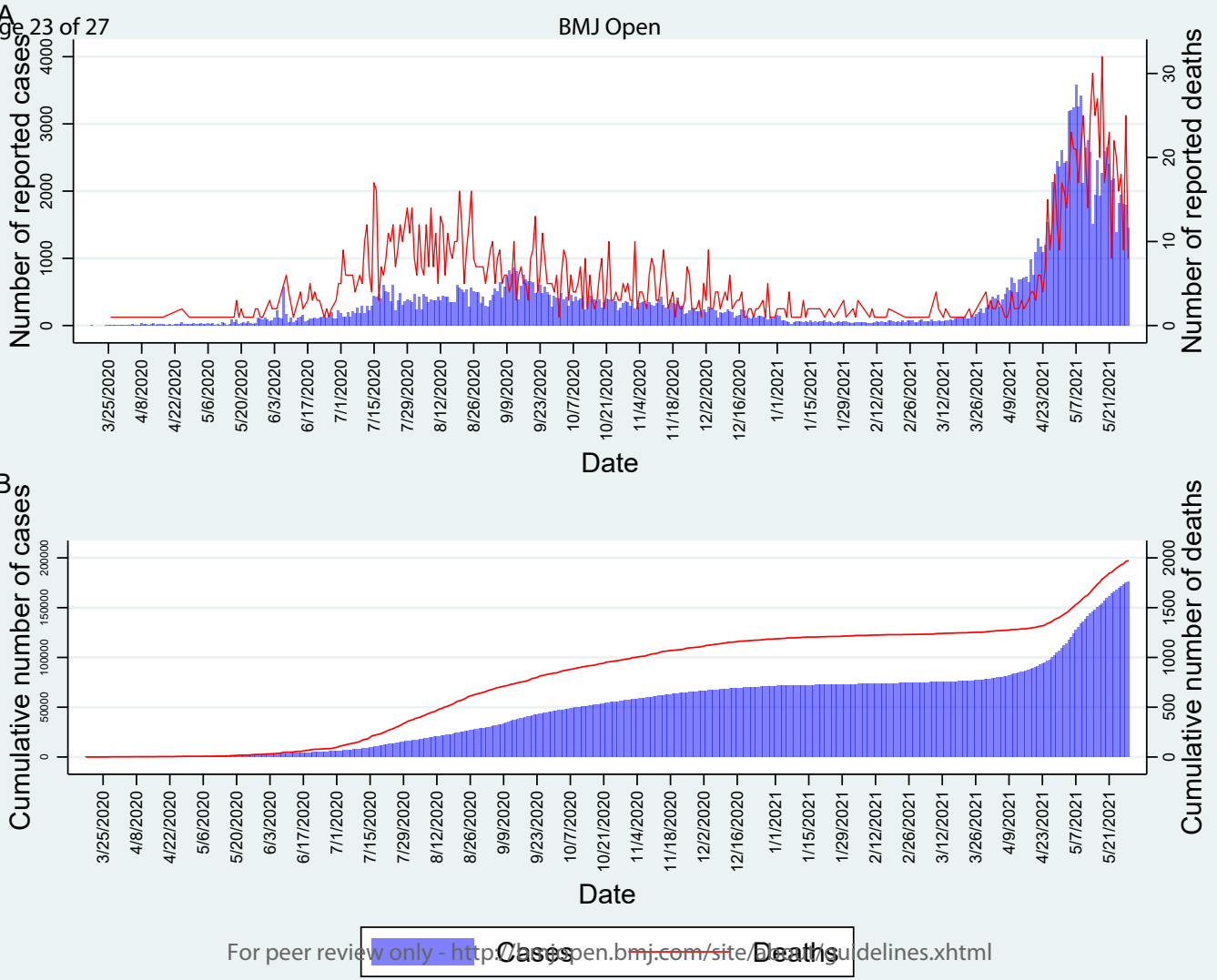
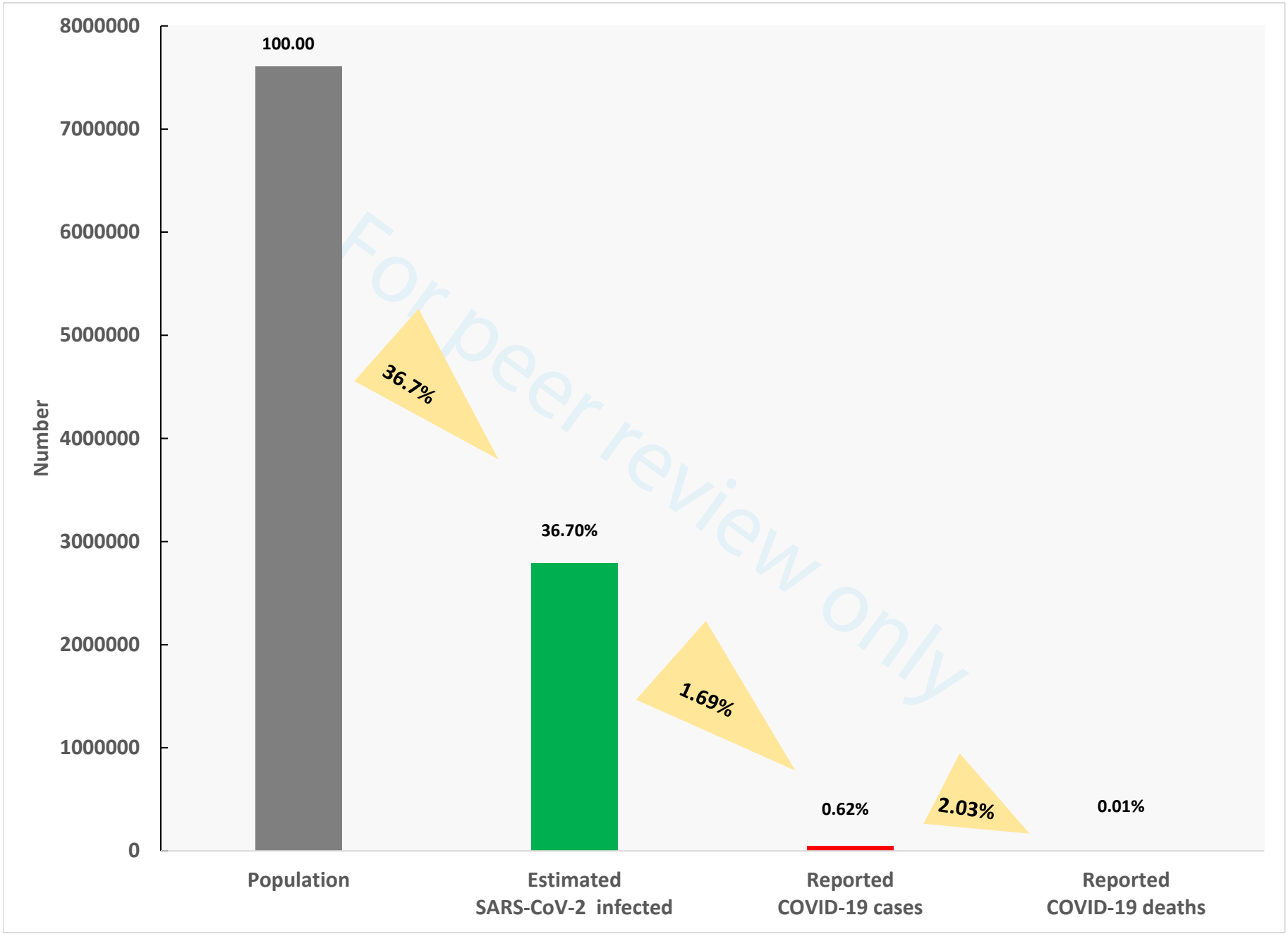
28.5 (24.4-33.0)
Anantnag
35.2 (30.7-39.8)




A
Total participants
6230
History of COVID-19 like symptoms
Symptomatic
474
Asymptomatic
5756
Ever tested for COVID-19 (RT-PCR)
Yes
233*
No
241
Yes
859†
No
4897
COVID-19 RT-PCR result
Positive
87
Negative
145
Positive
89
Negative
767
Seropositivity, Number (%)
74
(85.1)
68
(46.9)
105
(43.6)
66
(74.2)
277
(36.1)
1825
(37.3)
*Result unknown for 1 participant; †Result unknown for 3 participants


B
Total participants
6230
Seropositivity
Seropositive
2415
Seronegative
3815
Ever tested for COVID-19 (RT-PCR)
Yes
485
No
1930
Yes
607*
No
3208
COVID-19 RT-PCR result
Positive
140
Negative
345
Positive
36
Negative
567
Symptomatic, Number (%)
74
(52.9)
68
(19.7)
105
(5.4)
13
(36.1)
77
(13.6)
136
(4.2)
*Result unknown for 4 participants





Supplemental Table 1: Participant characteristics by district

District	Total	Age (years)				Gender		Residence	
		<30	30-49	50-69	70+	Female	Male	Rural	Urban
Anantnag	421	84	197	113	27	214	207	295	126
Budgam	442	113	190	105	34	263	179	354	88
Bandipora	424	106	174	114	30	227	197	341	83
Baramulla	405	113	176	98	18	214	191	325	80
Ganderbal	442	92	210	123	17	233	209	346	96
Kulgam	428	102	194	113	19	257	171	346	82
Kupwara	400	81	171	105	43	215	185	360	40
Pulwama	443	102	176	126	39	218	225	396	47
Shopiyan	407	119	152	90	46	211	196	368	39
Srinagar	2418	601	1032	656	129	1052	1366	233	2185
Total	6230	1513	2672	1643	402	3104	3126	3364	2866

Supplemental Table 2: Seroprevalence (unadjusted) by district and participant characteristics

