Seroprevalence of immunoglobulin G antibodies against SARS-CoV-2 in children and adolescents in Delhi, India, from January to October 2021: a repeated cross-sectional analysis

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ABSTRACT

Objectives: The aim of this study was to assess changes in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) immunoglobulin G (IgG) seroprevalence among children and adolescents in Delhi, India from January 2021 to October 2021.

Methods: This was a repeated cross-sectional analysis of participants aged 5 to 17 years from 2 SARS-CoV-2 seroprevalence surveys conducted in Delhi, India during January 2021 and September to October 2021. Anti-SARS-CoV-2 IgG antibodies were detected by using the VITROS assay (90% sensitivity, 100% specificity).

Results: The seroprevalence among 5- to 17-year-old school-age children and adolescents increased from 52.8% (95% confidence interval [CI], 51.3%−54.3%) in January 2021 to 81.8% (95% CI, 80.9%−82.6%) in September to October 2021. The assay-adjusted seroprevalence was 90.8% (95% CI, 89.8%−91.7%). Seropositivity positively correlated with participants’ age (p < 0.001), but not sex (p = 0.388). A signal to cut-off ratio ≥4.00, correlating with the presence of neutralization antibodies, was observed in 4,814 (57.9%) participants.

Conclusion: The high percentage of seroconversion among children and adolescents indicates the presence of natural infection-induced immunity from past exposure to the SARS-CoV-2 virus. However, the lack of hybrid immunity and the concomitant likelihood of lower levels of neutralization antibodies than in adults due to the absence of vaccination warrants careful monitoring and surveillance of infection risk and disease severity from newer and emergent variants.

Keywords: COVID-19; India; Infection; SARS-CoV-2
Introduction

People below 18 years of age comprise nearly 34% of the Indian population [1], but prior to the emergence of newer variants of concern accounted for <5% of the total burden of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections [2]. Global evidence is indicative of children and adolescents having less susceptibility to SARS-CoV-2 infection than adults, with much lower rates of severe coronavirus disease 2019 (COVID-19) [3,4]. A retrospective analysis of mortality data from the state of Odisha in India with a population of approximately 43 million reported only 36 COVID-19-related child deaths through August 2021 [5]. Furthermore, a small fraction of the pediatric population experiences the persistence of symptoms beyond several weeks post-recovery, signifying the need to contain transmission [6].

Worldwide seroprevalence studies in the general population have reported comparable infection rates between children and adults despite the lower incidence of confirmed cases in the former, signifying the asymptomatic or mild course of illness in children that may frequently go undetected [7,8]. However, the transmission dynamics of the virus in children are unclear, for which reason repeated cross-sectional serosurveys in the same geographic area may provide crucial insights into the spread of infection in this group [3,9]. Furthermore, COVID-19 vaccination coverage in the under-18 age group in most lower-middle-income countries is low, rendering them more vulnerable to infection and disease [10]. The objective of this study was to assess changes in SARS-CoV-2 immunoglobulin G (IgG) seroprevalence among children and adolescents from January 2021 to October 2021.

Materials and Methods

This was a repeated cross-sectional analysis of 5- to 17-year-old participants from 2 SARS-CoV-2 seroprevalence surveys conducted in Delhi, India during January 2021 and September to October 2021. Both serosurveys were conducted in the general population aged ≥5 years and had an identical sample size (approximately 43 million), sampling methodology and laboratory procedures [11]. The time intervals represent the period before and after the second wave of the COVID-19 pandemic in Delhi, India, which was predominantly caused by the SARS-CoV-2 Delta (B.1.617.2) variant.

