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Research article

Anti_spike and anti_nucleocapsid IgG responses to SARS-CoV-2 in children of Jordan

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ABSTRACT

Background: It is proven that children have significantly milder COVID-19 disease compared to adults. Various immunological characteristics influence this age-related difference in protection against COVID-19. Pediatric COVID-19 in Jordan is extremely under reported.
Objectives: The primary goal of this work is to identify the anti_S and anti_N antibody responses in a random group of children in Jordan and compare it to that of naturally infected-unvaccinated adults.
Methods: 151 unvaccinated children, 4 days to 18 years old, were screened for anti_S and anti_N antibodies. History of COVID-19 infection or exposure to infection and symptom severity were reported by parents on a special questionnaire.
Results: 78.9 % and 65.3 % of participants were seropositive for anti_S IgG and anti_N Abs, respectively. There was a remarkable association between age and anti_S IgG and anti_N IgG antibody titers, as children aged 12 years or older had increased anti_S IgG titers (mean = 19.3 BAU/mL) compared to younger groups (means of 10.15, 9.24, 7.91 BAU/mL for age groups 6–12, 1–6, less than 1 year, respectively). Gender did not show a statistically important role in anti_S and anti_N IgG seropositivity rates or titers. Children displayed significantly elevated anti_S titers (mean = 13.23 BAU/mL) compared to naturally infected adults (mean = 9.72 BAU/mL), in contrast, adults' anti_N titers (mean = 39.64 U/mL) were significantly higher compared to those of children (mean = 10.77 U/mL).
Conclusions: The current work provides evidence of distinctly robust and persistent humoral immunity displayed by high anti_S and anti_N IgG in children, even >12 months post-infection. Age was the only factor that had a significant statistical impact on anti_S and anti_N Ab levels among the pediatric group in this study. Children exhibited significantly higher anti_S titers than naturally infected adults. In contrast, adults' anti_N titers were significantly higher. Such

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information can assist direct pediatric SARS-CoV-2 immunization programs, with implications for creating age-targeted strategies for diagnostic and population protection measures.

1. Introduction

Since the emergence of the coronavirus disease in 2019 (COVID-19), data describing pandemic waves, vaccines' efficacy, disease and vaccine complications, detailed immune response and many other aspects of infectivity is still pouring in from all around the world. However, most of the data available is based on adult cases, children's response to COVID-19 is still being evaluated [1–5].

The spread of COVID-19 infection among children is significantly lower compared to adults. By May 2023, children represented 17.9 % of cumulative COVID-19 patients in the United States [4]. Up to April 2022, out of total cases, pediatric infection rates varied from 10 % in Brazil to 23 % in Italy [6]. Interestingly, a study from China reported no difference in infection rates among different children age groups [5], while infection rates in Ontario were found to be higher among children 15–19 years of age compared to younger groups [7].

Mortality rates because of COVID-19 in children are tremendously low, ranging from 0.005 % to 0.01 % [8], but a higher risk is surely expected in children suffering from health conditions and poverty [9]. Estimating probability of death in pediatric patients with COVID-19 is difficult, but overall it is likely below 1 % [10].

While most cases stay within relatively comforting disease manifestations, children's COVID-19 severity varies widely, ranging from completely asymptomatic to long COVID disease and multisystem inflammatory syndrome (MIS) [11]. The rate of asymptomatic infections in children is believed to be underestimated, as these are less likely to be tested. Based on antibody (Ab) screening, 50 % of pediatrics who were seropositive for SARS-CoV-2 infection showed no symptoms [12]. Mild symptomatic cases primarily display fever, cough, diarrhea, vomiting and sour throat [11,13]. Hospitalization and intensive care were only required for about 15 % of infected children who displayed risk factors such as obesity, diabetes mellitus, asthma, and young age, particularly in the neonatal period. Such severe cases showed lower respiratory tract signs and longer illness duration [14].

The prevalence of long COVID-19, manifested by having one or more symptoms for longer than a month after infection [15], is not consistent, ranging from 0 to 27 % across different research settings [15]. The most prevalent clinical signs of long COVID in pediatrics include mood symptoms, fatigue, muscle weakness, sleep problems, cognitive symptoms, headache, respiratory symptoms, loss of appetite and altered eating preferences [15,16].

On the other hand, MIS, where inflammation develops in different body parts [17], happens in $<$0.01 % of infected children where 68 % of cases require intensive care support in 68 % [18]. Cardiac dysfunction was seen in up to 5 % of children requiring intensive care, in addition to encephalitis and other neurological findings displayed in hospitalized children [19,20].

The reasons why pediatric COVID-19 is less severe than that of adults lie in both arms of immunity [21]. From the innate side, in healthy children, frequent viral infections and vaccination prepare the immune system to confront the 1st exposure to SARS-CoV-2 [22]. Type 1 interferon is more readily produced in children compared to adults, expediting viral clearance and restricting viral spread. Also, in adulthood, the increased presence of Abs against type 1 interferon favors the spread of infection [23]. Adult innate immune cells display increased expression of cell adhesion molecules, leading to enhanced lung infiltration and pneumonia [24]. From the adaptive side, children have more naive and regulatory cells causing a less vigorous cytokine response that is less likely to reach a cytokine storm manifesting severe COVID-19 inflammation in adults [25]. Children have been proven to produce a more neutralizing Ab response linked to improved disease outcomes. Severe disease in adults is displayed by more non-neutralizing Ab response leading to what is called antibody-dependent enhancement (ADE) of infection [26], facilitating viral entry through cellular Fc receptors.