District	Overall	Age (years)				Gender		Residence	
		<30	30-49	50-69	70+	Male	Female	Urban	Rural
Anantnag	35.2 (30.7-39.8)	29.8 (21-40.4)	34.5 (28.2-41.4)	38.9 (30.4-48.2)	40.7 (24.2-59.7)	36.2 (30-43)	34.1 (28.1-40.7)	42.9 (34.5-51.6)	31.9 (26.8-37.4)
Budgam	43 (38.4-47.7)	44.2 (35.4-53.5)	37.9 (31.3-45)	48.6 (39.2-58.1)	50 (33.8-66.2)	41.9 (34.9-49.3)	43.7 (37.8-49.8)	38.6 (29.1-49.2)	44.1 (39-49.3)
Bandipora	39.6 (35.1-44.4)	37.7 (29-47.3)	42 (34.8-49.4)	40.4 (31.8-49.6)	30 (16.4-48.3)	37.6 (31.1-44.5)	41.4 (35.2-47.9)	55.4 (44.6-65.7)	35.8 (30.9-41)
Baramulla	34.6 (30.1-39.3)	27.4 (20-36.4)	32.4 (25.9-39.6)	44.9 (35.4-54.8)	44.4 (24-67)	39.3 (32.6-46.4)	30.4 (24.6-36.9)	36.3 (26.5-47.3)	34.2 (29.2-39.5)
Ganderbal	39.1 (34.7-43.8)	34.8 (25.8-45)	40.5 (34-47.3)	39.8 (31.6-48.7)	41.2 (21-64.8)	39.2 (32.8-46)	39.1 (33-45.5)	42.7 (33.2-52.8)	38.2 (33.2-43.4)
Kulgam	28.5 (24.4-33)	27.5 (19.7-36.9)	26.8 (21-33.5)	31 (23.1-40.1)	36.8 (18.7-59.7)	25.1 (19.2-32.2)	30.7 (25.4-36.6)	37.8 (28-48.7)	26.3 (21.9-31.2)
Kupwara	42.3 (37.5-47.2)	33.3 (24-44.2)	39.8 (32.7-47.3)	50.5 (41-59.9)	48.8 (34.4-63.4)	41.6 (34.7-48.9)	42.8 (36.3-49.5)	50 (35-65)	41.4 (36.4-46.6)
Pulwama	43.1 (38.6-47.8)	35.3 (26.7-45)	42.6 (35.5-50)	45.2 (36.8-54)	59 (43.2-73.1)	39.6 (33.4-46.1)	46.8 (40.3-53.4)	40.4 (27.5-54.9)	43.4 (38.6-48.4)
Shopiyan	31.9 (27.6-36.6)	28.6 (21.2-37.3)	29.6 (22.9-37.3)	41.1 (31.4-51.5)	30.4 (18.9-45.1)	31.1 (25-37.9)	32.7 (26.7-39.3)	38.5 (24.7-54.4)	31.3 (26.7-36.2)
Srinagar	40.7 (38.8-42.7)	39.1 (35.3-43.1)	39.2 (36.3-42.3)	41.9 (38.2-45.7)	53.5 (44.9-61.9)	37.7 (35.2-40.3)	44.6 (41.6-47.6)	40.8 (38.7-42.9)	39.9 (33.8-46.3)

Chronic disease and SARS-CoV-2 infection in the study population

Of the 6230 participants, 1145 reported a history of at least one chronic disease. Two hundred ninety-eight reported a history of more than one chronic disease.

Supplementary Table 3: Chronic disease in the study population

Chronic disease (n = 1145)	Number (%)
Hypertension	815 (13·1%)
Diabetes	314 (5·0%)
Chronic Obstructive Pulmonary Disease	39 (0·6%)
Coronary Heart Disease	35 (0·6%)
Cerebrovascular Disease	16 (0·3%)
Asthma	15 (0·2%)
Chronic Kidney Disease	10 (0·2%)
Chronic Liver Disease	5 (0·1%)
Cancer	4 (0·1%)

Supplementary Table 4: History of COVID-19 like symptoms and seropositivity vis-à-vis history of chronic disease

		History of COVID-19 like symptoms	
		Yes	**No**
Reported history of chronic disease (n=1145)	**Seropositive**	78	417
	Seronegative	63	587
Did not report any history of chronic disease (n=5085)	**Seropositive**	169	1751
	Seronegative	164	3001

Of the 1154 participants who reported a history of chronic disease 78 (6.8%) reported a history of COVID-19 like symptoms within three months of the interview date and were seropositive for SARS-CoV-2 specific IgG (symptomatic infection). The proportion of symptomatic infection among participants who did not report any history of chronic disease was 3.3% (169/5085).

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3 and Figure 1
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	3, 4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	4, 5
Study size	10	Explain how the study size was arrived at	3
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4, 5
		(*b*) Describe any methods used to examine subgroups and interactions	5
		(*c*) Explain how missing data were addressed	5
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	4, 5
		(*e*) Describe any sensitivity analyses	6, Table 3
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure 2, and page 5
		(b) Give reasons for non-participation at each stage	Figure 2
		(c) Consider use of a flow diagram	Figure 2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1, Figure 2
Outcome data	15*	Report numbers of outcome events or summary measures	Table 2

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2
		(*b*) Report category boundaries when continuous variables were categorized	Table 1, 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Table 2, Table 3
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11, 12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9, 10, 11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for exposed and unexposed groups.

BMJ Open

Seroprevalence of SARS-CoV-2 specific IgG antibodies in Kashmir, India, seven months after the first reported local COVID-19 case: results of a population-based seroprevalence survey from October-November, 2020

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-053791.R4
Article Type:	Original research
Date Submitted by the Author:	04-Sep-2021

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Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Epidemiology
Keywords:	COVID-19, EPIDEMIOLOGY, Epidemiology < INFECTIOUS DISEASES

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Seroprevalence of SARS-CoV-2 specific IgG antibodies in Kashmir, India, seven months after the first reported local COVID-19 case: results of a population-based seroprevalence survey from October-November, 2020

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Word Count: 3727 (excluding abstract, tables, and references)

ABSTRACT

Objectives: We designed a population-based survey in Kashmir to estimate the seroprevalence of SARS-CoV-2 specific IgG antibodies in the general population aged 18 years and above.

Setting: The survey was conducted among 110 villages and urban wards across ten districts in Kashmir from 17 Oct 2020 to 04 Nov 2020.

Participants: Individuals aged 18 years and above were eligible to be included in the survey. Serum samples were tested for the presence of SARS-CoV-2 specific IgG antibodies using the Abbott SARS-CoV-2 IgG assay.

Primary and secondary outcome measures: We labeled assay results equal to or above the cut-off index value of 1·4 as positive for SARS-CoV-2 specific IgG antibodies. Seroprevalence estimates were adjusted for the sampling design and assay characteristics.

Results: Out of 6397 eligible individuals enumerated, 6315 (98.7%) agreed to participate. The final analysis was done on 6230 participants. Seroprevalence adjusted for the sampling design and assay characteristics was 36.7% (95% CI 34.3%-39.2%). Seroprevalence was higher among the older population. Among seropositive individuals, 10.2% (247/2415) reported a history of COVID-19 like symptoms. Out of 474 symptomatic individuals, 233 (49.2%) reported having been tested. We estimated an infection fatality rate of 0.034%.

Conclusions: During the first seven months of the COVID-19 epidemic in Kashmir valley, approximately 37% of individuals were infected. The reported number of COVID-19 cases was only a small fraction of the estimated number of infections. A more efficient surveillance system with strengthened reporting of COVID-19 cases and deaths is warranted.

ARTICLE SUMMARY

Strengths and limitations of this study

- The findings of our study are based on a representative sample of the population.
- The laboratory test used for the detection of SARS-CoV-2 specific IgG antibodies in serum samples provides valid results.
- We report seroprevalence estimates adjusted for sampling design and test performance.
- Even though we adjusted the weighted seroprevalence estimates for test performance using manufacturer-provided sensitivity and specificity (100% and 99·63%, respectively), we did not quantify the test validity in-house.
- Because of lack of age- and gender-specific mortality data, we could not estimate age- and gender-specific infection fatality rates.

INTRODUCTION

On 11 Feb 2020, the World Health Organization announced that the disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) would be named coronavirus disease (COVID-19).[1] In Kashmir, the first case of COVID-19 was confirmed in Srinagar city on 18 Mar 2020.[2] The government imposed the first phase of the lockdown in Kashmir on 24 March 2020. During this phase, inter-state travel remained suspended. People were barred from moving outside except in an

emergency. Except for essential services, all government and private offices were advised to work from home. Universal masking was made mandatory. The lockdown was extended till 31 May 2020 and later relaxed in a phased manner.

Mild or asymptomatic infections are common in Coronavirus disease 2019 (COVID-19) and are an important source of infection transmission.[3,4] Such cases are less likely to be detected by a surveillance system based on reverse-transcriptase polymerase chain reaction (RT-PCR) testing. Therefore, the number of reported RT-PCR positive cases are an underestimate of the true number of infections in a population.

Seroprevalence surveys have been conducted in various countries at different stages of the current epidemic among various population groups.[5–14] Seroprevalence surveys provide a more accurate estimate of past infection, improve understanding of the infection transmission dynamics, and guide public health response.[15]

We designed this survey with the primary objective to estimate the seroprevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) specific IgG antibodies in the adult population of Kashmir valley.

METHODS

We designed a population-based cross-sectional study. The study covered all the ten districts of Kashmir, a valley in northern India. (Figure 1) We completed data collection in three weeks, from 17 Oct 2020 to 04 Nov 2020.

Ethics

We obtained written informed consent from all study participants. The study was approved by the Institutional Ethics Committee of Government Medical College Srinagar (reference number: 1004/ETH/GMC). We used anonymized participant data for analysis.