Delhi is a city and union territory of India with a population of roughly 19 million distributed across 11 districts and 280 wards with 5 major types of residential settlements, comprising planned colonies, urban slums, resettlement colonies, unauthorized colonies, and rural areas [12]. Each ward has a median population of approximately 70,000. A total of 4,286 participants aged between 5 to 17 years were included in the September to October serosurvey round. This sample size was adequate considering an expected seroprevalence of 53%, as in the January 2021 round [11], with 95% confidence levels, 2% absolute precision, a design effect of 1.5, and a 20% non-response rate. Two-stage sampling was used. Within each ward, a line-list of settlements was made, and each settlement was classified as 1 of the 5 types: (1) planned colonies, (2) resettlement colonies, (3) urban slums/ JJ clusters, (4) unauthorized colonies, and (5) villages. Next, the proportion of population belonging to each settlement type for every ward was tentatively estimated. One hundred participants were then selected from each ward, stratified according to the settlement type, with the probability proportional to the (settlement type) population size estimated in the previous step. The sampling areas within each ward were then selected from the line-list of available settlement types, with a preference for selecting 2 areas per settlement type using the simple random sampling method. Within each selected sampling area, households were selected through systematic random sampling. Finally, from each household, a single participant was selected using the age-order procedure, wherein all the eligible members were listed in ascending order of their ages, with subsequent application of the lottery method.

Data in the January 2021 round were collected on paper, while in the September to October 2021 round, data were collected electronically using a customized Android tablet application. The data collection for each of these rapid serosurveys lasted 12 to 15 days. The field volunteers were trained in several batches through virtual (online) training sessions on the sampling strategy, selection of participants, data entry, labeling of vials, and validation rules for the generation and assignment of a unique identification number to each participant.

From each participant, 3 to 4 mL of venous blood was collected under aseptic precautions by a trained phlebotomist and transported for processing to a single designated laboratory. The VITROS assay on VITROS 3600 (Ortho Clinical Diagnostics, Raritan, NJ, USA), which is based on chemiluminescence technology, was used for the screening and detection of anti-SARS-CoV-2 IgG antibodies [13]. This assay was reported as having a specificity of 100% and a sensitivity of 90%, which meets the World Health Organization’s prescribed guidelines for conducting SARS-CoV-2 serosurveys [14]. A signal to cut-off (S/CO) ratio of ≥1 was considered as reactive and <1 as non-reactive. Using the current assay, the presence of SARS-CoV-2 neutralizing antibodies is strongly correlated with an S/CO ratio ≥4.0 [15].
Statistical Analysis
The data were analyzed using IBM SPSS ver. 25.0 (IBM Corp., Armonk, NY, USA). The seroprevalence estimates were reported as proportions with 95% confidence intervals (CIs). The adjusted seroprevalence was estimated after application of the Rogen-Gladen estimator, which allows a statistical correction based on the test assay's sensitivity and specificity using the formula, true prevalence = apparent prevalence + (specificity−1)/(specificity+sensitivity−1) [16].

The results were expressed as frequency and proportions for categorical variables. Continuous variables were reported as mean and standard deviation for those with a normal distribution, and as median and interquartile range for those with a non-normal distribution. The chi-square test was used to assess associations between categorical variables. A p-value of <0.05 was considered to indicate statistical significance.

Ethics
The study was approved by the Institutional Ethics Committee of Maulana Azad Medical College & Associated Hospitals, New Delhi (vide F1/IEC/MAMC/85/03/2021/No428, dated 21.08.2021). We enrolled children aged <7 years after electronically obtaining parental consent, while for those aged from 7 to 17 years, both participant assent and parental consent were obtained electronically.

Results
Participant Characteristics (September to October 2021 Round)
A total of 4,286 participants were initially recruited of which blood samples were successfully processed in 4,211 cases including 2,165 males (51.4%) and 2,046 (48.6 %) females. The mean ± standard deviation age of the participants was 12.7 ± 3.4 years. The participants were enrolled from the following housing settlement types: planned colonies, 896 (23.2%); urban slums, 1,780 (46.0%); resettlement colonies, 454 (11.7%); unauthorized colonies, 211 (5.4%); and villages, 527 (13.6%) (n = 3,868, missing = 343).

Anti-SARS-CoV-2 IgG antibodies were detected in 3,445 participants. The crude SARS-CoV-2 IgG seroprevalence was 81.8% (95% CI, 80.9%–82.6%), and after further adjustment for assay characteristics, the seroprevalence was estimated as 90.8% (95% CI, 89.8%–91.7%).