Levels of anti_S Abs against COVID-19 differ in pediatrics compared to adults, but reports have been inconsistent in this concern. Some studies reported that children had significantly higher and longer persisting anti_S IgG titers compared to adults [27], while others stated that anti_S titers were increased in adults compared to children early post infection but reached similar levels 6 months later [28]. Moreover, some studies have proved that adults display a higher concentration of neutralizing Abs and effective Ab-dependent cell cytotoxicity compared to pediatric patients [29], while others showed completely contrasting results [26]. In general, children displayed lower levels of anti_N Abs that decreased more quickly compared to adults [28]. In adults, the extent of anti_N response positively correlated with the severity of the disease [30].

Another major difference in the specific humoral immunity to COVID-19 between children and adults is the correlation between symptom severity and corresponding Ab titers. In contrast to adults, children with asymptomatic infection showed an earlier and higher anti_S response compared to mildly and moderately symptomatic ones [31]. Interestingly, 25 % of convalescent pediatric cases and 12 % of and MIS-C children were found to be seronegative. Similarly, during acute infection, children with severe disease did not show any detectable Ab response, in contrast to those with asymptomatic, mild and moderate disease [32]. This was consistent with low numbers of circulating T follicular helper cells and a higher concentration of inflammatory cytokines [32].

Pediatric COVID-19 in Jordan is extremely under reported in all aspects of infection rate, symptom severity, immune response, vaccination rates … etc. As a first attempt, the main objective of this study is to determine the anti_S and anti_N Ab response in a random group of children in Jordan and compare it to that of naturally infected-unvaccinated adults.

2. Materials and methods

2.1. Participants, setting, and ethical consideration

This Work used a cross-sectional design and convenience sampling methodology. 151 Jordanian children voluntarily participated in the study, after their guardians' agreement, at health centers in Central Jordan. The study took place between July and November 2022. Serum samples were collected from participating children and a questionnaire was filled by their guardians. Serum samples were stored for later screening of anti_s IgG and anti_n IgG in November and December 2022. Socio-demographic data and previous history of COVID-19 infection were collected for each child. The study adhered to the guidelines of the Deceleration of Helsinki and was approved by the institutional review board (IRB) committee at the Hashemite University (No.22/4/2021/2022) and Prince Hamza Hospital (PHH).

For comparison, adults' serum samples were included in the study. These samples were previously collected early after the onset of the COVID-19 pandemic in 2020 and before the start of the vaccination campaign. Hence, the Ab response detected in this group is coming from natural infection with SARS-CoV-2.

2.2. Demographic and COVID-19 history of population study

The study enlisted 151 children of various age groups who have NOT BEEN VACCINATED against COVID-19. Guardians of participating children were asked to fill a questionnaire composed of four sections. The first section represented an invitation to participate in the investigation by displaying its aim, assuring the parents' agreement to participate their children in the study, along with their right of withdrawal and anonymity. The second section was concerned with the demographic characteristics of the participants; age and gender.

The third section involved questions to reveal the participant's history of COVID-19 infection. The section started with yes or no questions concerning whether they have ever been infected with COVID-19, the number of times they have been infected, the timing of the last infection, and the method of COVID-19 infection confirmation. Moreover, a subjective question about the severity of COVID-19 infection(s) was asked where participants chose the answer mild, moderate, severe, or hospitalized.

The final section of the questionnaire covered study subjects contact with people infected with COVID-19. The section incorporated yes or no questions regarding the possible contact of participants with infected individuals whether it was their parents, siblings, schoolmates, friends, or teachers. In infants below 1 year of age, their mothers were asked about COVID-19 infection history and timing in relation to pregnancy. Last but not least, mothers were questioned about their COVID vaccination history and timing.

2.3. Sample collection

Serum samples from pediatric participants were collected at central hospitals and private laboratories of Central Jordan, between July and October 2022, after obtaining informed consent from their parents. Samples from naturally infected, unvaccinated adults were collected at PHH after obtaining signed consent forms. These were collected up to 6 months post RT-PCR confirmation of COVID-19 infection in 2020. All samples were stored at $-20\circ$C until further use.

2.4. Anti_spike IgG measurement

IgG antibodies specific to SARS-CoV-2 S protein in human serum/plasma were detected using an Enzyme Linked Fluorescent Assay (ELFA) technique (VIDAS®, Biomerieux inc., Hazelwood, MO, USA), where fluorescence intensity is directly proportional to the concentration of Ab in the sample. The ratio between relative fluorescence value (RFV) measured in the sample and RFV obtained for the calibrator (humanized recombinant SARS-CoV-2 antibody) was calculated and used as index for result interpretation. The results were considered positive if index $\geq$1 or negative if index $<$1,A standard equation that complies with the World Health Organization standards was used to convert readings into binding antibody units per milliliter (BAU/mL).