Sample size

Based on the results of a previous study conducted in July 2020, we speculated that, by October 2020, the prevalence would have increased to around 20%.[16] We calculated the minimum sample size based on an anticipated seroprevalence of 20%, an absolute precision of 2%, and a design effect of 2. We used OpenEpi to make sample size calculations.[17] We adjusted the sample size for a possible non-response of 10% to obtain a minimum size of 3376. We decided to select 3600 individuals from nine of the ten districts (except district Srinagar). To obtain precise estimates for district Srinagar, sample size estimation was made for the district separately. We used a design effect of 1.5, an anticipated seroprevalence of 20%, and absolute precision of 2% to obtain a sample size of 2302 for the district, further increasing to 2400 to account for non-response. We thus targeted a total sample size of 6000 (3600 + 2400).

Participants

All adults ≥18 years of age were eligible to participate in the study. We selected eligible participants using a three-stage stratified cluster sampling technique. We listed all clusters in the valley using the Census 2011 data.[18] Within each of the ten districts in the valley, clusters were stratified into urban

and rural clusters. We selected clusters within each of the 20 strata by probability proportionate to size (PPS) sampling. Except for district Srinagar which we oversampled to obtain precise seroprevalence estimates for, ten clusters were randomly selected from each district. We selected 20 clusters from district Srinagar. We divided each selected cluster into four equal areas and chose a central location within each of the four areas as the starting point. Thereafter, we approached consecutive households to enroll at least ten eligible participants. We thus identified a total of 440 random locations within 110 clusters in ten districts. We invited all eligible adults in a household for participation.

Variables

The primary outcome variable of interest was SARS-CoV-2 specific IgG antibodies. In addition, we obtained information from participants about their age, gender, history of COVID-19 like symptoms in the three months before the interview date, history of contact with a known COVID-19 patient, and history of COVID-19 testing.

Procedure

We informed eligible adults about the purpose and the procedure of the study. Study participation was voluntary. Participants were interviewed by health personnel specifically trained for the interview. Interview responses were recorded in an Epicollect5 form.[19] Once the interview was completed, a trained phlebotomist collected 3-5 mL of venous blood from the antecubital vein under aseptic precautions into a red-top collection tube containing a clot activator. The tube was left standing, undisturbed, for at least 30 minutes for clot formation. The sample was later transported to a central facility for centrifugation. Centrifuged samples were transported to a central laboratory for further processing and analysis. Serum samples were tested for the presence of SARS-CoV-2 specific IgG antibodies using the Abbott SARS-CoV-2 IgG assay. The assay uses chemiluminescence to detect IgG antibodies against the SARS-CoV-2 nucleocapsid protein. The reported sensitivity and specificity of the assay are 100% (95% CI 95.89-100.00)and 99·63% (99.05-99.90), respectively.[20] As recommended by the manufacturer, we labeled assay results equal to or above the cut-off index value of 1·4 as positive for SARS-CoV-2 specific IgG antibodies.

Statistical methods

We report unweighted seroprevalence estimates in percentages. We used the Agresti-Coull procedure to calculate a 95% confidence interval (CI) for seroprevalence estimates.[21] A weighted estimate of seroprevalence is provided. To calculate survey weights (inverse of sampling probability), we used the estimated population of the districts. We used the census 2011 data and growth rates from Sample Registration System to estimate the population of the districts in 2020.[18,22] Survey weights obtained were further adjusted for non-response and age and sex structure (post-stratification weights). We further adjusted the weighted seroprevalence estimates for test performance to calculate "weighted seroprevalence adjusted for test performance". We did this using the formula:

$$Weighted\ seroprevalence\ adjusted\ for\ test\ performance = (Weighted\ seroprevalence + Test\ specificity - 1)/(Test\ sensitivity + Test\ specificity - 1).$$[23]

We used the manufacturer-provided sensitivity and specificity in the above formula.[20] We used the extremes of the manufacturer-provided 95% CI of the test sensitivity and specificity (upper limit of

sensitivity, lower limit of specificity; and lower limit of sensitivity, upper limit of specificity) to report sensitivity analyses.

We analyzed the difference in seroprevalence estimates across levels of a categorical variable using a Chi-square test adjusted for the sampling design.

We estimated the number of SARS-CoV-2 infections by multiplying the weighted seroprevalence adjusted for test performance with the estimated population of the valley. To estimate the number of infections per reported case, we divided the estimated number of SARS-CoV-2 infections by the reported number of COVID-19 cases two weeks before the survey date. We calculated the infection fatality rate by dividing the reported number of deaths by the number of estimated infections, assuming a three-week lag time from infection to death.[24]

We analyzed the data using Stata version 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LP.).

Patient and Public Involvement

No patients or members of the public were involved in this study.

RESULTS

We enumerated 6 397 individuals ≥18 years from 3 077 households and 110 clusters (34 urban clusters and 76 rural clusters) between 17 Oct 2020 and 04 Nov 2020. Of the 6 397 eligible individuals, 6 315 (98·7%) agreed to participate and were enrolled. The final analysis was done on a sample of 6 230 participants. (Figure 2)

Of the 6 230 participants, 1 513 (24·3%) were between 18 and 30 years of age, 2 672 (42·9%) were aged 30-49 years, 1 643 (26·4%) were aged 50-69 years, and 402 (6·4%) were 70 years and older. (Table 1). There was equal representation from males and females, and 3 364 (54·0%) resided in a rural area. Of the 3 104 females, 56 (1·8%) reported being pregnant at the time of the survey. Four hundred seventy-four (7·6%) reported COVID-19 like symptoms in the three months preceding the survey, and 439 (7·0%) reported to have ever come in contact with a known COVID-19 case. One thousand ninety-two (17·5%) reported having been tested for COVID-19 using RT-PCR or a Rapid Antigen Test (RAT) previously, of whom 176 (16·2%) reported to have tested positive for the disease.

Table 1: Characteristics of study participants

	Frequency	Percent
Total	6230	
Age, years		
18-29	1513	24·3
30-49	2672	42·9
50-69	1643	26·4
≥70	402	6·5
Gender		
Male	3126	50·2
Female	3104	49·8

Residence		
Urban	2866	46·0
Rural	3364	54·0
Pregnant (n=3104)	56	1·8
Self-reported history of chronic disease	1145	18.4
History of COVID-19 like symptoms	474	7·6
History of contact with a known COVID-19 case	439	7·0
Ever tested for COVID-19 (RT-PCR)	1092	17·5
RT-PCR result (n=1088*)		
Positive	176	16·2
Negative	912	83·8

COVID-19: Coronavirus disease; RT-PCR: Reverse transcriptase-polymerase chain reaction
*RT-PCR result not known in four participants

We found an overall unweighted seroprevalence of 38·8% (95% CI 37·6 – 40·0). The seroprevalence ranged from 28·5% in district Kulgam to 43·1% in district Pulwama. (Figure 1 and online supplemental file 1) The overall weighted seroprevalence (adjusted for sampling design) was 36·9% (95% CI 34·5 – 39·4). The weighted seroprevalence adjusted for test performance was 36·7% (95% CI 34·3 – 39·2). (Table 2) Upon sensitivity analyses, the weighted seroprevalence adjusted for test performance ranged from 36·3% (95% CI 33·9 – 38·8) to 38·4% (95% CI 35·9 – 41·0). (Table 3)

Table 2: Seroprevalence of SARS-CoV-2 specific IgG antibodies by participant characteristics

	Number tested	Number seropositive	Unweighted seroprevalence, % (95% CI)	Weighted seroprevalence, % (95% CI)	Weighted seroprevalence adjusted for test performance, % (95% CI)	Design-based F, p-value
Total	6230	2415	38·8 (37·6-40·0)	36·9 (34·5-39·4)	36·7 (34·3-39·2)	
Age, years						
18-29	1513	538	35·6 (33·2-38·0)	33·7 (30·1-37·6)	33·5 (29·8-37·4)	6·42, 0·0006
30-49	2672	1000	37·4 (35·6-39·3)	36·3 (33·5-39·3)	36·1 (33·3-39·1)	
50-69	1643	691	42·1 (39·7-44·5)	42·5 (38·8-46·2)	42·3 (38·6-46·0)	
≥70	402	186	46·3 (41·5-51·2)	45·3 (37·8-53·0)	45·1 (37·6-52·8)	
Gender						
Male	3126	1166	37·3 (35·6-39·0)	36·1 (33·5-38·9)	35·9 (33·3-38·7)	0·94, 0·34
Female	3104	1249	40·2 (38·5-42·0)	37·8 (34·5-41·3)	37·6 (34·3-41·1)	
Residence						
Urban	2866	1180	41·2 (39·4-43·0)	40·2 (36·3-44·1)	40·0 (36·1-43·9)	3·43, 0·07

Rural	3364	1235	36·7 (35·1-38·4)	35·5 (32·5-38·7)	35·3 (32·2-38·5)	
Self-reported history of chronic disease						
Yes	1145	495	43·2 (40·4-46·1)	41·9 (37·4-46·6)	41·7 (37·2-46·4)	6.14, 0.02
No	5085	1920	37·8 (36·4-39·1)	36·2 (33·7-38·9)	36·0 (33·5-38·7)	
History of COVID-19 like symptoms						
Yes	474	247	52·1 (47·6-56·6)	47·4 (37·9-57·1)	47·2 (37·7-56·9)	5·53, 0·02
No	5756	2168	37·7 (36·4-38·9)	36·3 (33·9-38·8)	36·1 (33·7-38·6)	
History of contact with a known COVID-19 case						
Yes	439	219	49·9 (45·2-54·5)	45·2 (38·3-52·2)	45·0 (38·1-52·0)	7·13, 0·01
No	5791	2196	37·9 (36·7-39·2)	36·5 (34·1-39·0)	36·3 (33·9-38·8)	
Ever tested for COVID-19 (RT-PCR)						
Yes	1092	485	44·4 (41·5-47·4)	41·0 (35·4-46·9)	40·8 (35·2-46·7)	2·17, 0·14
No	5138	1930	37·6 (36·2-38·9)	36·2 (33·5-39·0)	36·0 (33·3-38·8)	
RT-PCR result (n=1088*)						
Positive	176	140	79·5 (73·0-84·9)	81·8 (74·8-87·1)	81·7 (74·7-87·1)	74·93, <0·0001
Negative	912	345	37·8 (34·7-41·0)	38·8 (33·3-44·7)	38·6 (33·1-44·5)	

