

Original Research Article

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Seroprevalence of SARS CoV 2 specific Ig G antibodies in District Srinagar, Kashmir: a population based study

Taha Ayub¹, Malik Waseem Raja^{1*}, S. M. Salim Khan¹, Inaamul Haq¹, Mariya Amin Qureshi¹, Sabhiya Majid², Iqra Nisar Chowdri¹, Tanzeela Bashir Qazi¹, Rafiya Kousar¹, Abdul Aziz Lone¹, Iram Sabah¹, Shahroz Nabi¹, Ishtiyaq Ahmad Sumji¹, Misbah Ferooz Kwoosa¹, Shifana Ayoub¹, Mehwish Afzal Khan¹, Shaista Ismail¹, Anjum Asma¹, Munaza aziz¹

¹Department of Community Medicine, ²Department of Biochemistry, Government Medical College Srinagar, Jammu and Kashmir, India

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***Correspondence:**

Dr. Malik Waseem Raja,

E-mail: rajwsm.raja@gmail.com

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ABSTRACT

Background: The seroprevalence studies are conducted with the aim of estimating the extent of SARS CoV2 infection in any community. A cross sectional population based study was conducted in Srinagar district of Kashmir, India so as to provide relevant information on the proportion of people who have experienced a recent or past infection.

Methods: An estimated sample size of 2400 was calculated based on anticipated seroprevalence of 20% and an absolute error of 2.5%. 20 clusters were selected using 2 stage cluster sampling. A set of questions on a mobile based application i.e Epicollect 5 was used to collect information on various variables and 3-5 ml of venous blood was taken for Ig G antibody testing. Chemiluminiscent Microparticle Immunoassay (CMIA) procedure using fully automated analyser by Abbott with sensitivity of 100% and specificity of 99.6% was used to detect IgG antibodies against SARS COVID-2.

Results: 2480 eligible individuals participated in the study. The overall seroprevalence of Ig G antibodies against SARS-CoV 2 in the current study was 40.6% (95% CI 38.7-42.6), with seroprevalence being significantly higher among females (44.7% as compared to 37.5%). Age standardized seroprevalence revealed that seroprevalence increased with the increasing age.

Conclusions: The findings of Seroprevalence study may fail to reveal the true picture of covid-19 infection as there were certain participants who were positive for COVID on RTPCR but were IgG negative. Thus, the individual variation of immune response to the virus, role of mucosal Ig A antibodies and T cell mediated immunity cannot be ruled out.

Keywords: COVID 19, IgG antibodies, SARS CoV2, Seroprevalence, Srinagar

INTRODUCTION

COVID-19 is a novel viral disease caused by SARS-CoV-2 that was first detected in Wuhan, China, in December 2019. Given the alarming levels of spread, severity of disease, and number of affected countries, the

World Health Organization (WHO) declared COVID-19 as a pandemic on March 11th, 2020.¹

The clinical syndrome caused by SARS-CoV-2 ranges from very mild symptomatology to severe pneumonia, acute respiratory distress syndrome, and death.² However,

there are several report findings that many individuals might carry the virus without presenting any symptoms for several weeks.³ Generally, mildly affected or asymptomatic individuals are not screened. As a result, the number of confirmed SARS-CoV-2 infections is largely underestimated. In this context, seroprevalence surveys are of utmost importance to assess the proportion of the population that has already developed antibodies against the virus and might potentially be protected against subsequent infection.⁴ The presence of specific antibodies is currently being investigated to assess the induction of an immune response in patients and to assess the degree of exposure and immunity in the general population. As it is a recently emerged corona virus variant, the kinetics and degree of immunity induced following contact with the virus and COVID-19 disease are largely unknown. Estimates of the prevalence of seroconversion as proxy for protection of the general population may support health decision making.⁵

Detection of antibodies to SARS-CoV-2 in a person's blood likely indicates that they were infected at some point since the start of the pandemic. Thus, seroprevalence studies can provide relevant information on the proportion of people who have experienced a recent or past infection. They are relevant when conducted in the community.³

