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Serum ACE2 and Anti-MMR Antibody Profiles in Pediatric Patients with and without SARS-CoV-2 Infection

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ABSTRACT

Background: Coronavirus Disease 2019 (COVID-19) typically manifests with milder symptoms and lower mortality rates in children when compared to adults.

Objective: To investigate potential mechanisms underlying this age-related protection, we examined whether serum levels of angiotensin-converting enzyme 2 (ACE2) and IgG antibody titers against Measles, Mumps, and Rubella (MMR) vaccines are associated with susceptibility to SARS-CoV-2 infection in the pediatric population.

Methods: In this case-control study, conducted before the introduction of mass COVID-19 vaccination, we enrolled children aged 1–15 years. The cases were hospitalized children with confirmed COVID-19, while the control group consisted of outpatients with non-infectious, non-immunodeficient conditions and no documented history of COVID-19. The COVID-19 status was confirmed using RT-PCR. Serum levels of ACE2 and anti-MMR IgG antibodies were assessed using ELISA.

Results: Eighty-three patients including 39 cases with COVID-19 infection and 44 controls were enrolled in this study. The median serum ACE2 levels were 3.6 in COVID-19 cases and 3.8 ng/mL in control cases (p=0.440). Similarly, antibody levels against Mumps (p=0.788), Measles (p=0.281), and Rubella (p=0.083) did not differ significantly between the groups, although Rubella seropositivity was more frequent in COVID-19 cases than in controls (p=0.039).

Conclusions: Our findings did not support a significant association between serum ACE2 levels or MMR antibody titers and protection against COVID-19 in children. The higher prevalence of Rubella seropositivity among infected cases may suggest possible cross-reactivity, but causal relationships could not be established in this study.

Keywords: ACE2, COVID-19, Measles, MMR vaccine, Mumps, Rubella, SARS-CoV-2

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INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of the *Betacoronavirus* genus, which also includes SARS-CoV and MERS-CoV (1). Although infection can occur at any age, global reports consistently show that children experience milder disease and markedly lower mortality compared with adults (2). When mortality does occur in the pediatric population, it is usually associated with underlying immunocompromising conditions. Several mechanisms have been proposed to explain this apparent age-related protection. One explanation is that children have a lower prevalence of comorbidities such as immunosuppression or chronic lung disease, which are known to increase the severity of COVID-19 in adults (3). Additionally, age-related differences in the development and regulation of innate and adaptive immune responses may play a key role in modulating SARS-CoV-2 pathogenesis and disease severity in children (4). Another proposed mechanism involves the differential expression of the angiotensin-converting enzyme 2 (ACE2), the functional receptor for SARS-CoV-2 that enables viral entry into host cells (5). Lower levels of ACE2 expression in children may limit viral binding and replication Furthermore, recent bioinformatic studies have identified sequence homology between the structural proteins of SARS-CoV-2 and those of the Measles and Mumps viruses. This observation has led to the hypothesis that prior immunization with the Measles-Mumps -Rubella (MMR) vaccine confers partial protection through cross-reactive immune responses (6, 7). Specifically, similarities in protein domains—such as RNA polymerase, helicase, and surface glycoproteins– have been reported between SARS-CoV-2 and paramyxoviruses. Such structural overlap raises the possibility that individuals previously exposed to non-coronavirus vaccines, such as MMR, may mount a more effective immune response against SARS-CoV-2 via cross-reactive epitopes (8). This hypothesis could partly explain why children, who are more recently vaccinated, tend to experience milder disease. This study was designed to evaluate two of these proposed mechanisms of protection in children: (1) whether serum levels of ACE2 differ between COVID-19-infected and uninfected pediatric patients, and (2) whether levels of anti-MMR IgG antibody are associated with susceptibility to SARS-CoV-2 infection in this age group.

MATERIALS AND METHODS

This case-control study was conducted at the Children's Medical Center Hospital in Tehran, Iran, between September 2020 and January 2021, before the initiation of nationwide COVID-19 vaccination campaigns. We enrolled pediatric patients aged between 1 and 15 years. The case group consisted of children hospitalized with symptomatic COVID-19 confirmed by RT-PCR of nasopharyngeal swabs. The control group included outpatient children referred for non-COVID-related complaints, such as hormonal or metabolic disorders, pre-operative evaluations, or routine follow-up visits. All controls had no documented history of COVID-19 infection in themselves or their immediate family and tested negative for SARS-CoV-2 by RT-PCR. Importantly, none of the control participants had an active infectious or immunosuppressive condition at the time of sampling.All children enrolled had previously received the MMR vaccine. Written informed consent was obtained from the parents or legal guardians of all participants. The study protocol and informed consent documents were reviewed and approved by the Ethics Committee of Aja University of Medical Sciences (Approval Code: IR.AJAUMS.REC.1399.171).