COVID-19: Coronavirus disease; RT-PCR: Reverse transcriptase-polymerase chain reaction
*RT-PCR result not known in four participants

Table 3: Sensitivity analyses for seroprevalence at extremes of test performance

	Weighted seroprevalence, % (95% CI)	**Weighted seroprevalence adjusted for test performance, % (95% CI)** ***[Sensitivity 100·00%, Specificity 99·63%]***	**Weighted seroprevalence adjusted for test performance, % (95% CI)** ***[Sensitivity 95·89%, Specificity 99·90%]***	**Weighted seroprevalence adjusted for test performance, % (95% CI)** ***[Sensitivity 100·00%, Specificity 99·05%]***
Overall	36·9 (34·5-39·4)	36·7 (34·3-39·2)	38·4 (35·9-41.0)	36·3 (33·9-38·8)
Age, years				
18-29	33·7 (30·1-37·6)	33·5 (29·8-37·4)	35·1 (31·3-39·1)	33·1 (29·4-37·0)

30-49	36·3 (33·5-39·3)	36·1 (33·3-39·1)	37·8 (34·9-40·9)	35·7 (32·9-38·7)
50-69	42·5 938·8-46·2)	42·3 (38·6-46·0)	44·3 (40·4-48·1)	41·9 (38·2-45·7)
≥70	45·3 937·8-53·0)	45·1 (37·6-52·8)	47·2 (39·4-55·2)	44·8 (37·2-52·5)
Gender				
Male	36·1 (33·5-38·9)	35·9 (33·3-38·7)	37·6 (34·9-40·5)	35·5 (32·9-38·3)
Female	37·8 (34·5-41·30	37·6 (34·3-41·1)	39·4 (35·9-43.0)	37·2 (33·9-40·7)
Residence				
Urban	40·2 (36·3-44·1)	40·0 (36·1-43·9)	41·9 (37·8-45·9)	39·6 (35·7-43·6)
Rural	35·5 (32·5-38·7)	35·3 (32·2-38·5)	37·0 (33·8-40·3)	34·9 (31·9-38·1)
Self-reported history of chronic disease				
Yes	43·2 (40·4-46·1)	41·9 (37·4-46·6)	43·6 (38·9-48·5)	41·3 (36·8-46·1)
No	37·8 (36·4-39·1)	36·2 (33·7-38·9)	37·7 (35·1-40·5)	35·6 (33·1-38·3)
History of COVID-19 like symptoms				
Yes	52·1 (47·6-56·6)	47·4 (37·9-57·1)	49·4 (39·5-59·5)	46·9 (37·3-56·7)
No	37·7 (36·4-38·9)	36·3 (33·9-38·8)	37·8 (35·3-40·4)	35·7 (33·3-38·2)
History of contact with a known COVID-19 case				
Yes	49·9 (45·2-54·5)	45·2 (38·3-52·2)	47·1 (39·9-54·4)	44·7 (37·7-51·7)
No	37·9 (36·7-39·2)	36·5 (34·1-39·0)	38·0 (35·5-40·6)	35·9 (33·5-38·4)
Ever tested for COVID-19 (RT-PCR)				
Yes	44·4 (41·5-47·4)	41·0 (35·4-46·9)	42·7 (36·9-48·9)	40·4 (34·8-46·4)
No	37·6 (36·2-38·9)	36·2 (33·5-39·0)	37·7 (34·9-40·6)	35·6 (32·9-38·4)
RT-PCR result (n=1088*)				
Positive	79·5 (73·0-84·9)	81·8 (74·8-87·1)	85·3 (78·0-90·8)	81·6 (74·6-87·0)
Negative	37·8 (34·7-41·0)	38·8 (33·3-44·7)	40·4 (34·7-46·6)	38·2 (32·7-44·2)

Seroprevalence was lowest among participants aged 18-29 years [33·5% (95% CI 29·8 – 37·4)] and was higher in older age groups. Seroprevalence was highest in those aged 70 years and above [45·1% (95% CI 37·6 – 52·8)]. Seroprevalence was not significantly different among males and females (p=0·34). The seroprevalence among urban residents was 40·0% (95% CI 36·1 – 43·9), slightly but not significantly, higher than rural residents [35·3% (95% CI 32·2 – 38·5), p=0·07]. (Table 2)

One in five participants (1145/6230, 18·4%) self-reported history of at least one chronic disease (Table 1). Hypertension (815/6230, 13.1%) and diabetes mellitus (314/6230, 5.0%) were the most commonly reported chronic diseases (online supplemental file 2). Seroprevalence was significantly higher in participants who self-reported history of chronic disease (41·7%, 95% CI 37·2 – 46·4) as compared to those who did not report a history of chronic disease (36·0%, 95% CI 33·5 - 38·7) (Table 2).

Among participants who reported a history of COVID-19 like symptoms, seroprevalence was 47·2% (95% CI 37·7 – 56·9) compared with 36·1% (95% CI 33·7 – 38·6) among participants who did not report such symptoms. Seroprevalence was higher among those who reported contact with a known COVID-19 case [45·0% (95% CI 38·1 – 52·0)] than participants who did not report any history of such contact [36·3% (95% CI 33·9 – 38·8)]. (Table 2)

Seroprevalence was not significantly related to being tested for COVID-19 (RT-PCR). However, those who reported a positive RT-PCR COVID-19 test had significantly higher seroprevalence (81·7%, 95% CI 74·7 – 87·1) as compared to those who reported a negative RT-PCR COVID-19 test (38·6%, 95% CI 33·1 – 44·5). (Table 2)

Among 2 415 seropositive individuals, only 247 (10·2%) reported a history of COVID-19 like symptoms. Only 20.1% (485/2415) of the seropositive individuals were tested for COVID-19 (RT-PCR). Among 474 who reported a history of COVID-19 like symptoms, 233 (49·2%) were tested for COVID-19 (RT-PCR). Among 4897 individuals who did not report a history of COVID-19 like symptoms and were never tested for COVID-19 (RT-PCR), 1825 (37.3%) were seropositive. (Figure 3)

Among 36 participants who reported a positive RT-PCR COVID-19 test but were seronegative, the duration between COVID-19 RT-PCR test and serological testing ranged from 9 days to 101 days. In only four of these 36 participants, the duration between the COVID-19 RT-PCR test and the serological test was 14 days or less. Of the remaining 32 participants, 21 did not report a history of CVOID-19 like symptoms, nine did not report a history of contact with a known COVID-19 case, and eight reported neither a history of COVID-19 like symptoms nor a history of contact with a known COVID-19 case.

We estimated that there were 1 673 484 (95% CI 1 564 047 – 1 787 482) cumulative number of infections among adults aged ≥18 years in the valley by 03 Oct 2020, two weeks before the start of the survey. If we assume that the seroprevalence was similar to the overall seroprevalence in the population not included in our study (<18 years of age) then the estimated cumulative number of infections in the valley by 03 Oct 2020 was 2 791 933 (95% CI 2 609 354 – 2 982 119). Considering that the cumulative number of reported COVID-19 cases was 47 071 by 03 Oct 2020 (Figure 4), we estimate the number of infections per reported case as 59·3 (95% CI 55·4 – 63·4). The number of reported COVID-19 deaths after a three-week lag period (on 24 Oct 2020) was 955. Thus, we estimated the infection fatality rate as 0.034% (95% CI 0.032 – 0.037). Of the total estimated SARS-CoV-2 infected persons, only 1.69% (47 071/2 791 933) were reported. Of the total reported COVID-19 cases, 2.03% (955/47 071) died.

DISCUSSION

We report the results of a seroprevalence survey conducted in Kashmir from October-November 2020, seven months after the appearance of the first local COVID-19 case. The COVID-19 pandemic is rapidly evolving worldwide. In Kashmir, several important events happened since we completed our survey. From 16 Jan 2021, COVID-19 vaccination was introduced in a phased manner. Healthcare workers were given preference during the first phase. From 01 Mar 2021, the vaccine was made available for people ≥60 years of age and those with chronic diseases in the age group of 45-59 years. However, especially during the early phases of the COVID-19 vaccination campaign, many people were hesitant to receive the vaccine doses. During the same time, SARS-CoV-2 Variants of Concern began to emerge and circulate. The daily number of COVID-19 cases started to rise again. The 'second wave' in April 2021 was more explosive than the 'first wave' at the beginning of the pandemic. The fear of the disease had

diminished, and COVID appropriate behaviour was no more a norm. The government and the people were caught unawares. There were several reports of a possible 'second infection' and reports of cases among previously vaccinated individuals. Given these developments, the current seroprevalence in Kashmir will be higher than what we report in this study.

The results of our study indicate that by the first week of October 2020, nearly seven months after the appearance of the first laboratory-confirmed COVID-19 case on 18 March 2020, close to 37% of the valley's population aged ≥18 years had been infected. Our results suggest that the cumulative number of SARS-CoV-2 infections by the first week of October 2020 was nearly 2·8 million, with an estimated infection fatality rate of 0.034%. Seroprevalence did not differ by gender but was higher in older age groups.