The first case of COVID-19 was reported in India when one of the medical students returning from Wuhan University was tested positive in Kerala on January 30, 2020.⁶ Now India is emerging as the world's biggest hotspot for severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) infection, second only to the United States of America, with more than 8.8 million recorded infections.⁷ Kashmir, a northern territory of India, reported its first positive case of novel corona virus on 18th March 2020 from a central district Srinagar which is currently the most affected place within the territory from where presently more new cases are reported each day. On 22nd March 2020, the Jammu and Kashmir government ordered the shutdown of all non-essential activities, commercial and business establishments, and educational institutions, except essential commodities and services to prevent the spread of SARS-CoV-2 infection. The health authorities started extensive case-detection and contact-tracing activities. Case-detection was based on the testing of nasopharyngeal samples by reverse transcriptase-polymerase chain reaction (RT-PCR).⁸ Many countries have started testing for the presence of antibodies against SARS-CoV-2 infection, both at the population level and in specific groups like healthcare workers. Seroepidemiological studies are crucial in understanding the dynamics of SARS-CoV-2 infection. World Health Organization, in its scientific briefing on 24th April, encouraged the member states to conduct seroepidemiological studies in the context of corona virus disease (COVID-19) for a better understanding of the extent of infection.⁹ In order to get the insight about the

extent of SARS-CoV-2 infection in our community, the current study was planned.

METHODS

A cross sectional study to find out the presence of Ig G antibodies against SARS COV-2 among adults (>18 years) of Srinagar district was conducted from 17 October to 20 October 2020. A two- stage cluster random sampling was done. In the first stage, 20 clusters (Wards/ village) were selected using PPS sampling and in each selected cluster, a minimum of 120 persons >18 years of age were randomly selected for participation in study. An estimated sample size of 2400 was calculated based on anticipated seroprevalence of 20% and an absolute error of 2.5%. This was the minimum sample size needed for conducting the study after making adjustments for sample design and possible non-response from subjects.

A survey team comprising of doctors, paramedics and laboratory technicians (phlebotomist) was constituted and each member was assigned a specific role in the team. They were trained for administration of questionnaire, pre-requisites of sample collection and transportation of samples. The training of survey teams was conducted by consultants from department of Community Medicine, GMC Srinagar on 15 October 2020. Ten teams were assigned a job of data and sample collection for 4 days with random allotment of 2 clusters per team. On the first day of survey, the team visited the selected cluster and randomly selected households in all possible 4 directions, with centre of road being the start point. From each household, all the individuals >18 years were interviewed only if they consented to participate in the study. All the participants were informed about the purpose of study and written consent was taken from them as well. From each household, atleast 3 samples were taken and this process continued till 120 samples were collected from a cluster. A set of questions on a mobile based application i.e Epicollect 5 was used to collect information about demographic variables, history of any Influenza like symptoms, history of being in contact with covid positive case or ever being tested for covid.¹⁰ After collecting information from the participant, a unique 6 digit Identification code was assigned to that particular individual. Under standard aseptic precautions, 3-5 ml of venous blood was taken by trained phlebotomist and was then immediately transferred to red top serum tube with a clot activator. In order to avoid hemolysis, red top tubes were left to stand for atleast 30 minute interval. Once the required number of samples was collected, they were transported to the central laboratory of Government Medical College, Srinagar for centrifugation maintaining the cold chain requirements through out. The samples were then cross checked for labelling and were arranged sequentially before shifting them to Department of Bio-Chemistry, GMC, Srinagar for Ig G testing.

Chemiluminiscent Microparticle Immunoassay (CMIA) procedure using fully automated analyser by Abbott with

sensitivity of 100% and specificity of 99.6% was used to detect IgG antibodies against SARS COVID-2.¹¹ The test result was considered positive for SARS COV-2 Ig G if index value was ≥ 1.40 as fixed by manufacturer.

The data from laboratory was entered in the duplicate Epicollect 5 forms by two independent trained personnel and any discrepancy found by independent 3rd individual was rectified by referring to original data source. All the information of the participant was inter linked using unique 6 digit identification number allotted earlier.

The data was entered into XL sheet and was summarized as frequency, mean and percentage. Difference in seroprevalence across age groups, gender, presence of comorbidity, individuals having history of ILI symptoms in

previous 3 months and history of contact with known COVID case or ever been tested for COVID was also calculated.

RESULTS

During the study period, 2480 eligible individuals participated in the study. The refusal rate was 2.05% (51 out of 2480). Information and blood sample was collected from 2429 participants but analysis was done on 2418 participants only because of some missing data or data entry errors.

Among the participants, there was slight predominance of males over females (56.5%), with mean age of participants being 41.7 years as depicted in Table 1.