Blood samples were collected from each participant and stored at –80°C until analysis.

Serum levels of ACE2 were measured using ELISA kits (ZellBio GmbH, Germany). Anti-Measles and anti-Mumps IgG antibodies were quantified using Vircell ELISA kits, while anti-Rubella IgG titers were assessed using ARCHITECT kits. All assays were performed in accordance with the manufacturers' instructions.Based on the kit protocols, seropositivity was defined as follows:
Anti-Measles IgG: >11 IU/mL
Anti-Mumps IgG: >11 IU/mL
Anti-Rubella IgG: ≥10 IU/mL

Data were analyzed using IBM® SPSS® Statistics version 20.0 (SPSS Inc., Chicago, IL, USA). The normality of data distribution was evaluated using the Shapiro–Wilk test. Quantitative variables were reported as means with standard deviations (SD) or medians with interquartile ranges (IQR), depending on distribution. Independent t-tests or Mann-Whitney U tests were used for continuous variables, and chi-square or Fisher's exact tests were used for categorical data. A p-value<0.05 was considered statistically significant.

RESULTS

Demographic Characteristics

Eighty-three patients, including 39 cases with COVID-19 and 44 controls, were enrolled in this study (Table 1). There was no significant difference between the groups in terms of mean age (p=0.093) and gender distribution (p=0.596). COVID-19 cases had a higher percentage of children aged ≤2 years old (30.8% vs. 11.6%, p=0.033). Among COVID-19 cases, gastrointestinal symptoms (82.1%) were the most common presentation following respiratory symptoms (inclusion criterion).

Serum Anti_MMR Antibody and ACE2 Levels

The serum levels of IgG antibodies against Measles, Mumps, and Rubella, as well as serum ACE2 concentrations, were compared between COVID-19 cases and controls (Table 2, Fig. 1).

Measles IgG: No significant difference was observed between COVID-19 cases and controls (mean±SD: 12.35±6.12 vs. 13.84±6.34 IU/mL; p=0.281).

Table 1. Demographic characteristics of patients

Variable	COVID-19 PCR test		*p*
	Positive (N=39)	Negative (N=44)	
Age (years), median (IQR)	4 (2-9)	6 (3-11)	0.093
Gender, No. (%)			
Male	19 (48.7)	24 (54.5)	0.596
Female	20 (51.3)	20 (45.5)	
Age Group, No. (%)			
≤2 years	12 (30.8)	5 (11.6)	0.033
>2 years	27 (69.2)	38 (88.4)	
Reason for referral, No. (%)			
Respiratory symptoms	39 (100)	-	
COVID-related GI symptoms	32 (82.1)	-	
Hormonal disorder	-	7 (15.9)	
Hematologic disorder	-	6 (13.6)	
GI disorder	-	6 (13.6)	
Pre-op assessment	-	5 (11.4)	
Metabolic disorder	-	4 (9.1)	
Growth retardation	-	4 (9.1)	
Neurologic disorder	-	3 (6.8)	
Dermatologic disorder	-	2 (4.5)	
Miscellaneous	7 (17.9)	7 (15.9)	

†COVID-19: Coronavirus disease 2019, N: total number of patients in each group, IQR: interquartile range, GI: gastrointestinal.

