Seroprevalence of SARS-CoV-2 infections among children visiting a hospital

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ABSTRACT
Importance: In this study, we retrospectively investigated the seroprevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies within serum samples from children in Beijing, China. These findings provide preliminary guidance regarding population susceptibility to SARS-CoV-2, which will aid in establishing policy toward coronavirus disease 2019 (COVID-19) prevention and control.

Objective: To understand the seropositivity of anti-SARS-CoV-2 IgM/IgG antibodies among children in Beijing, China, evaluate the susceptibility of children in Beijing to SARS-CoV-2, and provide prima facie evidence to guide SARS-CoV-2 prevention and control.

Methods: IgM/IgG antibody kits (colloidal gold) were used to conduct preliminary screening of SARS-CoV-2 IgM/IgG antibodies in serum samples of children who presented to Beijing Children’s Hospital, Capital Medical University, having fever or requiring hospitalization, from March 2020 to August 2020. Statistical analysis of anti-SARS-CoV-2 antibody seropositivity was performed according to the children’s general demographic characteristics, timing of admission to hospital, presence of pneumonia, and viral nucleic acid test results.

Results: The study included 19 797 children with both IgM and IgG antibody results. Twenty-four children had anti-SARS-CoV-2 IgM-positive results (positive rate of 1.2‰), twelve children had anti-SARS-CoV-2 IgG-positive results (positive rate of 0.6‰). Viral nucleic acid test results were negative for the above-mentioned children with positive antibody findings; during the study, two children exhibited positive viral nucleic acid test results, but their anti-SARS-CoV-2 IgM/IgG antibody results were negative. Anti-SARS-CoV-2 IgM antibody seropositivity was higher in the <1-year-old group than in the ≥6-year-old group. The rates of anti-SARS-CoV-2 IgM seropositivity was highest in August from March to August; IgG results did not significantly differ over time. The rates of anti-SARS-CoV-2 IgM or IgG seropositivity among children with and without suspected pneumonia did not significantly differ between groups.

Interpretation: During the study period, the rates of anti-SARS-CoV-2 IgM/IgG antibody seropositivity were low among children who presented to Beijing Children’s Hospital, Capital Medical University. The findings suggest that children in Beijing are generally susceptible to SARS-CoV-2 infection; COVID-19 prevention and control measures should be strengthened to prevent disease in children.

KEYWORDS
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Coronavirus disease 2019 (COVID-19), Antibody, Seroprevalence, Children
INTRODUCTION

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been a global public health problem. Accordingly, the World Health Organization declared that COVID-19 constitutes a “public health emergency of international concern.” As of November 26, 2020, the cumulative number of confirmed cases worldwide has exceeded 60.42 million, while the cumulative number of deaths has exceeded 1.42 million (https://covid19.who.int). The main clinical manifestations of SARS-CoV-2 infection are fever, fatigue, and dry cough. Some patients exhibit nasal congestion, runny nose, sore throat, diarrhea, and other symptoms. In patients with severe disease, breathing difficulties and/or hypoxemia may occur; these manifestations rapidly progress to acute respiratory distress syndrome, cytokine storm, and septic shock.¹

Unlike adult patients with COVID-19, the severity of disease in pediatric patients is relatively mild.²⁻⁴ The cause of this difference is unclear and may be related to several factors. First, seasonal coronavirus infections already occur in many children⁵,⁶ and the resulting antibodies may have a cross-protective effect against SARS-CoV-2.⁷⁻¹⁰ Second, compared with adults, the activity of angiotensin converting enzyme 2 is weaker in children, which may cause reduced susceptibility to SARS-CoV-2.¹¹ Third, the immune system is not fully established in children; severe illnesses caused by microbial infection may be avoided. For instance, primary Epstein–Barr virus infection usually causes only subclinical or mild symptoms in young children, whereas it is more likely to cause infectious mononucleosis in older children and adolescents.¹²⁻¹⁴ Furthermore, there are significantly fewer pediatric patients with COVID-19, compared with adult patients.¹⁵,¹⁶ This is presumably because children have had fewer environmental exposures to SARS-CoV-2.¹²,¹⁵ However, pediatric patients may not undergo laboratory tests because they exhibit few or no symptoms, thus, there are relatively few laboratory confirmed cases.¹⁷⁻²⁰ Some epidemiological studies have indicated that SARS-CoV-2 susceptibility and spread characteristics are less robust in children than in adults.¹⁷⁻²¹,²² To the best of our knowledge, there have been few evidence-based reports concerning pediatric susceptibility to SARS-CoV-2. Pan et al.²³ suggested that the detection of anti-SARS-CoV-2 antibodies in specific populations could aid in evaluation of population susceptibility.