Change in SARS-CoV-2 IgG Seroprevalence and the Predictors of Seropositivity (January to October 2021)
The seroprevalence of SARS-CoV-2 infection in the 5 to 17 years age-group increased from 52.8% in January 2021 to 81.8% in September to October 2021. The IgG seroprevalence increased from 48.4% to 75.9% in the 5 to 9 years age group, from 54.5% to 82.8% in the 10 to 14 age group, and from 52.0% to 83.8% in the 15 to 17 years age group (Table 1). In the adjusted analysis, older (15−17 years) adolescents had significantly higher odds of infection (adjusted odds ratio, 1.6; 95% CI, 1.4–1.9) than younger children (5−9 years), but no statistically significant association was observed with participants’ sex (Table 2). Residence in slums and resettlement colonies was independently associated with higher seropositivity in January 2021, but not during the September to October 2021 round (Table 3).

Distribution of the Signal to Cut-Off Ratio in the Seropositive Participants (September to October 2021)
The median (interquartile range) S/CO ratio in the SARS-CoV-2 seropositive (IgG) subgroup was 6.7 (3.5−10.8). A S/CO ≥4.00 was observed in 58% (n = 4,211) participants and in 70.8% of the seropositive (IgG) subgroup (n = 3,445). Among

| Variable | Sample size | Crude seroprevalence (%) (95% CI) | After assay adjustment (%) (95% CI) |
|----------|-------------|-----------------------------------|-----------------------------------|
| 5−9 y    |             |                                   |                                   |
| January 2021 | 701          | 48.4 (44.6−52.1)                  | 53.7 (49.6−57.8)                  |
| October 2021| 823          | 75.9 (72.9−78.7)                  | 84.3 (81.0−87.5)                  |
| 10−14 y  |             |                                   |                                   |
| January 2021 | 1,757        | 54.5 (52.2−56.9)                  | 60.6 (58.0−63.2)                  |
| October 2021| 1,836        | 82.8 (81.0–84.4)                  | 92.0 (90.0−93.8)                  |
| 15−17 y  |             |                                   |                                   |
| January 2021 | 1,879        | 52.0 (49.7−54.3)                  | 57.8 (55.2–60.3)                  |
| October 2021| 1,552        | 83.8 (81.8−85.5)                  | 93.1 (90.9–95.0)                  |

IgG, immunoglobulin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; CI, confidence interval.

On crude seroprevalence.
the SARS-CoV-2 seropositive (IgG) participants, there was a statistically significant difference in the S/CO ratio between school going adolescents (12−17 years) and younger children (5−11 years; \( p < 0.001 \)), but not between male and female participants (\( p = 0.114 \)) (Table 4).

## Discussion

The present study shows that approximately 4 out of 5 children and adolescents aged below 18 years had evidence of past exposure to SARS-CoV-2 infection, which, after adjustment for the tests' imperfections, was estimated to correspond to a true seroprevalence of over 90% \([11]\). The high percentage of seroconversion among unvaccinated children and adolescents in this study indicates the presence of natural infection–induced immunity from past exposure to SARS-CoV-2. In comparison, the SARS-CoV-2 IgG seroprevalence in the adult population in Delhi increased from 50.3% (95% CI, 49.7%−51.0%) in January 2021 to 91% (95% CI, 90.6%−91.4%) in September to October 2021 (Figure 1) due to natural infection, COVID-19 vaccination, or hybrid immunity \([11,17]\).

The nationwide serosurveys conducted by the Indian Council of Medical Research also reported the SARS-CoV-2 seroprevalence to have increased from 27.2% (95% CI, 24.9%−29.4%) in December 2020 to 60.1% (95% CI, 59%−61.1%) in July 2021 in the 10−17 and 6−17 age groups, respectively \([7,18]\). The increased seroprevalence in Delhi was probably due to the severe impact of the second wave of the COVID-19 pandemic in Delhi; this wave caused over 0.73 million cases,