Table 1: Seroprevalence of SARS COV-2 specific Ig G antibodies among the study participants of District Srinagar (n=2418).

Characteristics	Number of participants	Number of seropositive individuals	Seroprevalence (95% CI)
Overall	2418	984	40.6% (38.7-42.6)
Gender			
Male	1366	515	37.7% (35.1-40.3)
Female	1052	469	44.5% (41.5-47.6)
Age (years)			
≤ 30	731	291	39.8% (36.3 – 43.4)
31-50	1047	413	39.4% (36.5 – 42.4)
51-70	573	245	42.8% (38.7 – 46.8)
≥ 71	67	35	52.2% (40.2 – 63.9)
Comorbidity			
Present	577	272	47.1% (43.0 – 51.2)
Not present	1841	712	38.6% (36.4 – 40.9)
ILI symptoms in past 3 months			
Yes	274	161	58.7% (52.8 – 64.4)
No	2144	823	38.3% (36.3 – 40.4)
Contact with a known COVID- 19 case			
Yes	262	142	54.1% (48.1 – 60.1)
No	2156	842	39.0% (37.0 – 41.1)
Ever been tested			
RAT/RTPCR			
Yes	566	261	46.1% (42.0 – 50.2)
No	1852	723	39.0% (36.8 – 41.2)
RAT/RTPCR result* (n= 562)			
Positive	121	100	82.7% (74.7 – 88.6)
Negative	441	162	36.8% (32.6 – 41.5)

*RTPCR result was awaited in 4 participants.

Atleast one co-morbidity like hypertension, Diabetes mellitus, Cardiovascular diseases, Chronic kidney diseases was documented in 16% of the total participants. A small percentage of participants (11.3% and 10.8%) had ILI symptoms in previous 3 months before the date of interview and history of contact with known COVID-19 case. Around one-fourth of the participants were tested for RTPCR/RAT in the past, out of which only 46.1% were reported to be COVID positive. The overall

seroprevalence of Ig G antibodies against SARS-CoV 2 in the current study was 40.6% (95% CI 38.7-42.6), with seroprevalence being significantly higher among females (44.7% as compared to 37.5%). Further, the analysis revealed that seroprevalence increased significantly with the age of participants (39.8% among participants aged 18-50 years, 42.8% among 51-70 year old participants and 52.2% among those aged ≥ 70 years). High seroprevalence of Ig G antibodies against

SARS COVID-2 was reported among those participants with either single or multiple co-morbidity. The individuals who had history of ILI like symptoms in past 3 months or who had contact with known COVID case had higher seroprevalence of Ig G antibodies against SARS CoV 2. A significantly higher seroprevalence was seen among the participants who tested positive for SARS CoV-2 by either RAT or RTPCR tests.

Table 2: Odds ratio of seropositivity as per participant characteristics.

Characteristics	Odds ratio (95% CI)	P value
Age (years)		
≤30	1 (reference)	-
31-50	0.9 (0.8-1.1)	0.87
51-70	1.1 (0.9-1.4)	0.28
≥71	1.6 (1.0-2.7)	0.049
Female	1.3 (1.1-1.5)	0.001
With Co-morbidity	14 (1.1-1.7)	<0.001
ILI symptoms	2.2 (1.7-2.9)	<0.001
History of contact with known COVID 19 case	1.8 (1.4-2.3)	<0.001
Ever been tested RAT/RTPCR	1.3 (1.1-1.6)	0.003
Tested positive on RTPCR/RAT	8.1 (4.8-13.7)	<0.001

DISCUSSION

The current study was conducted to assess the extent of infection of SARS CoV 2 in general population of district Srinagar. In addition, sex specific and age specific seroprevalence estimates were also calculated. Seroprevalence studies are of immense use in providing key epidemiological and serological characteristics of COVID 19 virus in general population. The current study revealed that 4 in 10 individuals of Srinagar district has developed Ig G antibodies against the SARS COVID-2. Considerable variation has been observed in the results of SARS-CoV-2 seroprevalence studies.^{4,12-21} It is reported in one of the reviews, on literarature regarding seroprevalence that range varies from <0.1% to more than 20% in different regions.²²