The findings of our study are based on a representative sample of the population. The laboratory test used for the detection of SARS-CoV-2 specific IgG antibodies in serum samples provides valid results.[20,25] We report seroprevalence estimates adjusted for sampling design and test performance.

The overall adjusted seroprevalence of around 37% indicates that, by October 2020, a large proportion of the valley's population had been infected with the virus. Easing of lockdown, being fed up with the restrictions, and non-adherence to prevention norms are the possible reasons. Using several assumptions about the test sensitivity and specificity to calculate adjusted seroprevalence estimates yielded small differences.

Several factors potentially influence the seroprevalence rates. These include population density, social and demographic structure of the population, governmental policies and the extent of their implementation, immunity level of the population, time since the start of infection transmission, adherence to infection prevention guidelines, quality of contact tracing and quarantine, and possibly the geography and environment of an area. The emergence of several Variants of Concern and the introduction of COVID-19 vaccination will also influence population immunity. Herd immunity in the context of COVID-19 is a matter of debate as reports of a second infection continue to pour in.[26]

Comparison with previous reports suggests that, by October 2020, the seroprevalence had increased almost ten-fold since July 2020.[16,27] The second of the three nationwide seroprevalence surveys in India conducted in August-September 2020 reports an overall seroprevalence of 6.6%, ranging from 5.2% in rural areas to 16.9% in urban slums.[28] A nationwide survey conducted in December 2020-January 2021 reported an overall seroprevalence of 24.1% ranging from 4.9% - 44.4% across districts.[29] Kashmir is thus not a low-infection area. Being an oft-visited tourist area, Kashmir is at an increased risk of infection transmission. Adherence to COVID appropriate behavior (use of face masks in public, frequent handwashing, physical and social distancing) has been poor. The experience of a 'second wave' of COVID-19 in April-June 2021, the appearance of virus variants, and the introduction of vaccination programs warrant robust surveillance of the epidemic.

The seroprevalence of SARS-CoV-2 IgG antibodies was higher in older age groups. During the early period of the pandemic, people were adherent to social distancing and other non-pharmaceutical interventions because of a fear of the disease and administrative restrictions. With time, administrative restrictions were relaxed, fear of the disease attenuated, and people became fed up with the social restrictions. This led to an increase in the number of reported COVID-19 cases and provided the population, including older age groups, an opportunity to contract the infection. That older people have

an increased risk of symptomatic and more severe disease is now well known.[30,31] However, age-based differential susceptibility to SARS-CoV-2 infection antibody response and the reasons thereof are still a grey area and need further understanding. Existing literature might suggest that the more mobile and socially active young have a higher risk of infection.[6,7] However, this should not imply that the elderly have a decreased susceptibility to SARS-CoV-2 infection or a decreased antibody response.[32] On the contrary, several studies suggest that the seroprevalence of SARS-CoV-2 specific IgG antibodies is higher in the older age groups and particularly so in more dense population groups.[4,5,8–11,13] Furthermore, SARS-CoV-2 antibody levels have been reported to be higher in older people.[12]

The seroprevalence of SARS-CoV-2 specific IgG antibodies did not differ significantly by gender, though the figure was slightly higher for females. These findings are consistent with the available literature.[6,13] Difference in seroprevalence by gender has been suggested by some studies, and females have been reported to have lower antibody levels.[5,7,9,11,12,14,33]

Urban areas are more densely populated than rural areas, accelerating the transmission of infections in the population. The seroprevalence of SARS-CoV-2 specific IgG antibodies is thus expected to be higher in urban areas, especially during the early phases of an epidemic. However, as the epidemic progresses, the seroprevalence gap between urban and rural areas will wane off. We estimated an adjusted seroprevalence of 40·0% (95% CI 36·1 – 43·9) in urban areas as compared to 35·3% (95% CI 32·2 – 38·5) in rural areas (p=0·07).

People with a chronic disease experience more severe COVID-19 symptoms and are more likely to die when compared to people with no chronic disease.[34] We found a higher proportion of symptomatic infection among participants with a self-reported history of chronic disease (78/1145, 6·8%) as compared to participants with no chronic disease (169/5085, 3·3%) (online supplemental file 3). Little is, however, known about the risk of infection in chronic disease patients. We found a significantly higher seroprevalence among participants with a self-reported history of chronic disease (Table 2). This finding needs further research for corroboration and possible explanations.

People with a self-reported history of COVID-19 related symptoms, contact with a known COVID-19 case, or a positive COVID-19 RT-PCR had a higher seroprevalence as compared to their complement. Among seropositive individuals, only 10·2% reported being symptomatic. The percentage of asymptomatic infections, thus, was 90%. However, only 49% of individuals with a history of COVID-19 like symptoms were tested using RT-PCR. We also estimated that only one out of almost 59 infections gets reported. This reflects the necessity of improving the efficiency of RT-PCR testing so that more symptomatic individuals receive the test. Not all individuals with a known RT-PCR positive result showed the presence of IgG antibodies. Around 20% of RT-PCR positive individuals were seronegative, and in a large majority of them (32 out of 36), the duration since RT-PCR positivity was more than two weeks. This may be attributed to a poor B cell response or a false negative antibody test.[35] Around 38% of RT-PCR negative individuals were seropositive, suggesting a false-negative RT-PCR or infection acquisition at a date later than the RT-PCR test.

We estimated an infection fatality rate of 0.034% (95% CI 0.032 – 0.037). The infection fatality rate in SARS-CoV-2 infection has been reported to range from as low as 0.00% to 1.63%.[36] Our estimates of the infection fatality rate are low as compared to estimates from several Indian studies.[5,28,37] Under-reporting COVID-19 deaths because of the non-uniform definition for a 'COVID-19 death' may falsely lower the infection fatality rates.[38] Many other factors can influence the infection fatality rate in SARS-

CoV-2 infection – the quality of available health facilities, the age structure of the population, and COVID-19 epidemic intensity.[39,40] Developing countries usually have a younger population as compared to the developed countries, and Kashmir is not an exception. However, because of the possibility of under-reporting of COVID-19 deaths, the true infection fatality rate in Kashmir may be higher than our estimates. The infection fatality rate is, however, known to be lower in developing nations.[30,41] In developed nations like the United States and many European countries, a higher infection fatality rate has been reported.[30,42]

Limitations

One important limitation of our study is that even though we adjusted the weighted seroprevalence estimates for test performance using manufacturer-provided sensitivity and specificity (100% and 99·63% respectively), we did not quantify the test validity in-house. Another limitation of our study estimates is that we excluded people <18 years of age. The results of our study may not thus be generalizable to this group of the population.

Our estimated seroprevalence was much higher than we anticipated at the designing stage. This has impacted the precision of our estimates to some extent. However, we believe we still have been able to estimate the seroprevalence with reasonable precision.

Lack of reliable death counts is another potential limitation. This may have led to an underestimation of the infection fatality rate. We did not perform any adjustment for death counts. Further, because of lack of age- and gender-specific mortality data, we could not estimate age- and gender-specific infection fatality rates.

CONCLUSIONS

We conclude that nearly 37% of individuals aged 18 years and above were infected with SARS-CoV-2 in Kashmir by October 2020. The infection fatality rate in the valley is around 0.034%. A majority of cases go unreported. For every reported case, there are 59 unreported infections in the population. Since almost half of the symptomatic individuals go unreported, testing of symptomatic individuals and effective contact tracing needs to continue. Given the emergence of mutant Variants of Concern, increasing the population immunity through augmented and sustained vaccination is necessary. We further recommend that adherence to COVID-19 prevention measures should be ensured until a large proportion of the population gets vaccinated.

ACKNOWLEDGMENTS

We thank Prof. Samia Rashid for her support. We acknowledge the help from Divisional COVID-19 Control Room Kashmir, especially from Dr. Rauf Hussain Rather. We thank the following coordinating center teams and the laboratory support team for their support and coordination:

Coordinating center teams: Dr. Sabira Aalia Dkhar, Dr. Kouser Sideeq, Dr. Uruj Qureshi, Dr. Mehraj u Din Sofi, Dr. Yasmin Kango, Dr. Suhail Manzoor Shah, Dr. Bashir Ahmad Khan, Dr. Nabeel Ishtiyaq, Dr. Tajamul Hussain Khan, Dr. Lahoot Hassan Khan, Dr. Muzaffer Ahmad, Dr. Ramesh Kumar Malla, Dr. Ruqaya Rasool Kapta, Dr. Mohammad Yousuf Naikoo, Dr. Khalid Abbass, Dr. Zarar Ahmad Bhat, Dr. Sumair Rashid, Dr. Zubair Ahmad, Dr. Shalender Singh Bali, Dr. Abdul Rouf, Dr. Abdul Hamid, Dr. Khalid Bashir, Dr. Qazi Najeeb Ahmad, Dr. Syed Irtiza, Dr. P Sharik Shaheen Khaki, Dr. Sheikh Mohammad Shafi,

Dr. Mohammad Yousuf Zagoo, Dr. Mukhtar Ahmad Shah, Dr. Imtiyaz-ur-Rehman Bhat, Dr. Showkat Parray, Dr. Fazil Ali Kochak, Dr. Muzaffar Ahmad Zargar, Dr. Abdul Rashid Shah, Dr. Shagufta Saleem, Dr. Abdul Gani, Dr. Nighat Nasreen, Dr. Asima Nazeer, Dr. Zahoor Ahmad Lone.

Laboratory support team: Ishtiyaq Ahmad Handoo, Rahiba Makhdoomi, Iyman Wani, Romana Rasool, Asiya Rehman, Insha Rehman, Zahoor Ahmad, Gulzar Ahmad Wani, Javid Ahmad Bhat, M Saleem Malik, Naveed Manzoor, Mubeena.