To further implement the requirements of the Joint Prevention and Control Mechanism for COVID-19, Beijing Children’s Hospital has carried out SARS-CoV-2 nucleic acid and serum antibody tests for children who have a fever or require hospitalization. In this study, we retrospectively analyzed the anti-SARS-CoV-2 antibody results of these children to understand the epidemiological characteristics of serum antibodies against SARS-CoV-2 among children in Beijing. We also aimed to provide a preliminary assessment of population susceptibility to SARS-CoV-2 infection.

METHODS

Ethical approval

The study was approved by the Ethics Committee of Beijing Children’s Hospital (2020-Z-158). This was a retrospective study and was exempt from informed consent from patients.

Study participants

This study included 19,979 children presented to Beijing Children’s Hospital between January 1, 2020, and August 31, 2020. Information was collected regarding general demographics, timing of admission to hospital, presence of pneumonia, and nucleic acid test results. If a single child was treated ≥2 times and their antibody or nucleic acid test results were consistent, only the results of the first visit were included in the analysis. The initial diagnosis of pneumonia excluded non-infectious types of pneumonia (e.g., aspiration pneumonia, bronchitis obliterans, chronic bronchitis, asthmatic bronchitis, necrotizing pneumonia, and allergic pneumonia).

IgM/IgG antibody detection

Two milliliters of venous blood were collected from each patient by using vacuum blood collection tubes supplemented with EDTA-K2. The tubes were then incubated at room temperature for 2 hours. Subsequently, 10 μL of the serum layer was added to the sample hole of the reagent strip, along with 80 μL of sample diluent was added; the result was observed after incubation at room temperature for 15 minutes. The colloidal gold SARS-CoV-2 IgM and IgG antibody detection kits used in this study were produced by Beijing Innovita Biological Technology Co., Ltd. (Beijing, China); the antigen reagent coating included spike (S) and nucleocapsid (N) proteins.

Viral nucleic acid tests

All patients provided nasopharyngeal swabs for SARS-CoV-2 nucleic acid tests. Both the nucleic acid extraction kit and the automatic nucleic acid extraction instrument were purchased from Da An Gene Co., Ltd. (Guangzhou, China). The amplification kit targeting the conserved regions of the ORF1ab and N genes of SARS-CoV-2 was purchased from Sansure Biotech Inc. (Changsha, China). The amplification system included endogenous internal standard primers and probes; the resulting Ct values were determined in accordance with the manufacturer’s instructions. The ABI 7500 instrument (Thermo Fisher Scientific, Waltham, MA, USA) was used for real-time
quantitative polymerase chain reaction analysis.

Statistical analysis

SPSS version 16.0 (SPSS, Inc., Chicago, IL, USA) was used for data analysis. The chi-squared test was used for comparison of count data among groups. Differences with \( P < 0.05 \) were considered statistically significant.

RESULTS

Patient characteristics

There were 19,797 children in this study (9,026 males and 10,771 females) (Table 1). The male-to-female ratio was 1:1.19; the mean age was 5.5 years (range, 1 day to 17 years). The numbers of children aged <1 year, 1–<3 years, 3–<6 years, and ≥6 years were 2,461, 4,383, 4,334, and 8,619, respectively. The numbers of children who presented in each month from March to August were 1,812, 2,243, 3,628, 3,529, 3,549, and 5,036, respectively. Only 390 children were initially diagnosed with pneumonia (excluding non-infectious pneumonia, as described above). Finally, only two children had positive viral nucleic acid test results (Table 1).

| Characteristics | Number of patients, n (%) |
|-----------------|--------------------------|
| **Sex**         |                          |
| Male            | 9,026 (45.6)             |
| Female          | 10,771 (54.4)            |
| **Age (years)** |                          |
| <1              | 2,461 (12.4)             |
| 1–<3            | 4,383 (22.1)             |
| 3–<6            | 4,334 (21.9)             |
| ≥6              | 8,619 (43.5)             |
| **Months of visiting** |                    |
| March           | 1,812 (9.2)              |
| April           | 2,243 (11.3)             |
| May             | 3,628 (18.3)             |
| June            | 3,529 (17.8)             |
| July            | 3,549 (17.9)             |
| August          | 5,036 (25.4)             |
| **Pneumonia**   |                          |
| Yes             | 390 (2.0)                |
| No              | 19,407 (98.0)            |
| **Viral nucleic acid** |                    |
| Positive        | 2 (0.0)                  |
| Negative        | 19,795 (100.0)           |

Pneumonia: patients with aspiration pneumonia, obliterative bronchitis, chronic bronchitis, asthmatic bronchitis, necrotizing pneumonia, or allergic pneumonia were excluded. COVID-19, coronavirus disease 2019.