2.5. Anti_nucleocapsid IgG measurement

All serum samples were tested for IgG and IgM against Coronavirus Nucleocapsid using COVID-19 IgG/IgM Duo for quantitative detection (NanoEntek/Korea). The protocol was run according to manufacturer instructions. System readings $>$1.00 U/mL were considered positive.

2.6. Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 22.0 from Chicago, IL, USA, was utilized for conducting the statistical analysis. Different variable categories were summarized in terms of percentages and frequencies, while numerical variables were stated as mean $\pm$ standard deviation (SD). A one-way ANOVA and post-hoc analysis (Turkey's) was performed to analyze the contrast in mean values of positive anti_N IgG titers (excluding negative results) among the four age groups, as well as the contrast in mean values of positive anti_N IgG titers for these same four age groups. Unpaired *t*-test was conducted to explore the relationship between males and females as well as between adults and pediatric age groups in terms of mean anti_S titer and anti_N titer levels. In all

instances of statistical analysis, a significance level of $P \leq 0.05$ was deemed as statistically significant. Figures were generated utilizing GraphPad Prism version 8.0.0 software, (San Diego, CA, USA). The software was accessed on 20 January 2024, www.graphpad.com.

3. Results

3.1. Study participants

As shown in Table 1, a total of 151 children with ages ranging from 4 days to 18 years who have not received COVID-19 vaccination were involved in the study. Females consisted 55.6 % of the participants. The largest group fell in the 1–6 years (39.7 %) age category followed by the 13–18 (31.8 %) and 7–12 (23.8 %) categories, whereas only 7 participants (4.6 %) were under 1 year of age. History of COVID-19 infection was reported by the parents in only 46 (30.5 %) participants, of which only 3 (2 %) reported 2 infections. 18 (39.1 %) of the participants reported to have had infections confirmed by PCR, while the rest relied on signs and symptoms for the diagnosis. At the time of data collection, 78.3 % (36/46) of those with a history of COVID-19 infection had reported having their infection at least a year ago. Most of the infections were reported to be mild (56.5 %) or moderate (37 %). Of all participants, 132 (87.4 %) reported a history of contact with infected personnel.

3.2. Anti_S IgG levels in study subjects

147 of the pediatric participants were screened for the presence of anti_S IgG in their sera. Of these, 78.9 % (116/147) tested seropositive for COVID-19 infection (Table 2). Interestingly, 81/116 (69.8 %) of the seropositive subjects were reported to have not had any previous COVID-19 infections, neither by signs and symptoms nor by RT-PCR. On the other side, 32.3 % of all seronegative subjects (31/116) reported a history of COVID-19 infection, but none was confirmed by a PCR test.

In all 147 subjects, the mean of anti_S IgG titers was 10.5 BAU/mL (median = 8.26 BAU/mL) compared to a mean of 13.2 BAU/mL (median = 11.32 BAU/mL) when only those with positive anti_S samples were considered.

3.3. Anti_N IgG levels in study subjects

Out of all the 147 subjects tested for anti_N IgG, 65.3 % (96/147) were anti_N IgG positive as demonstrated in Table 2. Parents of 71.9 % (69/96) of those seropositive subjects stated that their children had never previously contracted COVID-19, as confirmed by PCR or through the appearance of COVID-19 like symptoms. In contrast, 35.3 % of all seronegative individuals (18/51) reported having been infected with COVID-19 either through the appearance of symptoms (13/18) or by PCR (5/18).

When only those with positive anti_N samples were taken into account, the mean anti_N IgG titer was 10.8 U/mL (median = 9.7 U/mL), as opposed to 7.1 U/mL (median = 4.88 U/mL) for all 147 individuals.

3.4. Effects of age, gender, prior COVID-19 infection history, number of COVID-19 infections, and timing of last infection on Anti_S and Anti_N IgG responses

The seropositivity of anti_S IgG displayed significant age-related variations (p = 0.0019). Specifically, participants aged more than

Table 1
Characteristics of study participants.

	Variable	Number (%)
Age (Years)	Less than 1	7 (4.6)
	1–6	60 (39.7)
	>6–12	36 (23.8)
	>12–18	48 (31.8)
Gender	Male	67 (44.4)
	Female	84 (55.6)
COVID-19 infections	No infection	105 (69.5)
	One	43 (28.5)
	Two	3 (2)
Timing of last infection (N = 46)	1–3 months	1 (2.2)
	>3–6 months	3 (6.5)
	>6–12 months	5 (10.9)
	>1 year	36 (78.3)
	NA	1 (2.2)
Infection confirmed by PCR (N = 46)	Yes	18 (39.1)
	No	25 (54.4)
	NA	3 (6.5)
History of contact with infected individual(s)	Yes	132 (87.4)
	No	18 (11.9)
	NA	1 (0.7)

n: number, NA: not available, PCR: Polymerase Chain Reaction.

Table 2
Anti_S & Anti_N IgG seropositivity.

	Variable	Number (%)	Age (Years)	Seropositivity (%)	P value
Anti_S IgG[a]	Positive	116 (78.9)	Less than 1	6/7 (85.7)	0.0019
	Negative	31 (21.1)	1–6	37/56 (66.1)	
			>6–12	29/36 (80.6)	
			>12–18	44/48 (91.7)	
Anti_N IgG[a]	Positive	96 (65.3)	Less than 1	4/7 (57.1)	0.0028
	Negative	51 (34.7)	1–6	35/58 (60.3)	
			>6–12	27/35 (77.1)	
			>12–18	30/47 (63.8)	

[a] 4 out of 151 subjects were not tested.

