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Original Research Article

Asymptomatic SARS-COV-2 carriage and sero-positivity in high risk contacts of COVID-19 cases^{*}Ayan Kumar Das^a, Kailash Chandra^b, Mridu Dudeja^{a,*}, Mohd Khursheed Aalam^c^a Department of Microbiology, HIMSR & HAHC Hospital, New Delhi, 110062, India^b Department of Biochemistry, HIMSR & HAHC Hospital, New Delhi, 110062, India^c Department of Community Medicine, HIMSR & HAHC Hospital, New Delhi, 110062, India

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ABSTRACT

Purpose: Identifying asymptomatic SARS-COV-2 carriage is one of the crucial factors in controlling the COVID 19 pandemic. The relationship between the asymptomatic viral carriage and the rate of seroconversion needs better understanding. The present study was conducted to identify the asymptomatic COVID-19 infection and seropositivity in high-risk contacts in the southern district of Delhi, India.

Methods: Following the screening of 6961 subjects, a total of 407 asymptomatic high-risk subjects were selected. Demographic data, socioeconomic status, and history of COVID-19 related symptoms in the last 4 months were recorded. Blood samples and Nasopharyngeal/oropharyngeal swabs were collected for the detection of SARS-COV-2 RNA and anti-SARS-COV-2 antibodies.

Results: 55 asymptomatic high-risk subjects (13.5%) tested positive for SARS-COV-2 infection and among them, 70.9% remained asymptomatic throughout their course of infection. The seropositivity among the subjects was 28.9% (n = 118) and was found significantly higher among lower-middle socioeconomic strata ($p = 0.01$). The antibody levels were significantly higher ($p = 0.033$) in individuals with a previous history of COVID-19 like symptoms as compared to the subjects, who had no such history. Asymptomatic healthcare workers showed a significantly increased rate of SARS-COV-2 infection ($p = 0.004$) and seropositivity ($p = 0.005$) as compared to the non-healthcare workers. Subjects, who were exposed to infection at their workplace (non-hospital setting) had the least RT-PCR positivity rate ($p = 0.03$).

Conclusions: A large proportion of SARS-COV-2 infection remains completely asymptomatic. The rate of asymptomatic carriage and seropositivity is significantly higher in healthcare workers as compared to the general population. The level of SARS-COV-2 antibodies is directly related to the appearance of symptoms. These observations may contribute to redefining COVID 19 screening, infection control, and professional health practice strategies.

1. Introduction

Asymptomatic carriage of SARS-COV-2 is perhaps one of the major challenges in the control of COVID 19 pandemic. SARS-COV-2 differs from previously known SARS-COV-1 and Middle East Respiratory Syndrome Coronavirus (MERS-COV) in terms of severity, the onset of symptoms, and transmissibility [1]. The role of asymptomatic infections was not understood previously and was acknowledged much later in the pandemic [2]. Asymptomatic individuals escape the quarantine or self-isolation mechanism and remain mobile to infect a large number of people silently for an extended period [1,3]. Alternatively, not all

contacts lead to infection, and the infection rate may depend on several associated factors [3,4].

Sero-conversion is known to happen within 2–3 weeks of infection and the presence of neutralizing antibodies is important for viral clearance and reduction in chances of re-infection [5]. The 3rd serosurvey conducted between December 17, 2020, and January 8, 2021, showed the spread of SARS-COV-2 to 21.5% population [6]. These numbers were quite high as compared to laboratory-confirmed COVID-19 positive cases (20 million by the end of May 2021) and escaping the screening mechanism in absence of symptoms can be one of the many reasons for it. Asymptomatic viral carriage is also important to diagnose as even in

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absence of overt disease, there is a possibility of development of silent changes in the lungs of the infected individuals that might require medical attention or manifest sequelae in later stages [3].

Very limited information is available from India outlining the asymptomatic carriage of SARS-CoV-2 in high-risk contacts and the factors associated with it. In a developing country like India, factors like population density, education, personal hygiene, living condition, accessibility to medical facilities and laboratory tests, etc may vary from region to region. These factors may directly or indirectly alter the rate of asymptomatic carriage and the spread of diseases like COVID-19. The present study was aimed to identify the prevalence of asymptomatic SARS-CoV-2 infection and to estimate the seropositivity rate amongst high-risk contacts including HCWs in the southern district of Delhi, India.