Table 2. Comparison of anti-MMR antibody titers and serum ACE2 levels between PCR-positive COVID-19 patients and PCR-negative patients

Variable		COVID-19 PCR-test		*p*
		Positive (N=39)	Negative (N=44)	
Measles IgG-Ab (IU/mL)	Mean±SD	12.35±6.121	13.84±6.335	0.281
Mumps IgG-Ab (IU/mL)	Median (IQR)	14.90 (12.95-17.15)	14.90 (12.62-17.40)	0.788
Rubella IgG-Ab (IU/mL)	Median (IQR)	45.65 (17.47-79.00)	26.30 (0.82-67.92)	0.083
ACE2 level (ng/mL)	Median (IQR)	3.60 (2.60-4.95)	3.80 (3.07-4.30)	0.440
Measles Ab index				
Seropositive	No. (%)	21 (53.8)	29 (65.9)	0.262
Seronegative	No. (%)	18 (46.2)	15 (34.1)	
Mumps Ab index				
Seropositive	No. (%)	31 (79.5)	35 (79.5)	0.995
Seronegative	No. (%)	8 (20.5)	9 (20.5)	
Rubella Ab index				
Seropositive	No. (%)	29 (85.3)	27 (65.8)	0.039
Seronegative	No. (%)	5 (14.7)	14 (34.2)	

†Normality testing confirmed the suitability of parametric analysis for Measles antibody titer and group differences were assessed using a T-test. All other variables were analyzed using non-parametric tests. ‡ COVID-19: Coronavirus disease 2019, PCR: polymerase chain reaction, N: total number of patients in each group Ab: Antibody, SD: standard deviation, IQR: Interquartile range, ACE2: angiotensin-converting enzyme 2, IU: international unit.

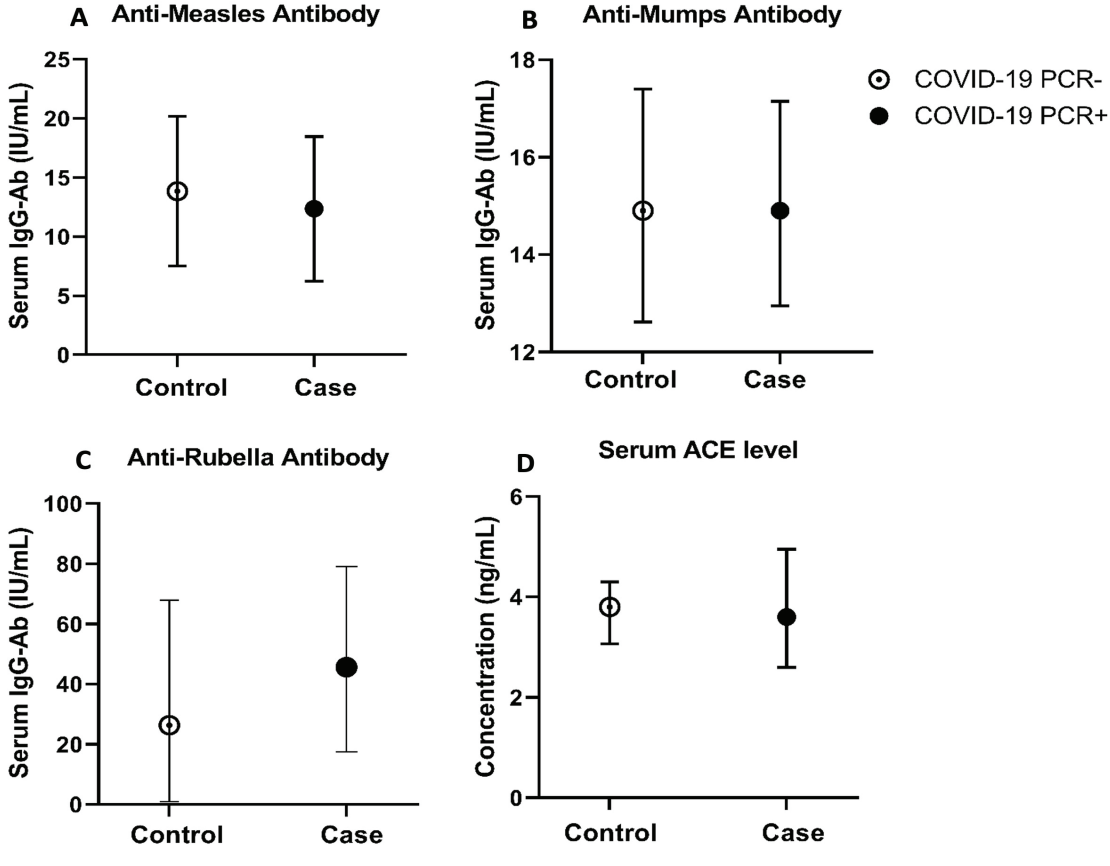

Fig. 1. Evaluation of serum ACE2 levels and anti-MMR antibodies in SARS-CoV-2 infected versus control pediatric subjects. A-D illustrate the central tendency and variability of Measles, Mumps, Rubella, ACE2 indices, respectively. No statistically significant differences were observed between COVID-19 cases and controls for any of these indices. MMR: Measles-Mumps -Rubella, ACE2: Angiotensin-converting enzyme 2