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### Table 2. Factors associated with SARS-CoV-2 seropositivity (September to October 2021)

| Variable                      | n (%)            | IgG seropositive (%) | Adjusted odds ratio (95% CI) | p-value |
|-------------------------------|------------------|----------------------|------------------------------|---------|
| **Age (y) (n = 4,211)**      |                  |                      |                              |         |
| 5−11                          | 1,493 (35.5)     | 1,164 (78.0)         | 1                            | <0.001  |
| 12−17                         | 2,718 (64.5)     | 2,281 (83.9)         | 1.5 (1.2−1.7)                |         |
| **Sex (n = 4,211)**           |                  |                      |                              | 0.18    |
| Male                          | 2,165 (51.4)     | 1,765 (81.5)         | 1                            |         |
| Female                        | 2,046 (48.6)     | 1,680 (82.1)         | 1.1 (0.9−1.3)                |         |
| **Settlement type (n = 3,868)** |            |                      |                              | 0.33    |
| Slum/resettlement             | 2,234 (57.8)     | 1,814 (81.2)         | 0.92 (0.9−1.1)               |         |
| Planned/unauthorized/village  | 1,634 (42.2)     | 1,345 (82.3)         | 1                            |         |
| **Diagnosed with COVID-19 (n = 3,865)** | | | 0.90 | |
| Yes                           | 822 (21.3)       | 674 (82.0)           | 1.0 (0.8−1.2)                |         |
| No                            | 3,043 (78.7)     | 2,482 (81.6)         | 1                            |         |

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; IgG, immunoglobulin G; CI, confidence interval; COVID-19, coronavirus disease 2019.

### Table 3. Factors associated with SARS-CoV-2 seropositivity (January 2021)

| Variable                      | n (%)            | IgG seropositive (%) | Adjusted odds ratio (95% CI) | p-value |
|-------------------------------|------------------|----------------------|------------------------------|---------|
| **Age (y)** (n = 4,338)       |                  |                      |                              |         |
| 5−11                          | 1,312 (30.2)     | 656 (50.0)           | 1                            | 0.03    |
| 12−17                         | 3,026 (69.8)     | 1,618 (53.5)         | 1.1 (1.0−1.3)                |         |
| **Sex**                       |                  |                      |                              | 0.049   |
| Male                          | 2,091 (48.2)     | 1,064 (50.9)         | 1                            |         |
| Female                        | 2,247 (51.8)     | 1,210 (53.9)         | 1.1 (1.0−1.3)                |         |
| **Settlement type**           |                  |                      |                              | 0.08    |
| Slum/resettlement             | 1,736 (40.0)     | 938 (54.0)           | 1.1 (1.0−1.3)                |         |
| Planned/authorized/village    | 2,602 (60.0)     | 1,336 (51.3)         | 1                            |         |
| **Diagnosed with COVID-19**   |                  |                      |                              | <0.001  |
| Yes                           | 102 (2.4)        | 77 (75.5)            | -                            |         |
| No (n = 4,301)                | 4,199 (97.6)     | 2,175 (51.8)         | -                            |         |

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; IgG, immunoglobulin G; CI, confidence interval; COVID-19, coronavirus disease 2019; -, not included in the regression (adjusted model).

*37 Values were missing.
including 11,075 deaths [19]. A similar large increase in SARS-CoV-2 seropositivity was reported in the aftermath of the Delta wave among children in England [20]. In this study, higher seroprevalence in those with a history of laboratory-confirmed COVID-19 was not observed. However, only 2.4% of the participants had a known history of COVID-19, a finding which agrees with previous studies suggestive of a significantly higher frequency of asymptomatic infections and undertesting in children than in adults [21,22]. A population-based study in Geneva also observed lower seroprevalence rates among younger (6–9 years old) children [23]. Moreover, in this study, high rates of seroconversion were observed in multiple pediatric age groups, a finding also reported in German and Danish studies [4,24]. However, a large cohort study in Canada observed that younger children were more likely than older children to transmit SARS-CoV-2 infection to other household members, suggestive of divergent dynamics of disease transmission [25].