Overall anti-SARS-CoV-2 IgM/IgG antibody test results

The overall anti-SARS-CoV-2 IgM antibody seropositivity rate was 1.2‰ (24/19,797), while the corresponding IgG antibody seropositivity was 0.6‰ (12/19,797). Children with positive IgM or IgG antibody test results were consulted by the hospital’s expert group in accordance with criteria including epidemiological investigations, clinical manifestations, and viral nucleic acid test results; all were excluded COVID-19.

Antibody seroprevalences stratified among distinct patient parameters

Sex

The rates of anti-SARS-CoV-2 IgM antibody seropositivity were 1.9‰ (17/9,026) in boys and 0.6‰ (7/10,771) in girls; the IgM antibody seropositivity rate was higher in boys than in girls (\( \chi^2 = 6.171, P = 0.014 \), Table 2). The rates of corresponding IgG antibody seropositivity were 0.6‰ (5/9,026) in boys and 0.6‰ (7/10,771) in girls; these rates did not significantly differ according to sex (\( \chi^2 = 0.075, P = 0.785 \), Table 2).

Age

The rates of anti-SARS-CoV-2 IgM antibody seropositivity in children aged <1 year, 1–<3 years, 3–<6 years, and ≥6 years were 2.4‰ (6/2,461), 1.8‰ (8/4,383), 0.7‰ (3/4,334), and 0.8‰ (7/8,619), respectively. The rate of anti-SARS-CoV-2 IgM antibody seropositivity was higher in children aged <1 year than in children aged ≥6 years (\( \chi^2 = 4.318, P = 0.048 \), Table 2); there were no significant differences among the other groups (data not shown). The rates of corresponding IgG antibody seropositivity in the above groups were 0.8‰ (2/2,461), 0.2‰ (1/4,383), 0.2‰ (1/4,334), and 0.9‰ (8/8,612), respectively; there were no significant differences among groups (data not shown).

Timing of admission to hospital

The rates of anti-SARS-CoV-2 IgM antibody seropositivity in children during the period from March 2020 to August 2020 were 0.0‰ (0/1,812), 0.0‰ (0/2,243), 0.3‰ (8/3,628), 0.0‰ (3/3,529), 2.3‰ (7/3,549), and 3.0‰ (15/5,036), respectively. All rates in July and August tended to be higher; the rate of IgM seropositivity was significantly higher in August than in March (\( \chi^2 = 5.409, P = 0.016 \), April (\( \chi^2 = 6.695, P = 0.009 \)), May (\( \chi^2 = 8.358, P = 0.004 \)), and June (\( \chi^2 = 10.530, P = 0.001 \)). The rate of IgM seropositivity was also significantly higher in July than in April (\( \chi^2 = 5.063, P = 0.027 \)), May (\( \chi^2 = 5.607, P = 0.020 \)), and June (\( \chi^2 = 7.964, P = 0.008 \)) (Table 2). From March to August, the rates of anti-SARS-CoV-2 IgG antibody seropositivity were 0.6‰ (1/1,812), 0.0‰ (0/2,243), 1.4‰ (5/3,628), 1.1‰ (4/3,529), 0.6‰ (2/3,549), and 0.0‰ (0/5,036), respectively (Table 2); these did not significantly...
TABLE 2 Anti-SARS-CoV-2 IgM/IgG antibody seroprevalences according demographic and clinical characteristics of pediatric patients screened for COVID-19