2. Materials and methods

This prospective cross-sectional study was conducted at a tertiary care hospital located in the south district of Delhi. Ethical clearance was obtained from the Institutional Ethics Committee (IEC) of Hamdard Institute of Medical Sciences and Research, New Delhi (letter no: HIMSR/IEC/001/2020, date: 07.08.2020). Post preliminary screening of 6961 subjects, a total of 407 asymptomatic healthy subjects, who had high-risk exposure with any laboratory-confirmed COVID-19 case were enrolled after obtaining written consent between 15th September 2020 and 15th February 2021. Any person who lives in the same household with a laboratory-confirmed COVID19 case, anyone within 1 meter of the confirmed case without precautions, Touched or cleaned the linens, clothes, or dishes of the patient, had direct physical contact with a positive patient without PPE, traveled in close proximity with a positive case or touched body fluids of the case without appropriate PPE were considered as High-risk contacts as defined by Integrated Disease Surveillance Programme, National Centre for Disease Control [7]. Individuals with symptoms consistent with COVID-19 in the last 2 weeks preceding sample collection or had tested positive for SARS-CoV-2 earlier, those vaccinated for SARS-CoV-2, those below 18 years and above 65 years of age, or had any co-morbidities were excluded from the study. Demographic details and the history of contact of each subject were collected by personal and telephonic interviews. History of any illness consistent with COVID 19 in the last 4 months was recorded. The socio-economic status (SES) of the subject was determined by using Kuppusswamy, 2019 socioeconomic scale [8].

5 ml of blood and Nasopharyngeal/oropharyngeal (NP/OP) swabs were collected from the subjects as per national guidelines [9]. Further follow-up of the subjects was done telephonically. The blood samples were analyzed for the presence of anti-SARS-CoV-2 total antibody (IgM + IgG) Elecsys Anti-SARS-CoV-2 kit, through chemiluminescent immunoassay (CLIA) on Cobas e411 analyzer, Roche Diagnostics. The cutoff index (COI; signal sample/cutoff) is generated and result is decided as: Non-reactive/Negative for anti-SARS-CoV-2 antibodies (COI <1.0) or Reactive/Positive for anti-SARS-CoV-2 antibodies (COI >1.0) [10].

For molecular detection of SARS-CoV-2, the Viral RNA was extracted from the collected NP/OP swabs in VTM using QIAamp Viral RNA mini kit, Qiagen, USA. RT-PCR was performed using COVIWOK RT-PCR Kit, Wockhardt Ltd (manufactured as Covid-19 Real-Time PCR Kit v1, SNP technologies, Turkey). The kit is validated and approved by ICMR and detects N gene and RdRp Gene of SARS-CoV-2 and prothrombin gene as an internal control. The CT < 35 was considered cut off for all the genes. The assay was run in the Agilent MX3005P RT-PCR system.

Statistical analysis: Demographic data of the subjects were represented as the mean \pm standard error of mean (SEM). The prevalence percentage and 95% CI was determined. The antibody test COI level for positive cases among symptomatic and asymptomatic subjects was compared with the help of Mann–Whitney *U* test. The level of significance was determined by unpaired, two-tailed *t*-test and χ^2 test where applicable. The level of significance was considered $p < 0.05$.

3. Results

Among the total of 407 subjects in this cross-sectional study, 218 (53.5%) were males. The median age/IQR of the study population was 28 years (24–35 years). The average period (mean \pm SEM) between exposure and collection of NP/OP swab was 4.8 ± 0.14 days. Most of the subjects (53.0%) were from lower middle SES and none were from the lower economic scale. Further 21.1% of subjects had reported a history of symptoms consistent with COVID19 in the last 4 months. 125 participants enrolled in the study were healthcare workers.