COMPETING INTERESTS

We declare no competing interests, financial or otherwise.

FUNDING

The National Health Mission Jammu & Kashmir funded the study. The funding agency was not involved in study designing, implementation, analysis, or interpretation of the study findings.

CONTRIBUTORS

S Muhammad Salim Khan: Conceptualization, Funding acquisition, Methodology, Writing-review & editing.

Mariya Amin Qurieshi, Inaamul Haq, Sabhiya Majid: Formal analysis, Data curation, Methodology, Project administration, Writing-original draft, Writing-review & editing.

Javid Ahmad, Taha Ayub, Anjum Bashir, Ashfaq Ahmad Bhat, Abdul Majeed Ganai, Yasmeen Jan, Rauf-ur-Rashid Kaul, Zahid Ali Khan, Muneer Ahmad Masoodi, Beenish Mushtaq, Fouzia Nazir, Muzamil Nazir, Malik Waseem Raja, Mahbooba Rasool: Project administration, Supervision, Investigation, Writing-review & editing.

Anjum Asma, Munazza Aziz, Shifana Ayoub, Arif Akbar Bhat, Iqra Nisar Chowdri, Shaista Ismail, Misbah Ferooz Kawoosa, Mehvish Afzal Khan, Mosin Saleem Khan, Rafiya Kousar, Ab Aziz Lone, Shahroz Nabi, Mohammad Obaid, Tanzeela Bashir Qazi, Iram Sabah, Ishtiyaq Ahmad Sumji: Supervision, Investigation, Writing-review & editing.

S Muhammad Salim Khan, Mariya Amin Qurieshi, Inaamul Haq, and Sabhiya Majid have verified the underlying data.

DATA SHARING

Anonymized data collected for the study, including individual participant data and a data dictionary defining each field in the set, will be made available to interested researchers on request by Inaamul Haq (haqinaam@yahoo.co.in) for two years from publication. The study protocol, statistical analysis plan, and informed consent forms are also available from Inaamul Haq.

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Figure 1 legend:

Figure 1: Location of the ten districts and seroprevalence (%) by district. Figures in parentheses indicate a 95% Confidence Interval for seroprevalence.

Figure 2 legend:

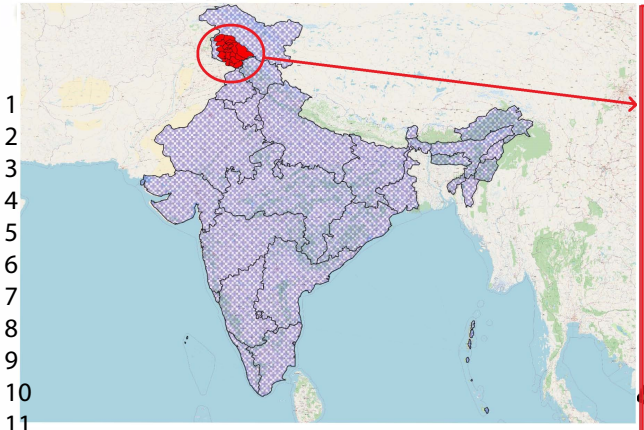
Figure 2: Participant flow.

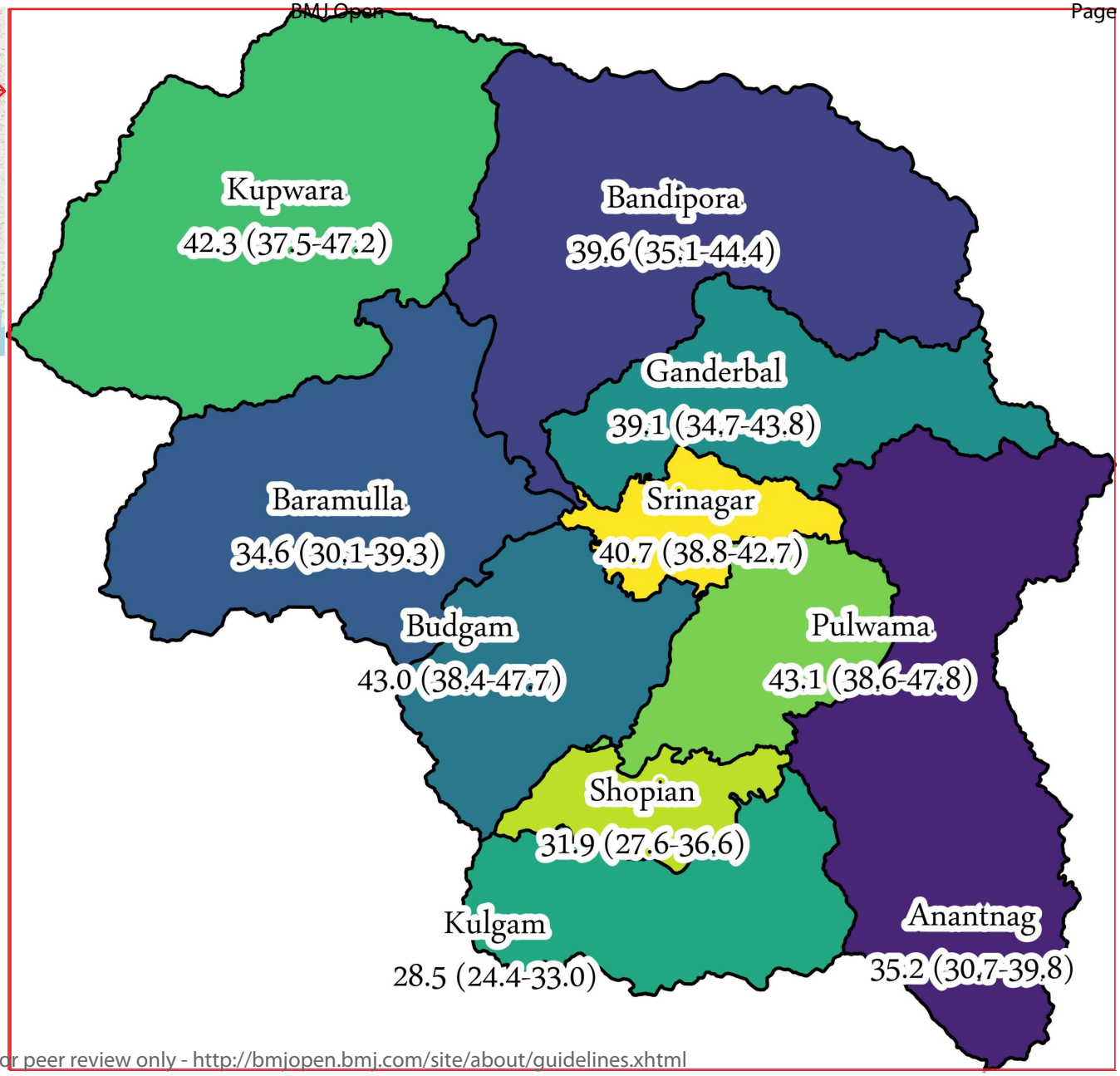
Figure 3 legend:

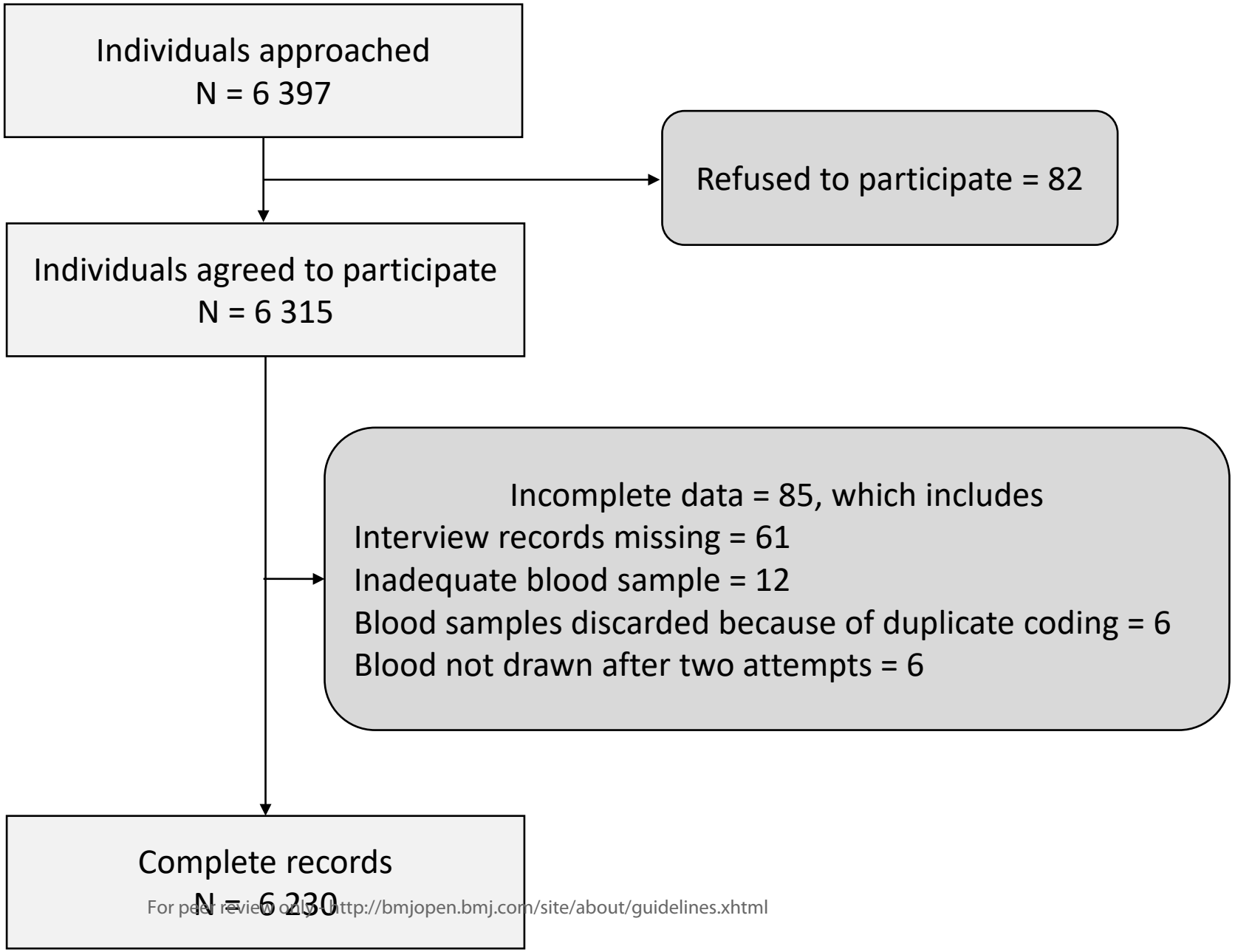
Figure 3: A. Seropositivity by the history of COVID-19 like symptoms, RT-PCR testing, and test result. B. History of COVID-19 like symptoms by seropositivity, RT-PCR testing, and test result.