| Characteristics | IgM | IgG |
|-----------------|-----|-----|
|                 | Positive, n (%) | Negative, n (%) | \(\chi^2\) | P   | Positive, n (%) | Negative, n (%) | \(\chi^2\) | P   |
| Sex             |     |     |     |     |     |     |     |     |
| Male            | 17 (1.9) | 9009 (998.1) | 6.171 | 0.014 | 5 (0.6) | 9021 (999.4) | 0.075 | 0.785 |
| Female          | 7 (0.6) | 10 764 (999.4) |       |       | 7 (0.6) | 10 764 (999.4) |       |       |
| Age (years)     |     |     |     |     |     |     |     |     |
| <1              | 6 (2.4) | 2455 (977.6) | Not shown | Not shown | 2 (0.8) | 2459 (999.2) | Not shown | Not shown |
| 1–<3            | 8 (1.8) | 4375 (998.2) | Not shown | Not shown | 1 (0.2) | 4382 (999.8) | Not shown | Not shown |
| 3–<6            | 3 (0.7) | 4331 (999.3) | 0.075 | 0.785 | 1 (0.2) | 4333 (999.8) |       |       |
| ≥6              | 7 (0.8) | 8612 (999.2) |       |       | 8 (0.9) | 8611 (999.1) |       |       |
| Months of visiting |     |     |     |     |     |     |     |     |
| March           | 0 (0.0) | 1812 (1000.0) |       |       | 1 (0.6) | 1811 (999.4) | Not shown | Not shown |
| April           | 0 (0.0) | 2243 (1000.0) |       |       | 0 (0.0) | 2243 (1000.0) |       |       |
| May             | 1 (0.3) | 3627 (999.7) | Not shown | Not shown | 5 (1.4) | 3623 (998.6) | Not shown | Not shown |
| June            | 0 (0.0) | 3529 (1000.0) | Not shown | Not shown | 4 (1.1) | 3525 (998.9) | Not shown | Not shown |
| July            | 8 (2.3) | 3541 (997.7) |       |       | 2 (0.6) | 3547 (999.4) |       |       |
| August          | 15 (3.0) | 5021 (997.0) |       |       | 0 (0.0) | 5036 (1000.0) |       |       |
| Pneumonia       |     |     |     |     |     |     |     |     |
| Yes             | 1 (2.6) | 389 (997.4) | 0.600 | 0.380 | 0 (0.0) | 390 (1000.0) | 0.241 | 0.623 |
| No              | 23 (1.2) | 19 384 (998.8) |       |       | 12 (0.6) | 19 395 (999.4) |       |       |
| Total           | 24 (1.2) | 19 773 (998.8) |       |       | 12 (0.6) | 19 785 (999.4) |       |       |

*Only the <1-year-old group had a higher rate of IgM seropositivity than the ≥6-year-old group, \(\chi^2 = 4.318, P = 0.038\). There were no significant differences among the other groups, \(P > 0.05\); \(\chi^2\) values not shown. *There were no significant differences in pairwise comparison between groups, \(P > 0.05\); \(\chi^2\) values not shown. The rate of IgM seropositivity was higher in July than in April (\(\chi^2 = 5.063, P = 0.027\)), May (\(\chi^2 = 5.607, P = 0.020\)), and June (\(\chi^2 = 7.964, P = 0.008\). The rate of IgM seropositivity was higher in August than in March (\(\chi^2 = 5.409, P = 0.016\)), April (\(\chi^2 = 6.695, P = 0.009\)), May (\(\chi^2 = 8.338, P = 0.004\)), and June (\(\chi^2 = 7.964, P = 0.001\). There were no significant differences among other groups, \(P > 0.05\); \(\chi^2\) values not shown. *There were no significant differences in pairwise comparison between groups, \(P > 0.05\); \(\chi^2\) values not shown. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019.

differ among groups (data not shown).

**Pneumonia or not**

The rates of IgM and IgG antibody seropositivity in children with suspected pneumonia were 2.6‰ (1/390) and 0.0‰ (0/390), respectively; in children diagnosed with non-pneumonia diseases, these rates were 1.2‰ (23/19 407) and 0.6‰ (12/19 407), respectively. When stratified according to pneumonia status, IgM (\(\chi^2 = 0.600, P = 0.380\)) and IgG (\(\chi^2 = 0.241, P = 0.623\)) antibody seropositivity rates were not significantly different between groups (Table 2).

**Viral nucleic acid test results**

Two children had positive SARS-CoV-2 nucleic acid test results, whereas their IgM/IgG antibody test results were negative (patient characteristics are shown in Table 3).