SARS-CoV-2 RNA was detected by RT-PCR in 55 of the otherwise asymptomatic study subjects; the point prevalence was 13.5% (95% CI: 10.3–17.2). The average cycle threshold (CT) value (mean \pm SEM) for the N and RdRp gene was 28.15 ± 0.84 and 27.83 ± 0.87 respectively. The subjects found positive for SARS-CoV-2 were advised for home quarantine. 16 subjects, who tested positive and were asymptomatic on day zero, showed mild symptoms consistent with COVID19 in the next 2 weeks and recovered without the requirement of hospitalization or any adverse outcome. The positivity was highest in the age group of 31–40 years (Table 1). Even though, the number of participants was more from lower-middle SES, the point prevalence of SARS-CoV-2 RNA was slightly higher in upper-lower SES (18.9%, 95% CI: 08.5–35.1) (statistically not significant, $p = 0.08$). Moreover, 45.4% of total RT-PCR positive cases were infected in the hospital settings as compared to only 4.7% (95% CI: 01.3–11.7) of the population, who had exposure at their work setting (non-HCWs/general population), and the difference was statistically significant ($p = 0.03$).

SARS-CoV-2 sero-positivity was 28.9% among the entire study population (Table 2). The percentage of seropositive females was slightly higher than males but the difference was statistically insignificant. The seropositivity was significantly higher among the lower middle SES (35.5%, 95% CI: 29.2–42.4) ($p = 0.01$). 75.0% (95% CI: 60.5–85.4) of subjects, who had a history of fever in the last 4 months preceding the sample collection, were seropositive. 6 out of the 7 subjects, who reported anosmia, were found to be seropositive. (Table 2). Out of the 407 asymptomatic subjects, 11 subjects (02.7%) who were RT PCR positive were also positive for SARS-CoV-2 antibody. Interestingly, none of them reported a history of COVID-19 like symptoms.

Sero positivity was compared between subjects, who reported a history of COVID19 like symptoms in the last 4 months with those, who were asymptomatic throughout. Among the previously symptomatic cases, the seropositivity was 44.1% (95%CI: 33.4–55.3), whereas the asymptomatic group reported seropositivity in only 24.9% (95%CI: 20.2–30.0) ($p = 0.00047$) (Fig. 1). The COI value of seropositive subjects with a history of COVID19 like symptoms and group of subjects with no history of symptoms was also compared. The antibody COI (mean \pm SEM) in previously symptomatic subjects and subjects, who had no such history were 52.25 ± 10.61 and 26.41 ± 04.52 respectively (Fig. 2). The difference was statistically significant ($p = 0.033$).

Further, the RT-PCR and seropositivity results were compared between HCWs ($n = 125$) and non HCWs ($n = 282$) (Table 3). The gender ratio within the HCWs was comparable. The RT-PCR positivity for SARS-CoV-2 in HCWs and non-HCWs were 20.8% (95% CI: 14.0–28.9) and 10.2%(95% CI: 07.0–14.4) respectively and the difference was statistically significant (p -value = 0.004). The average CT value for both N and RdRp gene and antibody COI (mean \pm SEM) was compared for HCWs with non-HCWs and no significant difference was found by unpaired two-tailed *t*-test ($p > 0.05$). However, the sero-positivity for SARS-CoV-2 antibodies among HCWs was significantly high at 38.4%(95% CI: 29.8–47.5) as compared to 24.8% (95% CI: 19.8–30.2) in the general population. (p value = 0.005).

4. Discussion

Detection of asymptomatic carriage of SARS-CoV-2 is an important step to arrest the rapid spread of the COVID19 pandemic [1]. In the

Table 1
Demographic details of RT-PCR positive asymptomatic subjects.

	Total subjects	RT-PCR positive	RT-PCR negative	Rate of positivity	95% CI	P value (significant <0.05)
n	407	55	352	13.5%	10.3–17.2	
Age (mean ± SEM)	30.9 ± 0.61	29.6 ± 1.32	31.3 ± 0.68			0.34 (t value:-0.94)
Positivity in different age groups in years						
18–30	249	32	217	13.2%	08.9–17.6	0.09 ($\chi^2 = 7.8$)
31–40	85	18	67	21.1%	13.0–31.3	
41–50	41	03	38	07.3%	01.5–19.9	
51–60	26	01	25	03.8%	0.10–19.6	
61–70	06	01	05	16.6%	0.42–64.1	
71 – above	00	00	00	–		
Gender						
Male	218	28	190	12.8%	08.7–18.0	0.67 ($\chi^2 = 0.18$)
female	189	27	162	14.2%	09.6–20.1	
Socio economic status (As per Kuppuswamy scale)						
Upper	55	09	46	16.3%	07.7–28.8	0.08 ($\chi^2 = 6.5$)
Upper middle	99	06	93	06.0%	02.2–12.7	
Lower middle	216	33	183	15.2%	10.7–20.7	
Upper lower	37	07	30	18.9%	08.5–35.1	
lower	00	00	00	–		
Place of exposure						
Home	123	16	107	13.0%	07.6–20.2	0.03($\chi^2 = 8.6$)
Community	50	10	40	20.0%	10.0–33.7	
Office ^a	84	04	80	04.7%	01.3–11.7	
Hospital	150	25 ^b	125	16.6%	11.0–23.6	
Relationship with contact						
Family member	123	16	107	13.0%	07.6–20.2	0.42($\chi^2 = 3.8$)
Colleague	191	22	169	11.5%	07.3–16.9	
Patient	43	07	36	16.2%	06.8–30.7	
Friend	34	08	26	23.5%	10.7–41.1	
Neighbor	16	02	14	12.5%	01.5–38.3	