The plausible explanation for this variation in seroprevalence could be the timing of estimation of antibodies in epidemic wave and the link between the magnitude of serological responses and severity of illness.²³ In Spain, despite a considerable proportion of population was affected by SARS CoV2, a small fraction of population was found positive for IgG antibodies. There is evidence that an individual variation in immune response to viral exposure happens, particularly in those with mild or asymptomatic disease.^{15,24}

The current study revealed a higher seroprevalence among females as compared to males, (OR of 1.3 and p value being <0.001). This observation is in contradiction with the sex specific seroprevalence elsewhere in the country or world.^{4,5,8,12-16,21} Similar contradictory results were observed when age specific seroprevalence was compared with other studies.^{4,6,8,12-16,25} The possible explanation for increased seroprevalence among females and in individuals aged above 70 years could be that we are at the tail of first epidemic wave and there has been mixing of population, thus the previously less susceptible segment of population i.e. female and elderly individuals who used to follow strict indoor restrictions so as to avoid being COVID positive, have been exposed to this deadly virus somewhere. However increased seroprevalence was reported with the increasing age of participants by one of the researches done in Italy.²⁶ A higher seroprevalence was seen among individuals with comorbidities, however higher among those with multiple comorbidities.^{8,21,27-30} This supports the evidence that individuals with pre existing co- morbidities are at increased risk of contracting severe COVID 19 infection, thus eliciting a strong IgG antibody response against this deadly virus. The study revealed certain significant factors which increased the probability of individual being positive for IgG antibodies against SARS CoV-2. They were presence of ILI symptoms in recent past 3 months, history of contact with known COVID 19 case and presence of co-morbidity.⁸ Those participants with history of ILI had 2.2 times higher chance of being IgG positive as compared to those with no such history. In addition, individuals with history of contact had 1.8 times higher chance of being IgG positive when compared to those with no such history. This supports the evidence that any individual complaining with ILI symptoms should get him/herself tested for RTPCR/RAT.

Interestingly, 21 out of 121 (17%) individuals who were declared COVID positive on RTPCR/RAT were found negative for IgG antibody titres. Further analysis regarding the time since RTPCR and serological testing revealed a time lapse of 1-4 months in all of them. It needs to be mentioned here that the data regarding the time period of sustenance of SARS CoV2 specific Ig G antibodies in people's body following infection is not known. However, there are some studies suggesting that the antibody titres declined during early convalescent phase.³¹⁻³³ Further the proportion of individuals mounting a T cell mediated immunity against this SARS CoV2 cannot be ruled out. A study from the Karolinska Institute reported that the individuals mounting T cell responses after mild covid-19 asymptomatic disease consistently exceeded those mounting a detectable IgG serological responses against the virus.³⁴ The role of Ig A antibodies in combating respiratory infections like that of COVID 19 like illness cannot be ignored^{35,36}. There are studies claiming that serum IgA antibody responses may be detectable earlier than IgG and IgM responses and can persist for at least 38 days in hospital patients recovering from covid.^{37,38} The role of Ig A antibody response is

further supported by a seroprevalence survey done in Ischgl, Austria, using a combined IgG and IgA approach wherein higher seroprevalence of SARS-CoV-2 antibodies were reported; far higher than rates in previous population based surveys.³⁹ In addition, the current study revealed that 36.8% individuals with negative RT PCR results were picked up as positive for Ig G antibodies indicating that a high proportion of asymptomatic cases are prevalent in our community. However, serological tests have their own limitations, with varied sensitivity and specificity. Further it cannot be ruled out the possible confounding by pre existing antibodies against SARS CoV, MERS-CoV or common cold coronavirus infections, thus giving false positive results.^{40,41}

CONCLUSION

The seroprevalence study was conducted with the aim of estimating the extent of SARS CoV2 infection in our settings and it revealed that only 4 out of 10 individuals were positive for antibody titres. The findings of study failed to reveal the true picture of covid-19 infection as there were certain participants who were positive for COVID on RTPCR but were IgG negative. Additionally individual variation of immune response to the virus cannot be ruled out. A considerable number of cases may be missed out in our community owing to the threshold levels being set for IgG titres. Specific consideration should be given to the role of mucosal antibody response and T cell mediated immune response as well. Thus a study which takes into consideration all these relevant features should be planned to reveal the true epidemiological characteristics of SARS CoV2 infection in our settings. Further our findings suggest that SARS CoV 2 IgG seroprevalence was found significantly high among females and it increases with increasing age. These findings have important implications on epidemiological modelling strategies and public health policy as well.

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