TABLE 3 Demographic and clinical characteristics of two children with positive SARS-CoV-2 nucleic acid test results

| Characteristics | Patient 1 | Patient 2 |
|-----------------|-----------|-----------|
| Sex             | Male      | Female    |
| Age (years)     | 1         | 1         |
| Months of visiting | June     | June     |
| †Pneumonia      | No        | No        |
| Fever           | Yes       | Yes       |
| Antibody        |           |           |
| IgM             | Negative  | Negative  |
| IgG             | Negative  | Negative  |

†Pneumonia: patients with aspiration pneumonia, obliterative bronchitis, chronic bronchitis, asthmatic bronchitis, necrotizing pneumonia, or allergic pneumonia were excluded. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
DISCUSSION

Thus far, there have been few reports concerning seroepidemiological analyses of serum anti-SARS-CoV-2 antibodies in children. Among children aged 5–19 years in Geneva, Switzerland, the rate of anti-SARS-CoV-2 IgG antibody seropositivity was 7.3%,19 while it was approximately 1% among children in Seattle (Washington, USA). Those findings indicated that children are susceptible to SARS-CoV-2 infection. To the best of our knowledge, there have been no reports concerning the seroprevalence of anti-SARS-CoV-2 antibodies among children in China. The present study showed that the seropositivity rates of anti-SARS-CoV-2 IgM and IgG antibodies were low among children in Beijing area (1.2‰ and 0.6‰, respectively); this implies that children in Beijing area are generally susceptible to SARS-CoV-2 infection. Therefore, COVID-19 prevention and control should be strengthened to prevent the disease in children.

The laboratory diagnosis of COVID-19 mainly relies on viral nucleic acid testing.24,25 antibody test results can only serve as auxiliary diagnostic data for COVID-19 (see Diagnosis and Treatment Protocol for COVID-19 Patients (Tentative 8th Edition), the National Health Commission of the People’s Republic of China, http://regional.chinadaily.com.cn/pdf/DiagnosisandTreatmentProtocolforCOVID-19Patients(Tentative8thEdition).pdf). In this study, 36 children with anti-SARS-CoV-2 antibody seropositivity had negative viral nucleic acid test results. After comprehensive consultation by an expert group, COVID-19 was ruled out in all 36 patients; however, some IgM antibody seropositivity results might have been false positives. False positive antibody seropositivity results are often caused by factors such as differences in kit sensitivity and specificity, as well as patient immune status.26,27 Furthermore, two patients had positive viral nucleic acid test results, but exhibited IgM and IgG antibody seronegativity. These patients were presumed to be in an early stage of disease, such that specific antibodies were not yet generated.28 These results support the current notion that it is difficult to achieve early diagnosis of COVID-19 (or to rule out the presence of COVID-19) on the basis of antibody test results alone.

In addition, the results of this study showed that the rate of anti-SARS-CoV-2 IgM antibody seropositivity was higher in males than in females. This is consistent with the previous findings that male children more frequently develop other respiratory virus infections, compared with female children. For example, among children with acute respiratory infections (e.g., respiratory syncytial virus or adenovirus), the prevalence is higher among boys than among girls.29-32 Second, the rate of IgM antibody seropositivity was significantly higher in children aged <1 year than in children aged ≥6 years. Yet, the underlying mechanisms are still not fully understood. Third, the IgM antibody seropositivity rates were highest among children who presented to hospital in August and July. The prevalence of some respiratory virus infections (e.g., influenza and respiratory syncytial virus) have obvious seasonal characteristics; the peaks of these infections in northern China occur in winter and spring. However, the findings in this study do not enough yet to illustrate any seasonal characteristics of COVID-19. The clinical significance of these findings requires further investigation. Additionally, since the end of July, the number of children presenting to hospital has substantially increased, which may have contributed to the enhanced seropositivity rate.

There were some limitations in this study. First, the SARS-CoV-2-specific neutralizing antibody was not tested among IgG antibody-positive samples; IgG seropositive patients with previously asymptomatic infections may have been included. Second, the study population were children visiting a hospital, rather than a random sample of children in the community. However, the sample size was large and the patients’ general demographic characteristics were wide-ranging; thus, the results are presumably representative of the region.

In summary, this retrospective study showed that the rates of anti-SARS-CoV-2 IgM/IgG antibody seropositivity were low in children who presented to Beijing Children’s Hospital from March 2020 to August 2020. The findings suggest that children in Beijing area are generally susceptible to SARS-CoV-2 infection; COVID-19 prevention and control measures should be strengthened to prevent the disease in children.

CONFLICT OF INTEREST

None.

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