Level of significance was calculated by unpaired 2 tailed t-test and chi square test.

^a For non HCWs.

^b All were healthcare workers.

Table 2
Demographic Details of sero positive subjects.

	Total subjects	Antibody positive	Antibody negative	Sero prevalence	95% CI	P value (significant <0.05)
n	407	118	289	28.9%	24.6–33.6	
Age in years (mean ± SEM)	30.9 ± 0.61	31.8 ± 1.22	30.8 ± 0.71			0.43 (t value: 0.78)
Gender						
Male	218	56	162	25.6%	20.3–32.0	0.11 ($\chi^2 = 2.4$)
female	189	62	127	32.8%	26.1–39.9	
Socio economic status (As per Kuppuswamy scale)						
Upper	55	13	42	23.6%	13.2–37.0	0.01 ($\chi^2 = 10.1$)
Upper middle	99	21	78	21.2%	13.6–30.5	
Lower middle	216	77	139	35.6%	29.2–42.4	
Upper lower	37	07	30	18.9%	07.9–35.1	
Lower	00	0	0	–	–	
History of symptoms in last 4 months						
fever	44	33	11	75.0%	60.5–85.4	
Cough	35	13	22	37.1%	23.1–53.6	
Dyspnoea	01	01	0	100%	–	
Sore throat	34	11	23	32.3%	19.1–49.1	
Body ache	20	8	12	40.0%	21.8–61.3	
Headache	28	8	20	28.5%	15.2–47.0	
Loss of smell/taste	7	6	1	85.7%	48.6–97.4	

Level of significance was calculated by unpaired 2 tailed t-test and chi square test.

present study, the point prevalence of asymptomatic RT-PCR positive cases was found to be 13.5% in the southern district of Delhi. Comparatively higher asymptomatic viral carriage at 20.5% was reported by *Wattal* et al. in a study conducted in the New Delhi district [11]. Even higher rate has been reported in a few other studies [3,12,13]. As it may take a while for an individual to develop overt symptoms, we followed all the subjects included in the study for 14 days. Among the 55 RT-PCR positive subjects, 70.9% remained completely asymptomatic during the study period. The finding is comparable to that of *Day* et al. from Italy [14].

There was no difference in terms of gender among the asymptomatic carriers ($p = 0.67$). This is in contrast to findings of both *Dai* et al. and *Abate* et al. The former reported that young females are more prone to be asymptomatic carriers [15], whereas higher prevalence among males was reported by the latter [16]. No significant difference in positivity was seen among the study age groups. However, among the RT-PCR positive cases, 58.9% of subjects were within 18–30 years of age. Various studies have reported that young adults tend to remain asymptomatic during the course of infection [3]. A study done at AIIMS Patna relates upper socio-economic level with high positivity [17]. Even though no