12 years displayed the highest seropositivity rate for anti_S IgG at 91.7 %, while those aged less than 1 year had the next highest rate at 85.7 %. Individuals aged 6–12 years showed an intermediate rate of 80.6 %, while those aged 1–6 years had the lowest rate at 66.1 %. Furthermore, as illustrated in Fig. 1a, a statistically significant association between anti_S titers and age is evident ($p = 0.00000075$). Mean titers in subjects with positive anti_S IgG test samples above the age of 12 years (19.33 BAU/mL) were higher than those aged less than 1 year (7.91 BAU/mL), 1–6 years (9.24 BAU/mL) and 6–12 years (10.15 BAU/mL) with P values of 0.0153, 0.000004 and 0.00012, respectively, according to Turkey's post-hoc analysis.

Gender had no statistically significant association neither with anti_S IgG seropositivity rates nor with Ab titers ($P = 0.357$) as seen in Fig. 1b.

Age exhibited a significant association with anti_N titer levels ($P = 0.0117$), particularly between individuals aged 1–6 years (mean = 13.6 U/mL) and those aged 12–18 years (mean = 8.25 U/mL) ($P = 0.0067$). The seropositivity rates of anti_N were generally less than anti_S but also displayed a statistically significant difference among age groups ($P = 0.0028$). The highest seropositivity rate for anti_N IgG among participants was observed in those aged 6–12 years, at 77.1 %. In contrast, the lowest rate was found among individuals aged less than 1 year, at 57.1 %. Participants aged more than 12 years displayed a rate of 63.8 %, while those aged 1–6 years had a rate of 60.3 %. Similar to anti_S titers, gender had no statistically significant association with anti_N IgG. Fig. 2a and b show the relation of anti_N IgG titers with age and gender, respectively. Interestingly, the reported history of infection had no statistically significant role ($P < 0.05$) neither in the seropositivity rate nor in the magnitude of the Ab response of participants as reflected by anti_S & anti_N IgG titers. The anti_S seropositivity rate among participants who reported a COVID-19 infection prior to serum collection (77.8 %) was close to those who did not (79.4 %).

In addition to that, the timing of the last infection according to the date of sample collection as well as the number of previous COVID-19 infections didn't have a statistically significant association with seropositivity or Ab titers of anti_S IgG and anti_N IgG.

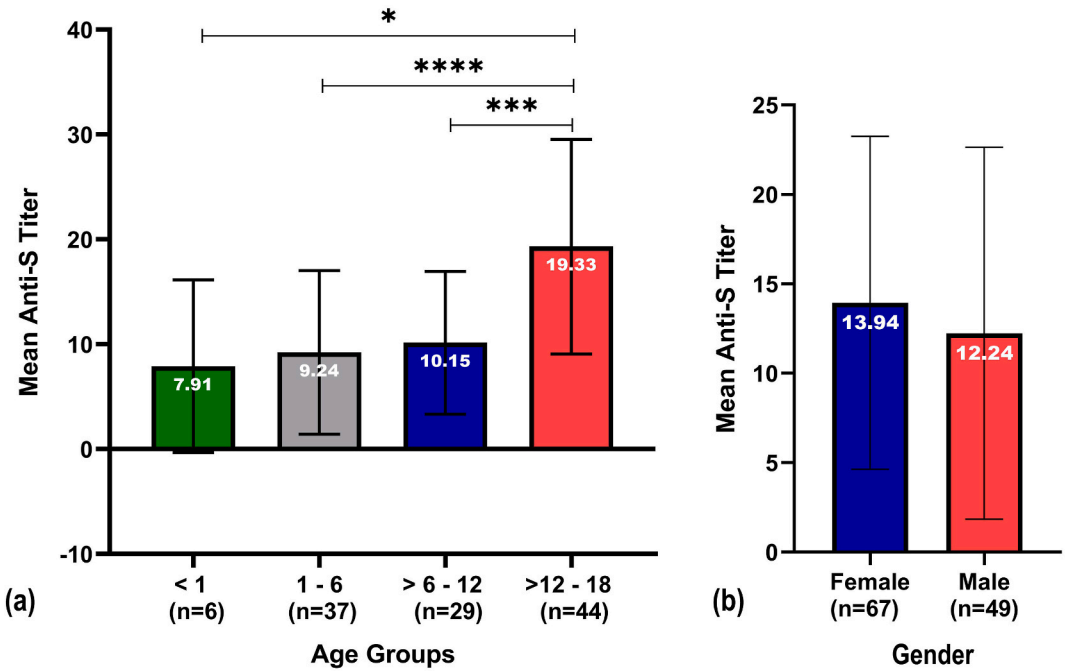

Fig. 1. Association of anti-S titer with age (a) and gender (b). Values represented mean + SD. P value > 0.05: ns; P value < 0.05: *, P value < 0.01: **, P value < 0.001***, P value < 0.0001****.