Table 3. Evaluation of anti-Rubella antibody index across age category

Age Group	Anti-Rubella Antibody Index	COVID-19 PCR test		*p*
		Positive (N=34)	Negative (N=41)	
<2 years	Seropositive, No. (%)	8 (80)	1 (25)	0.052
	Seronegative, No. (%)	2 (20)	3 (75)	
≥2 years	Seropositive, No. (%)	21 (87.5)	25 (67.6)	0.077
	Seronegative, No. (%)	3 (12.5)	12 (32.4)	

Mumps IgG: Median titers were identical in both groups (14.90 IU/mL in both; p=0.788).

Rubella IgG: Median antibody titers were higher in COVID-19 cases compared with controls (45.65 vs. 26.30 IU/mL), although the difference was not statistically significant (p=0.083).

Serum ACE2 levels did not differ significantly between groups (median 3.60 ng/mL vs. 3.80 ng/mL; p=0.440).

MMR Vaccine Neutralizing Antibody Index (Response Status)

Participants were categorized as seropositive or seronegative based on antibody index thresholds defined by the assay kits.

Measles: 53.8% of COVID-19 patients and 65.9% of controls were seropositive (p=0.262).

Mumps: Seropositivity rates were identical in both groups (79.5%; p=0.995).

Rubella: A significantly higher proportion of Rubella seropositivity was observed among COVID-19 cases (85.3%) compared to controls (64.3%; p=0.039). To account for potential confounding by age, given that younger children may have lower antibody titers following vaccination, Rubella seropositivity was analyzed separately in children aged <2 and ≥2 years. After stratification, no statistically significant differences were observed between groups in either age group (Table 3), although the same trend toward higher rubella seropositivity in cases persisted (Table 3). For measles and mumps, no significant differences in seropositivity were detected between case and control groups (p>0.05, Table 2).

DISCUSSION

The present study investigated the potential role of serum ACE2 levels and IgG antibody titers against MMR vaccines in resistance to SARS-CoV-2 infection among children. We found no significant difference in serum ACE2 levels between groups (p=0.440), although circulating ACE2 may not reflect tissue expression patterns. Although ACE2 serves as the viral receptor, our findings suggest that serum ACE2 levels are not directly related to disease susceptibility. Similarly, no significant associations were identified between serum IgG antibody levels against Measles, Mumps, or Rubella and SARS-CoV-2 infection status. A considerable proportion of children demonstrated subprotective antibody levels (Measles: 39.8%; Mumps: 20.5%; Rubella: 26.3%). However, rubella seropositivity was significantly higher among SARS-CoV-2 infected children, raising the possibility of cross-reactivity between SARS-CoV-2 and Rubella antigens.

The mechanism underlying the age- related differences in COVID-19 susceptibility remain incompletely understood. Proposed explanations include lower ACE2 receptor expression, reduced viral replication, and faster viral clearance due to prior immunization against antigens with structural homology, nonspecific induction of type I IFNs and overall hypo-inflammatory response in children.

ACE2 serves as the entry receptor for SARS-CoV-2, and reduced expression has been proposed to limit viral entry and disease severity. At the same time, ACE2 exerts protective effects by converting angiotensin

II into angiotensin-(1–7), thereby reducing inflammation, oxidative stress, and fibrosis in the lungs (9). Viral binding and downregulation of ACE2 during SARS-CoV-2 infection disrupt this balance, potentially worsening pulmonary injury (10). Clinical studies, however, have not consistently shown age-related differences in ACE2 expression (11).