The present study has the following key implications. The high percentage of seroconversion among children and adolescents indicates the presence of natural infection-induced immunity from past exposure to SARS-CoV-2. Although variants of concern, especially Omicron, can potentially bypass this immune response and cause reinfection and incident disease, the possibility of severe disease needing hospitalization is likely to remain low [26]. Careful monitoring and surveillance are needed to detect any possible effect of the absence of hybrid immunity because of a lack of vaccination in children compared to adults on their overall risk of infection and disease severity from newer and emergent variants. Nevertheless, evidence for prioritizing the vaccination of children at the expense of unvaccinated adult populations nationally and globally is lacking, since seroprevalence in children is comparable to that in adults.

Certain limitations of this study should be noted. First, the field volunteers did not adhere to the guideline for recording details of non-responding households in the data collection application, for which reason there was no audit trail for non-response estimates. We tentatively estimated the non-response rate to be <20% based on the feedback provided by the field volunteers and experiences from the previous rounds of the serosurveys. Most population-based seroprevalence studies in India have also reported high non-response rates, especially in the pediatric age group [17,27]. In this study, major reasons for non-response were parental concerns, fear of pain during blood sample collection, and the perceived lack of individual benefit. Nevertheless, considering the high seroprevalence, the non-response bias is unlikely to have significantly impacted the results of this study.

Second, the sex distribution of the 5 to 17 years age-group population according to the 2011 census estimates for Delhi is approximately 54% males and 46% females, compared to 51.4% and 48.6% in the study sample. Considering the observation of slightly higher seroprevalence in females than in males, the absence of adjustment for sex weights would have slightly underestimated the true seroprevalence of SARS-CoV-2 in the pediatric population. Third, SARS-CoV-2 neutralizing antibodies were screened in only a subset (approximately 10%) of the participants, and the correlation observed with the S/CO ratio was then generalized to the complete sample as an indirect predictor of immunological

Table 4. IgG SARS-CoV-2 seroprevalence and S/CO ratio in children, September to October 2021

| Age group (y) | Male (n = 1,765) | Female (n = 1,680) | Total (n = 3,445) |
|---------------|-----------------|--------------------|------------------|
|               | S/CO ≥4         | S/CO               | S/CO ≥4          | S/CO               | S/CO ≥4          | S/CO               |
| 5–11          | 383 (66.0)      | 6.4 ± 3.9          | 410 (70.2)       | 6.8 ± 3.9          | 793 (68.1)       | 6.6 ± 3.9          |
| 12–17         | 843 (71.1)      | 7.7 ± 4.7          | 807 (73.6)       | 7.9 ± 4.7          | 1,650 (72.3)     | 7.8 ± 4.7          |

Data are presented as n (%) or mean ± standard deviation.

IgG, immunoglobulin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S/CO, signal to cut-off.

*a) IgG seropositive only.

Figure 1. Comparison of immunoglobulin G (IgG) seroprevalence in under-18 and adult participants.
protection [15]. Fourth, there is growing recognition of the waning of anti-SARS-CoV-2 IgG antibodies, which may reduce the seroprevalence levels but may not necessarily have a detrimental impact on immune protection against reinfection because of existing cell media immunity and immunological memory [28,29]. Fifth, we were unable to estimate the durability of antibody levels because of the lack of prospective follow-up of the study participants.

In conclusion, nearly 9 in 10 children and adolescents in Delhi had IgG antibodies against SARS-CoV-2, with high proportions of seroconversion observed across multiple age-group groups and both sexes. Future studies should assess the real-world effectiveness of COVID-19 vaccines authorized for pediatric groups in preventing symptomatic infection, inhibiting disease transmission, protecting against severe disease, and avoiding long-COVID symptoms.

Notes

Ethics Approval
The study was approved by the Institutional Ethics Committee, Maulana Azad Medical College & Associated Hospitals, New Delhi (vide F.I./IEC/MAMC/85/03/2021/No428 dated 2108.2021).

Conflicts of Interest
The authors have no conflicts of interest to declare.

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Availability of Data
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors’ Contributions
Conceptualization: all authors; Data curation: SM; Formal analysis: SB; Investigation: PS; Methodology: all authors; Project administration: PS, SM; Resources: MMS; Supervision: PS, MMS; Validation: PS, MMS; Writing-original draft: SB; Writing-review & editing: all authors.

Additional Contributions
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