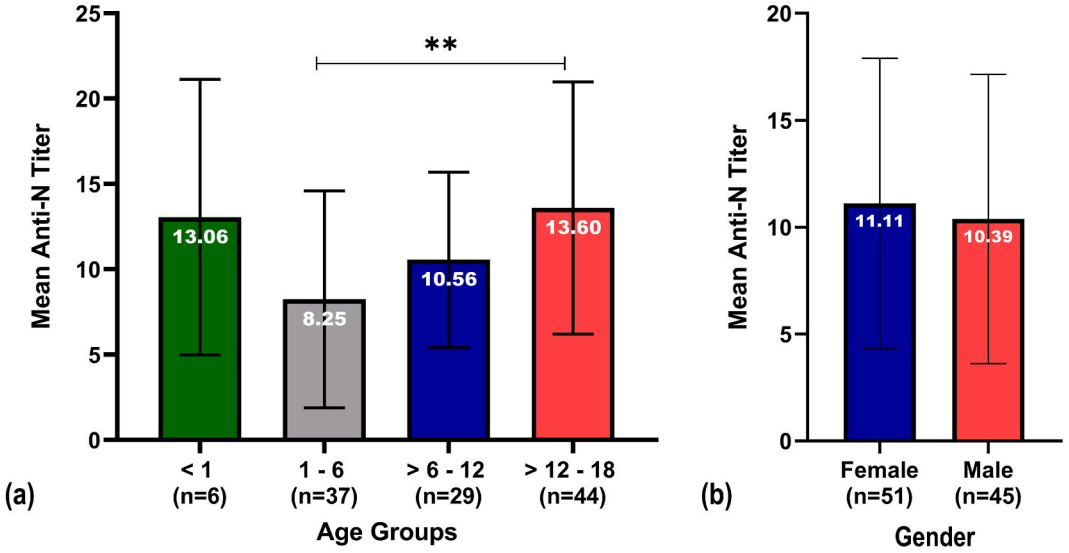

Fig. 2. Association of anti-N titers with age (a) and gender (b). Values represented mean + SD. P value > 0.05: ns; P value < 0.05: *, P value < 0.01: **, P value < 0.001***, P value < 0.0001****.

Table 3
Anti_S and Anti_N Mean Titers Association with Age, Gender, History of infection and the severity of symptoms.

Variable	Anti_S IgG	Mean titer[a]	Anti_N IgG	Mean titer[a]
Age				
Less than 1		7.9		13.06
Positive	6		4	
Negative	1		3	
1–6		9.24		8.24
Positive	37		35	
Negative	19		23	
7–12		10.15		10.56
Positive	29		27	
Negative	7		8	
13–18		19.3		13.6
Positive	44		30	
Negative	4		17	
Gender				
Male		12.24		10.39
Positive	49		45	
Negative	14		22	
Female		13.94		11.11
Positive	67		51	
Negative	17		29	
COVID-19 infection				
Yes		15.56		12.19
Positive	35		27	
Negative	10		18	
No		12.22		10.22
Positive	81		69	
Negative	21		33	
Severity of symptoms				
Mild		17.37		13.07
Positive	23		15	
Negative	3		10	
Moderate		13.82		11.99
Positive	9		9	
Negative	7		7	
Severe		8.78		11.65
Positive	2		2	
Negative	0		1	

[a] Positive samples only are used to calculate the Mean IgG titer levels. Anti_S titers are measured as BAU/mL. Anti_N titers are measured as U/mL.

Similarly, reported symptom severity had no association with anti_S IgG seropositivity and titers. This happened to be the same for anti_N IgG seropositivity and titers.

Individuals with a confirmed previous COVID-19 infection, as determined by PCR, demonstrated a 100 % seropositivity rate for anti_S IgG. However, this was not the case for anti_N IgG, as they only showed a seropositivity rate of 70 %.

Table 3 demonstrates the mean titer levels of anti_S and anti_N in study subjects based on age, gender, history of infection and severity of symptoms.

4. Comparison of Anti_S and Anti_N Ab responses between pediatrics and naturally infected unvaccinated adults

Pediatric anti_S IgG titers mean (13.2 BAU/mL) among those with positive test serum samples was found to be statistically higher than the mean levels (9.7 BAU/mL) present among the adult age group with positive test samples. Fig. 3a demonstrates the statistically significant difference (P = 0.018) between pediatric and adult anti-S IgG titers.

In contrast, anti_N IgG levels were much higher among the adult age group (39.64 U/mL) compared to pediatrics (10.77 U/mL) where only positive test samples were considered with a statistically significant difference (P = 0.00000013) as seen in Fig. 3b. Adult participants' characteristics are shown in Table 4.

5. Discussion

It is well established that, in contrast to other respiratory infections, children have significantly milder COVID-19 disease compared to adults [1–3]. Studying infection rates of SARS-CoV-2 in pediatric populations is important because a considerable ratio of children worldwide display asymptomatic disease. These act as serious silent reservoirs of the virus that can be transmitted to persons in contact, causing viral spread and perhaps serious complications in adults [33].

The adaptive immunity against pathogens is generated by both cellular (T cell) and humoral (Ab) responses. Helper and follicular T cell responses are crucial to support Ab production by virus specific B cells. Interestingly, there is evidence indicating T cell responses specific against SARS-CoV-2-specific may prevent infection without detectable anti_SARS-CoV-2 Abs. Still, investigation of virus-specific T cell responses is more intricate, difficult, time consuming and of greater cost compared to measurement of humoral responses [34].