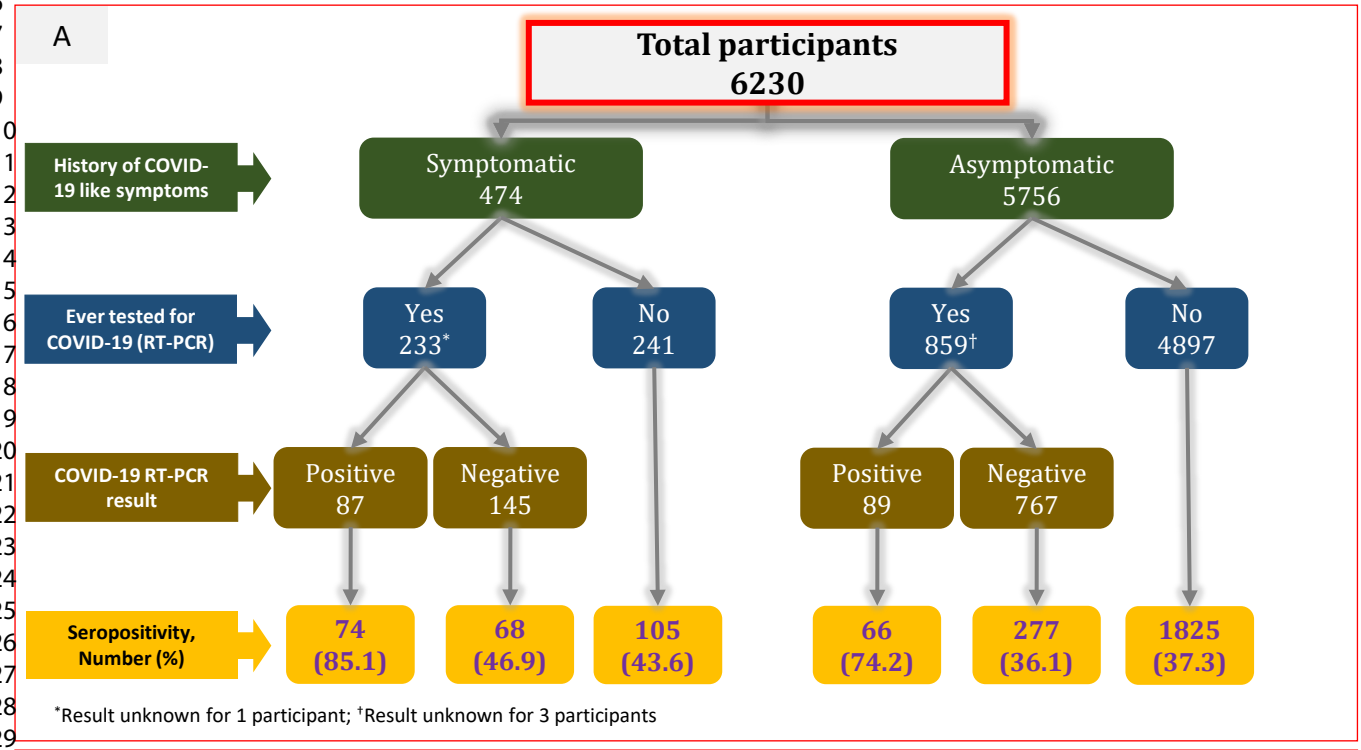
Figure 4 legend:

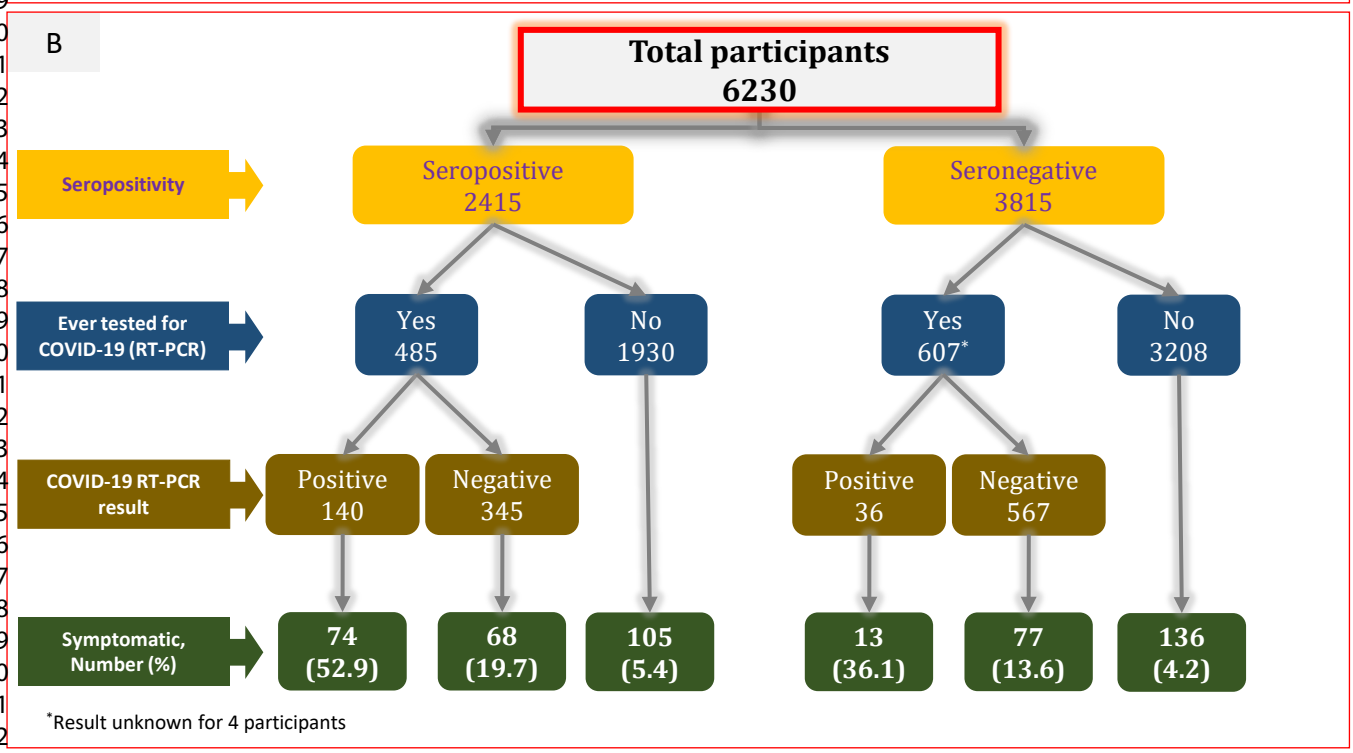
Figure 4: A. Daily cases and deaths in Kashmir since the start of the COVID-19 pandemic; B. Cumulative number of cases and deaths in Kashmir.