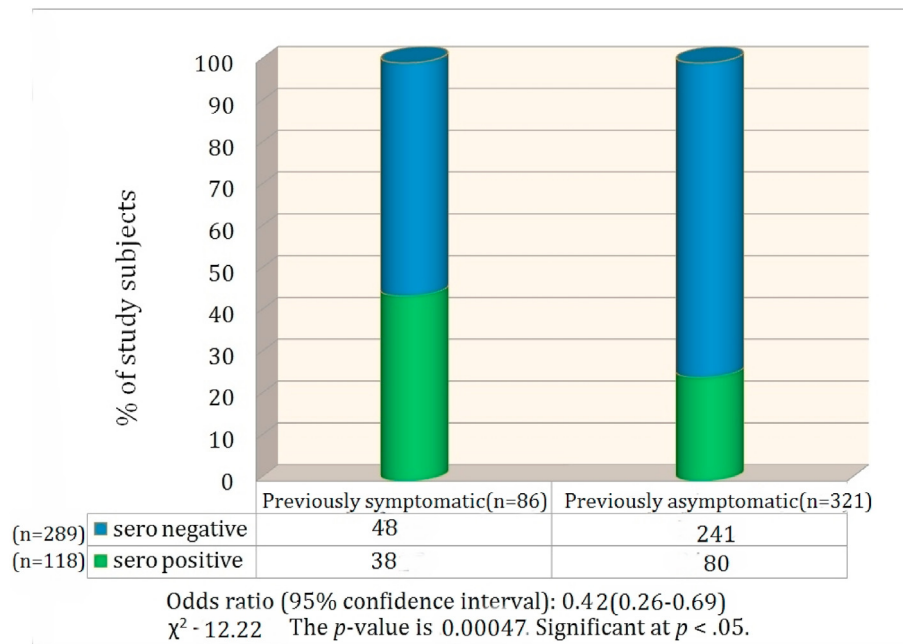


Fig. 1. Comparison of sero-positivity among asymptomatic subjects with or without past history of COVID 19 like symptoms.

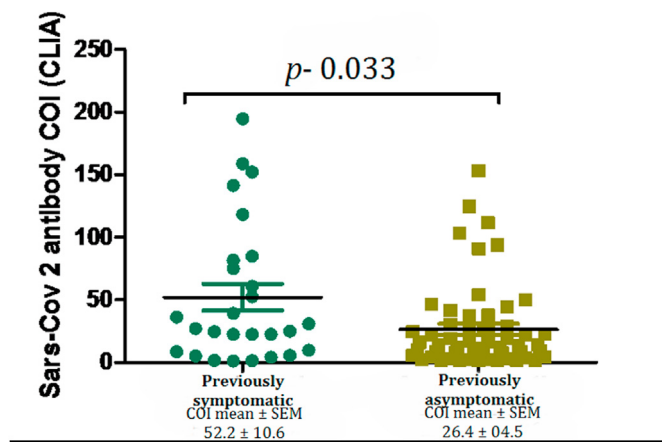


Fig. 2. Correlation of antibody COI level in asymptomatic subjects with or without past history of COVID-19 like symptoms (significant at *p* < 0.05).

significant difference in RT-PCR positivity rate among different socio-economic groups was found here, the upper-middle-class showed the least positivity. When the place of exposure was compared, a significantly lesser rate of RT-PCR positivity was found in those exposed at their work settings (non-healthcare) (*p* = 0.03). This finding may indicate that people tend to follow preventive protocols more stringently at their workplaces in comparison to their home environment or the community. No significant correlation was seen between the rate of positivity and the relationship with contacts (*p* = 0.42). However, we observed a higher positivity rate, when the subjects were exposed to an infected friend in the community (23.5%, 95% CI: 10.7–41.1). A similar association is seen in the case of healthcare workers. Twice the numbers of HCWs were infected, when exposed to their COVID 19 positive colleagues rather than when exposed to patients. This again can be attributed to lowering of their guards, in terms of preventive measures, in a more relaxed setting.

More than one-fourth of the subjects carried antibodies against SARS-COV-2. The finding is much higher than the 2nd national serosurvey

Table 3

Comparison of viral carriage and sero positivity between HCWs and non HCWs.