Measuring circulating IgG is a reliable approach to determine previous infection [35]. Anti_S IgG remains detectable even after 14 months post natural infection [36], which is important in our study as most of the pediatric participants reported a last infection period

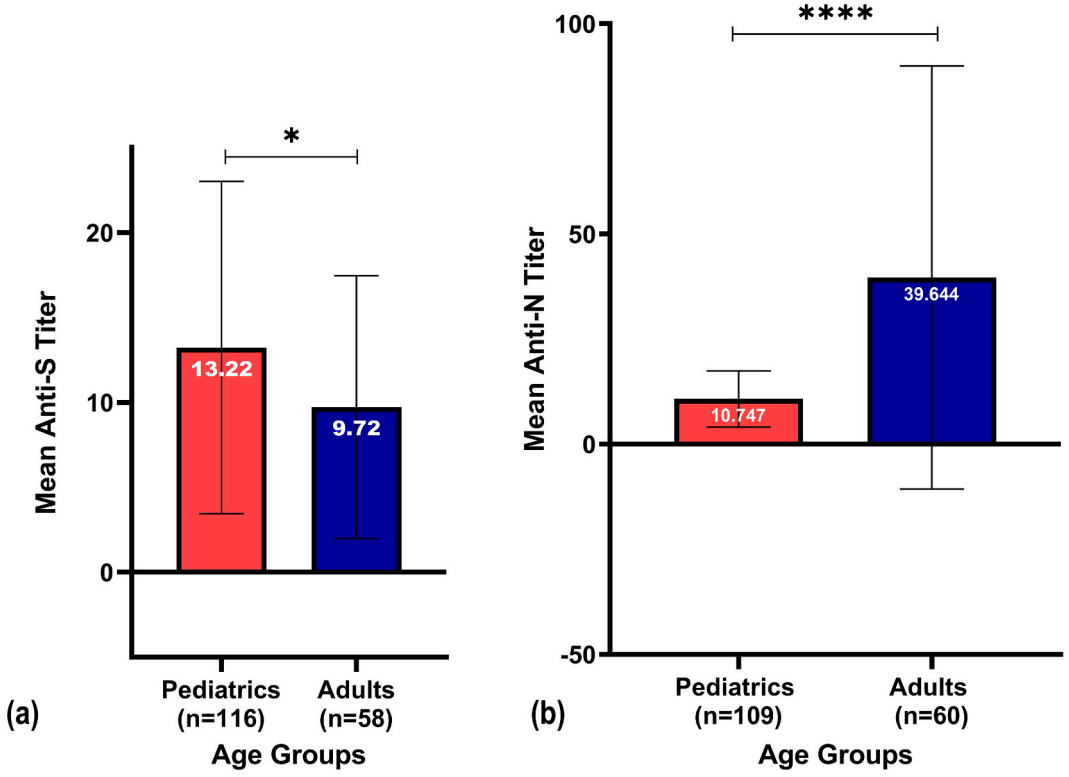

Fig. 3. Adults versus pediatrics anti-S titer (a) and Adults versus pediatrics anti-N titer (b). Values represented mean + SD. P value > 0.05: ns; P value < 0.05: *, P value < 0.01: **, P value < 0.001***, P value < 0.0001****.

Table 4
Adult participants' characteristics.

	Mean ± SD	
Age	39.6 ± 14.98	
Gender	Variable	Number (%)
	Male	60 (60.6)
	Female	39 (39.4)
Timing of last infection	1–3 months	52 (52.5)
	>3–6 months	37 (37.4)
	>6–12 months	5 (5.05)
	>1 year	0
	NA	5 (5.05)
Severity of symptoms	Asymptomatic	10 (10.1)
	Mild	30 (30.3)
	Moderate	39 (39.4)
	Severe	19 (19.2)
	NA	1 (1)

NA: not available.

of 1 year or longer. The kit we have used here is specific for measuring anti_S1 Abs. The receptor binding domain (RBD) that allows viral entry is part of the S1 domain of the S protein [37]. Although not confirmed here, anti-RBD Abs are good indicators of immune protection as they are known for their neutralizing activity, at least in part.

Although anti_N wanes at a faster rate compared to anti_S Abs [38], the presence of anti_N IgG has proved to correlate with more severe symptoms and more extensive viral spread [39] that could lead to the release of higher concentrations of N protein from virally infected cells.

Except for a report on hospitalization needs of COVID-19 infected cancer pediatric patients [40], pediatric COVID-19 in Jordan is extremely under-reported in all aspects of infectivity rate, symptom severity, long COVID disease, MIS-C, immune response, vaccination rates … etc [41]. This study represents the first to measure the Ab response against COVID-19 in a random sample of Jordanian children, and adds to the few international studies on this aspect of pediatric immune response to the disease.