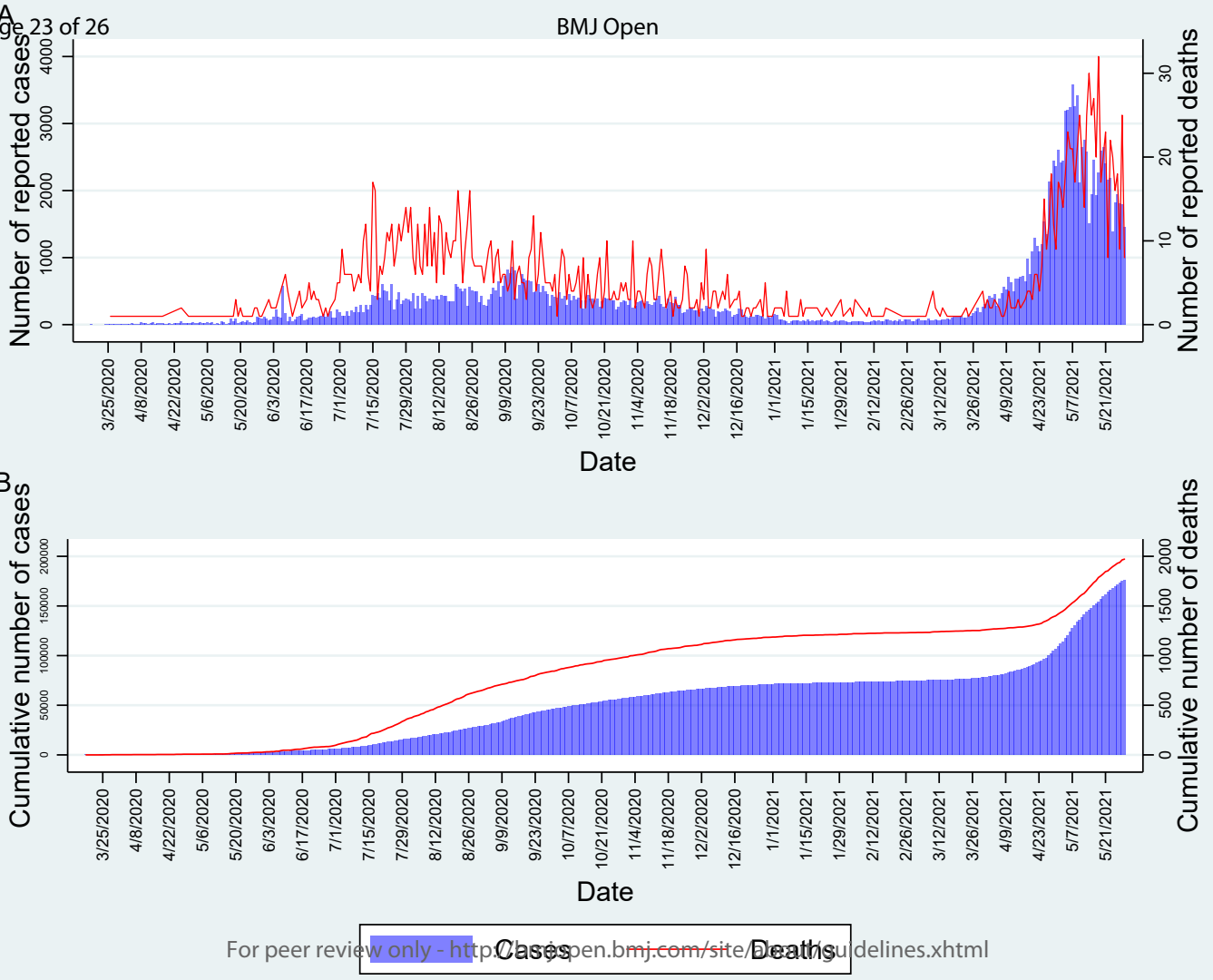



Kupwara
42.3 (37.5-47.2)
Bandipora
39.6 (35.1-44.4)
Ganderbal
39.1 (34.7-43.8)
Baramulla
34.6 (30.1-39.3)
Srinagar
40.7 (38.8-42.7)
Budgam
43.0 (38.4-47.7)
Pulwama
43.1 (38.6-47.8)
Shopian
31.9 (27.6-36.6)
Kulgam
28.5 (24.4-33.0)
Anantnag
35.2 (30.7-39.8)




A
Total participants
6230
History of COVID-19 like symptoms
Symptomatic
474
Asymptomatic
5756
Ever tested for COVID-19 (RT-PCR)
Yes
233*
No
241
Yes
859†
No
4897
COVID-19 RT-PCR result
Positive
87
Negative
145
Positive
89
Negative
767
Seropositivity, Number (%)
74
(85.1)
68
(46.9)
105
(43.6)
66
(74.2)
277
(36.1)
1825
(37.3)
*Result unknown for 1 participant; †Result unknown for 3 participants


B
Total participants
6230
Seropositivity
Seropositive
2415
Seronegative
3815
Ever tested for COVID-19 (RT-PCR)
Yes
485
No
1930
Yes
607*
No
3208
COVID-19 RT-PCR result
Positive
140
Negative
345
Positive
36
Negative
567
Symptomatic, Number (%)
74
(52.9)
68
(19.7)
105
(5.4)
13
(36.1)
77
(13.6)
136
(4.2)
*Result unknown for 4 participants



Supplemental Table 1: Participant characteristics by district

District	Total	Age (years)				Gender		Residence	
		<30	30-49	50-69	70+	Female	Male	Rural	Urban
Anantnag	421	84	197	113	27	214	207	295	126
Budgam	442	113	190	105	34	263	179	354	88
Bandipora	424	106	174	114	30	227	197	341	83
Baramulla	405	113	176	98	18	214	191	325	80
Ganderbal	442	92	210	123	17	233	209	346	96
Kulgam	428	102	194	113	19	257	171	346	82
Kupwara	400	81	171	105	43	215	185	360	40
Pulwama	443	102	176	126	39	218	225	396	47
Shopiyan	407	119	152	90	46	211	196	368	39
Srinagar	2418	601	1032	656	129	1052	1366	233	2185
Total	6230	1513	2672	1643	402	3104	3126	3364	2866

Supplemental Table 2: Seroprevalence (unadjusted) by district and participant characteristics

District	Overall	Age (years)				Gender		Residence	
		<30	30-49	50-69	70+	Male	Female	Urban	Rural
Anantnag	35.2 (30.7-39.8)	29.8 (21-40.4)	34.5 (28.2-41.4)	38.9 (30.4-48.2)	40.7 (24.2-59.7)	36.2 (30-43)	34.1 (28.1-40.7)	42.9 (34.5-51.6)	31.9 (26.8-37.4)
Budgam	43 (38.4-47.7)	44.2 (35.4-53.5)	37.9 (31.3-45)	48.6 (39.2-58.1)	50 (33.8-66.2)	41.9 (34.9-49.3)	43.7 (37.8-49.8)	38.6 (29.1-49.2)	44.1 (39-49.3)
Bandipora	39.6 (35.1-44.4)	37.7 (29-47.3)	42 (34.8-49.4)	40.4 (31.8-49.6)	30 (16.4-48.3)	37.6 (31.1-44.5)	41.4 (35.2-47.9)	55.4 (44.6-65.7)	35.8 (30.9-41)
Baramulla	34.6 (30.1-39.3)	27.4 (20-36.4)	32.4 (25.9-39.6)	44.9 (35.4-54.8)	44.4 (24-67)	39.3 (32.6-46.4)	30.4 (24.6-36.9)	36.3 (26.5-47.3)	34.2 (29.2-39.5)
Ganderbal	39.1 (34.7-43.8)	34.8 (25.8-45)	40.5 (34-47.3)	39.8 (31.6-48.7)	41.2 (21-64.8)	39.2 (32.8-46)	39.1 (33-45.5)	42.7 (33.2-52.8)	38.2 (33.2-43.4)
Kulgam	28.5 (24.4-33)	27.5 (19.7-36.9)	26.8 (21-33.5)	31 (23.1-40.1)	36.8 (18.7-59.7)	25.1 (19.2-32.2)	30.7 (25.4-36.6)	37.8 (28-48.7)	26.3 (21.9-31.2)
Kupwara	42.3 (37.5-47.2)	33.3 (24-44.2)	39.8 (32.7-47.3)	50.5 (41-59.9)	48.8 (34.4-63.4)	41.6 (34.7-48.9)	42.8 (36.3-49.5)	50 (35-65)	41.4 (36.4-46.6)
Pulwama	43.1 (38.6-47.8)	35.3 (26.7-45)	42.6 (35.5-50)	45.2 (36.8-54)	59 (43.2-73.1)	39.6 (33.4-46.1)	46.8 (40.3-53.4)	40.4 (27.5-54.9)	43.4 (38.6-48.4)
Shopiyan	31.9 (27.6-36.6)	28.6 (21.2-37.3)	29.6 (22.9-37.3)	41.1 (31.4-51.5)	30.4 (18.9-45.1)	31.1 (25-37.9)	32.7 (26.7-39.3)	38.5 (24.7-54.4)	31.3 (26.7-36.2)
Srinagar	40.7 (38.8-42.7)	39.1 (35.3-43.1)	39.2 (36.3-42.3)	41.9 (38.2-45.7)	53.5 (44.9-61.9)	37.7 (35.2-40.3)	44.6 (41.6-47.6)	40.8 (38.7-42.9)	39.9 (33.8-46.3)

Chronic disease and SARS-CoV-2 infection in the study population

Of the 6230 participants, 1145 reported a history of at least one chronic disease. Two hundred ninety-eight reported a history of more than one chronic disease.

Supplementary Table 3: Chronic disease in the study population

Chronic disease (n = 1145)	Number (%)
Hypertension	815 (13·1%)
Diabetes	314 (5·0%)
Chronic Obstructive Pulmonary Disease	39 (0·6%)
Coronary Heart Disease	35 (0·6%)
Cerebrovascular Disease	16 (0·3%)
Asthma	15 (0·2%)
Chronic Kidney Disease	10 (0·2%)
Chronic Liver Disease	5 (0·1%)
Cancer	4 (0·1%)

Supplementary Table 4: History of COVID-19 like symptoms and seropositivity vis-à-vis history of chronic disease

		History of COVID-19 like symptoms	
		Yes	**No**
Reported history of chronic disease (n=1145)	**Seropositive**	78	417
	Seronegative	63	587
Did not report any history of chronic disease (n=5085)	**Seropositive**	169	1751
	Seronegative	164	3001

Of the 1154 participants who reported a history of chronic disease 78 (6.8%) reported a history of COVID-19 like symptoms within three months of the interview date and were seropositive for SARS-CoV-2 specific IgG (symptomatic infection). The proportion of symptomatic infection among participants who did not report any history of chronic disease was 3.3% (169/5085).

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3 and Figure 1
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	3, 4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	4, 5
Study size	10	Explain how the study size was arrived at	3
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4, 5
		(*b*) Describe any methods used to examine subgroups and interactions	5
		(*c*) Explain how missing data were addressed	5
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	4, 5
		(*e*) Describe any sensitivity analyses	6, Table 3
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure 2, and page 5
		(b) Give reasons for non-participation at each stage	Figure 2
		(c) Consider use of a flow diagram	Figure 2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1, Figure 2
Outcome data	15*	Report numbers of outcome events or summary measures	Table 2

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2
		(*b*) Report category boundaries when continuous variables were categorized	Table 1, 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Table 2, Table 3
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11, 12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9, 10, 11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for exposed and unexposed groups.