	Health care workers	Non Health care workers	P value (significant <0.05)
Total subjects	125	282	
Age - (Mean ± SEM)	29.2 ± 0.80	31.5 ± 0.78	0.076 (t value:-1.7)
Gender			0.30 ($\chi^2 = 1.04$)
Male	59	159	
Female	66	123	
RT-PCR positive	26 (20.8%)	29 (10.2%)	0.004 ($\chi^2 = 8.19$)
95% CI	14.0–28.9	07.0–14.4	
N gene CT- (Mean ± SEM)	27.7 ± 1.27	27.6 ± 1.05	0.56 (t value:0.57)
RdRp gene CT- (Mean ± SEM)	27.1 ± 1.29	26.9 ± 0.95	0.53 (t value:0.63)
Antibody positive	48 (38.4%)	70 (24.8%)	0.005 ($\chi^2 = 7.7$)
95% CI	29.8–47.5	19.8–30.2	
Antibody COI ratio for positive subjects (Mean ± SEM)	38.4 ± 7.47	32.3 ± 6.25	0.53 (t value: 0.62)

conducted in August–September 2020 [18]. However, it must be considered that the present study was done on specific subjects of limited numbers and the findings do not necessarily represent the seroprevalence of the entire population of the region. While assays detecting antibodies against SARS-COV-2 nucleocapsid antigen, as used in the present study, has higher sensitivity during early stages of infection than antibodies against spike proteins, the former decay faster post recovery and can be a limiting factor. Similar studies have reported 39% seropositivity in Karnataka between June–August 2020 and 57.9% in a south Indian slum [19,20]. Though sero-positivity was slightly higher among female subjects, there was no significant difference in concurrence to most surveys done around the world [21,22]. Few studies have been done to establish the relationship between SES with COVID-19 seroprevalence. Peruvian and South African reports show a significant increase in seroprevalence with the decrease in SES [23,24]. In our study, significantly higher

seropositivity was seen among participants from lower-middle SES ($p = 0.01$). The seropositivity among the SARS-CoV-2 RT PCR positive subjects was 20%. The percentage is quite high to indicate re-infection and rather may suggest convalescent stage of asymptomatic infection in those individuals.

Among the presently asymptomatic study subjects, seropositivity was higher in those, who experienced fever and anosmia/ageusia in the preceding 4 months. Worldwide, fever, fatigue, and cough have been identified as the most common manifestations amongst many other known symptoms [11]. *Wattal* et al. reported fever in 59.8% of COVID19 patients [11]. But the presence of asymptomatic RT-PCR positive cases warrants that contacts should be screened irrespective of the presence or absence of symptoms. *Long* et al. found a poor immune response and lower level of pro and anti-inflammatory cytokines in asymptomatic cases [25]. We demonstrated significantly higher sero-positivity among subjects with a history of symptoms in comparison to those, who reported no such history ($p = 0.00047$). Though the comparison of COI between symptomatic and asymptomatic subjects is limited by the qualitative nature of the assay, higher antibody levels can be observed in the group of subjects with a history of COVID19 like symptoms than in the asymptomatic group. The finding was statistically significant ($p = 0.033$). A similar conclusion was drawn by *Shields* et al. [4]. However, the antibody response depends on a few other factors like nutrition, individual constitution, innate immunity, cell-mediated immunity, nature of infecting viral strain, etc, and further studies with larger group sizes are needed to understand the factors influencing post-infection antibody levels.

We also compared the laboratory findings among asymptomatic HCW and non-HCWs. A study from Maharashtra found only 1.6% asymptomatic HCWs as RT-PCR positive [26]. The seropositivity among HCWs detected by the study from eastern India was comparable to our findings [27]. We found a significantly higher level of seropositivity ($p = 0.005$) as well as viral carriage ($p = 0.004$) amongst the HCWs than in non-HCWs. Prolonged exposure to a high-risk environment can be considered as a major cause of such findings. Nevertheless, the mere presence of antibodies does not ensure future protection. Cases of re-infection are reported all around the world. Studies estimating the IgG decay rate and protective level of neutralization antibodies will help us to appreciate the mechanism of antibody response in COVID-19.

5. Conclusion

The study identifies the crucial role played by asymptomatic carriage in conjunction with seropositivity in the spread of COVID 19 infection thereby emphasizing the need for judicious screening of asymptomatic viral carriage and sero-positivity to control the COVID-19 pandemic. These observations may contribute to redefining COVID 19 screening, infection control, and professional health practice strategies.

CRedit authorship contribution statement

Ayan Kumar Das: Investigation, Software, Writing – original draft. **Kailash Chandra:** Methodology, Investigation, Validation. **Mridu Dudeja:** Conceptualization, Supervision, Writing – review & editing. **Mohd Khursheed Aalam:** Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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