Out of 147 participants, 78.9 % were seropositive for anti_S IgG Abs. This suggests a considerable exposure of these participants to SARS-CoV-2, which could be expected given that serum samples were collected in June–November 2022, after the greatest spread of the Omicron wave experienced in March 2022 [41]. In fact, parents reported contact with infected individuals to be the case for 83.6 % of the participants. Interestingly, 69.8 % of the seropositive subjects had no reported history of COVID-19 infection according to their parents, neither by appearance of symptoms nor by PCR confirmation. As an international trend, this indicates that most infections in children are asymptomatic or come with mild/unrecognizable symptoms [31,42]. In contrast, among the seronegative subjects, 32.3 % reported previous COVID-19 infection, although none of these cases were confirmed by PCR, suggesting an infection with symptoms similar to those of COVID-19 or an old infection, against which Abs declined to undetectable levels.

Similar to the anti_S response, 65.3 % of participants showed seropositivity for anti_N IgG, suggesting prior viral infection, replication and spread. Interestingly, 71.9 % of seropositive subjects had no reported history of previous SARS-CoV-2 infection. These children did not exhibit any symptoms associated with COVID-19 and had not undergone a COVID-19 PCR test. This could also be due to asymptomatic infections or a lack of awareness of mild or subclinical cases. Conversely, 35.3 % of seronegative individuals reported a history of COVID-19 infection. Only 9.8 % of these reported cases were confirmed by PCR, which may be attributed to false-positive self-reported cases, or the possibility of other respiratory infections being mistaken for COVID-19 [43]. It should be kept in mind that when performing serological analysis for anti_N Abs in children, the outcomes may not consistently provide an accurate representation of previous infections. This is because children who have been infected with COVID-19 tend to exhibit lower concentrations of anti_N Ab compared to adults. Additionally, these Ab levels diminish at a faster rate than in adults [28,44].

A considerable association between age and anti_S IgG seropositivity is demonstrated here, as children aged 13 years or older were more likely to have a positive anti_S IgG serum and significantly higher Ab titers compared to younger groups (Fig. 1a–Tables 2 and 3). This suggests that age plays a role in the development and extent of immunity to SARS-CoV-2 infection in children. In this concern, reports of the literature are inconsistent. While some reported a similar positive correlation between anti_S Ab titers and age to our findings [27], others reported a contrasting negative association. Yang et al. (2021) found out that younger children display a stronger anti_S response compared to older ones, not only in terms of magnitude, but also in terms of neutralizing activity and avidity of Abs produced [22]. Similarly, Weisberg et al. (2020) found that, in study children other than MIS-C patients, age and anti_S IgG titers showed significant negative correlation [44]. It is worth mentioning that the high seropositivity rate of the infant group (<1 year old) (85.7 %), in addition to a probable natural infection, could be coming from mother to fetus *trans*-placental transfer due to infection and/or vaccination.

Our findings show a significant moderate association between age and anti_N titers between the 13–18 and 6–12 years old groups (Fig. 2a–Tables 2 and 3). This is in contrast to a previous study that showed no correlation between anti_N levels and age in their pediatric group [44].

Gender showed no statistically significant role in anti_S and anti_N IgG seropositivity rates or titers (Figs. 1b and 2b). This observation implies that the role of gender in the formation of anti_S IgG Abs in childhood years may not be of substantial influence. This finding contradicts previous research conducted on adults, which demonstrated that female participants exhibited a greater

likelihood of possessing elevated levels of Ab titers [45].

This discrepancy between studies described above could be due to many factors including the timing of sample collection, the type of variant circulating at the time of blood draw, number of previous COVID-19 infections, the tested population (general community or hospital setting), geographical/racial differences, probability of previous cross-reactive Human Corona Virus (HCoV) infections, type of testing/antigens used for Ab titer measurement, and probably others.

The severity of the last reported COVID-19 infection, as subjectively assessed by parents, did not show any association with anti_S IgG seropositivity or titers (Table 3). It is noteworthy that out of the 46 participants whose parents reported a prior COVID-19 infection, only 3 reported severe symptoms that did not need hospitalization. Hence, most of our study subjects are considered asymptomatic or with mild symptoms. Consistent with our results, previous studies indicated children with varying degrees of disease severity demonstrated comparable patterns of Ab profiles, except for MIS-C and severely ill hospitalized children, who presented a deficient Ab response in all means of magnitude and neutralization activity [44,46]. The same lack of association was observed for anti_N IgG seropositivity and titers in association to infection severity since low levels of anti_N IgG Abs were observed in pediatric subjects with different disease severities, indicating that the generation of anti_N IgG does not depend on the presence or severity of symptoms [44, 46]. This aligns with a study conducted on adults, which revealed that individuals with mild to no symptoms did not necessarily exhibit positive anti_N IgG [47].

This study investigated the discrepancies in anti_S and anti_N IgG levels between pediatrics and adults. Our findings reveal significant variations in Ab levels between the two groups, assuring age driven differences in immunity to SARS-CoV-2 [48].

Among individuals with positive test serum samples, the mean anti_S IgG titers were remarkedly increased in pediatrics compared to adults, highlighting the influence of age on the magnitude of Ab responses to the virus [48,49]. This can explain immune protection against more severe COVID-19 in children. Such high anti_S titers might have come from previous cross-reactive HCoV and other viral infections in children, where SARS-CoV-2 acted as a booster effect to a recently generated cross-reactive humoral immunity [49].

In contrast, the anti_N IgG levels were considerably higher in adults than pediatrics among individuals with positive test samples. This observation aligns with the milder course of infection, probably due to restricted viral spread in younger persons, leading to reduced N protein production from virus-infected cells.

In general, studies in the literature state that the differences in anti_S and anti_N IgG levels between pediatric and adult age groups have different interpretations in terms of immune protection against SARS-CoV-2. The higher anti_S IgG titers in children correlate with stronger protective immune responses as manifested by asymptomatic and/or mild symptoms. In contrast, higher anti_S IgG titers in adults is associated with more severe infections including those requiring hospitalization and ICU admissions [39,50–52]. On the other hand, higher anti_N IgG levels in adults indicate a potentially more robust immune response against the N protein positively associated with symptom severity and older age [39,50]. Pediatric anti_N responses are weaker compared to adults [44,46]. This may reflect a more controlled viral spread, limiting the exposure of the immune system to the N protein, involved in viral replication. These findings may have implications for diagnostic tests targeting this viral protein [44,46].

Age was the only factor that had a significant statistical impact on anti_S and anti_N Ab levels among the pediatric group in this study (gender, prior COVID-19 infection history, number of COVID-19 infections, and duration from last infection had no significant effect). Among non-vaccinated adults with natural infection age, symptoms, needs for oxygen, admission department, and duration from infection had significant statistical effects on anti_S seropositivity and/or titers [50,51], while only admission department and duration from infection had a significant effect on anti_N IgG levels [50] using the same anti_S and anti_N Ab assays applied in this study [50,51]. Age was an important predictor of SARS-CoV-2 Ab response in infected children versus adults regardless of other confounding factors including time post-symptom, clinical syndrome, or gender [49]. Furthermore, age-driven variations in SARS-CoV-2 Ab dynamics were observed in mildly infected and non-vaccinated children compared to adults up to 52 weeks post symptoms [28], the important effect of age in children versus adults on SARS-CoV-2 Ab response was highlighted by other studies [27, 29]. Other factors related to the COVID-19 virus including viral load and viral variants, patients' health, risk factors, and immune state, and epidemiological factors have been reported to affect anti_SARS-CoV-2 Ab levels [27,28,49–51].

The current work provides evidence of distinctly robust and persistent humoral immunity displayed by high anti_S and anti_N IgG in children, even >12 months post-infection, that could be the cause of immune protection against COVID-19 infection. Age was the only factor that had a significant statistical impact on anti_S and anti_N Ab levels among the pediatric group in this study. Such information can be helpful to guide pediatric SARS-CoV-2 vaccination programs with implications for developing age-targeted strategies for testing and protecting the population.

6. Limitations

One major limitation of this study is the difference in sampling time between the pediatric and adult groups. All adults' samples were collected early to mid-2020 within 6 months of PCR-confirmed infection, whereas pediatric samples were collected in late 2022 mostly >1 year post COVID-19 infection. This difference in timing imposes differences in circulating viral variants as well as increased possibility of re-infection in the pediatric group, both of which could impact seropositivity rates and magnitude of anti_S and anti_N Ab titers. Another limitation of our study is that we did not compare total neutralizing versus non-neutralizing anti_S and anti_N Abs. Moreover, the sample number per group is low.

7. Conclusions

Pediatric humoral immunity against SARS-CoV-2 is displayed by high anti_S seropositivity rate and titers despite of lack of

symptoms or mild symptom severity, even after one year of infection. This comes in concordance with most similar studies and could explain high protection against COVID-19 in children. Within the 0–18 years old age range of participants, children in ages of puberty (>12 years old) showed significantly higher anti_S and, to a lower extent, anti_N Ab titers compared to younger age groups. This could indicate multiple infections with SARS-CoV-2 or cross-reactive viruses. Compared to naturally infected adults, children show significantly higher anti_S titers, explaining better immune protection against COVID-19. In contrast, adults' anti_N titers were significantly higher. As most children showed no or mild symptoms, lower anti_N titers could be a reflection of limited viral spread and less release of N protein from virally infected cells. Pre-COVID-19 children serum samples, if found in good storage conditions, could help explore the potential cross-reactive immunity resulting from recent HCoV infection that could explain higher anti_S titers and "no" or "mild" symptomatic COVID-19 infection. Further investigation is needed to dissect the mechanisms that drive these age-related differences in Ab levels and to explore their implications for vaccine efficacy, disease severity, and long-term immunity.

Data Availability

Data included in article/supp. material/referenced in article.

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Institutional review board statement

The study received approval from the institutional review board (IRB) of the Hashemite University under reference no. 22/4/2021/2022.

Informed consent statement

All subjects or their parents involved in this study provided Informed consent prior participation.

CRediT authorship contribution statement

Arwa Qaqish: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Manal Mohammad Abbas:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Mohammad Alkhateeb:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Mohammad Al-Tamimi:** Writing – review & editing, Validation, Funding acquisition, Conceptualization. **Minas Mustafa:** Methodology, Writing – original draft. **Abdel-Ellah Al-Shudifat:** Funding acquisition, Conceptualization. **Shahd Tarawneh:** Writing – review & editing, Writing – original draft. **Rand Dawoud:** Writing – review & editing, Writing – original draft. **Amel Mryyian:** Writing – review & editing, Writing – original draft. **Mu'ath Al-Ajaleen:** Writing – review & editing, Writing – original draft.

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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Appendix A. Supplementary data

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