Global epidemiological data suggest that live attenuated vaccines, such as MMR, might induce nonspecific immune protection, potentially reducing the severity of SARS-CoV-2 infection (12). In Iran, where ~90% of the population is vaccinated with MMR, COVID-19 mortality is high in adults but remains low in children (13-15). Several studies have explored structural homologies between SARS-CoV-2 and paramyxoviruses. Young A. et al. (6) demonstrated amino acid similarities between SARS-CoV-2 and Rubella fusion proteins and found that higher levels of Rubella antibody titer correlated with better COVID-19 prognosis. Additional analyses identified structural similarities between the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein and the fusion glycoproteins of Measles and Rubella viruses (7, 16, 17). Clinical studies have produced conflicting results: Hassani and his colleagues (18) reported significantly higher titers of anti-Measles antibody after COVID-19 recovery, while Gold et al., observed elevated Mumps virus-neutralizing antibody titers post- infection, but no changes in Measles or Rubella antibodies (19). Taken together, these contradictory findings complicate the interpretation make it difficult to attribute the observed protective effect to any single component of the trivalent MMR vaccine.

Different strains are used for vaccination across countries, which may influence both the immune response and the longevity of protection. The TAKAHASHI/HDC strain, used for Rubella immunization in Iran (20), may yield different outcomes compared to studies employing alternative strains.

Age is another confounding factor: younger children may have only received one dose of the MMR vaccine or may not yet have developed full immune maturity, which could explain differences in seropositivity. In our study, children ≤2 years were disproportionately represented among COVID-19 cases, potentially influencing antibody profiles. Although subgroup analysis was performed, the small sample size reduced statistical power.

Beyond humoral responses, MMR may exert protective effects against SARS-CoV-2 infection by nonspecific immune mechanisms. It has been proposed that the vaccine enhances innate immunity by inducing type I interferon (IFNs) and activating NK cells (16). Supporting this, Yengil et al. administered a booster dose of MMR vaccine to healthy subjects and reported a lower incidence of SARS-CoV-2 infection compared with unvaccinated healthy controls. (21). An observational study has also suggested that COVID-19 patients who have received the MMR vaccine may experience milder forms of the disease and a reduced risk of hospitalization (22). In pediatric populations, limited evidence indicates that prior Measles vaccination may reduce both the incidence and severity of SARS-CoV-2 (23, 24). These findings align with the concept of "trained immunity," in which live attenuated vaccines enhance innate immune responses against unrelated pathogens (25, 26). While our results did not confirm a significant association between MMR-induced humoral immune response and children's resistance to COVID-19, it is possible that innate immunity plays an important role in resistance to SARS-CoV-2 infection through non-specific training,

Severe inflammation in response to SARS-CoV-2 infection is a more likely mechanism of tissue damage in COVID-19 than direct cytopathic effects caused by viral replication (27). Unlike children, the physiological milieu in older adults is characterized by a heightened inflammatory state. Pro-inflammatory cytokines such as TNF-a and IL-6, are elevated in healthy older adults

compared with children (28). Furthermore, immune-senescence in aging is associated with a chronic low-grade inflammatory state termed "inflammaging" (29), which predisposes individuals to chronic diseases such as cardiovascular disease, diabetes, and metabolic syndrome, thereby increasing vulnerability to infections (30). However, the immunological profile of healthy children is non-inflammatory, which contributes to their lower susceptibility to underlying diseases and various infections.

This study was constrained by a small sample size (statistical power=0.188), absence of serologic testing to assess prior SARS-CoV-2 exposure (e.g., anti-nucleocapsid antibodies), and heterogeneity in post-vaccination immune responses among younger children. Furthermore, a subset of participants exhibited antibody titers below protective thresholds, suggesting incomplete immunization.

Further prospective, multi-center studies involving larger cohorts and detailed immune profiling are warranted to elucidate the complex relationship between prior vaccination, ACE2 expression, and pediatric resistance to SARS-CoV-2.

CONCLUSION

In this study, no significant differences were observed in serum ACE2 levels or in the quantitative titers of anti-Measles, Mumps, or Rubella IgG antibodies between children with and without SARS-CoV-2 infection. Notably, Rubella seropositivity was significantly more prevalent among infected children, suggesting the possibility of cross-reactive immune responses. However, this finding does not imply a protective or pathogenic role and should be interpreted with caution, particularly in light of the study's retrospective design and potential confounding factors such as age and vaccine strain. Our data indicate that serum ACE2 concentration may not serve as a reliable biomarker for susceptibility to COVID-19 in children. Furthermore, although MMR vaccination may contribute to trained immunity or cross-reactivity under certain conditions, we found no direct evidence supporting its role in preventing pediatric SARS-CoV-2 infection. Larger cohorts and comprehensive immune profiling are needed to elucidate the potential links between MMR vaccination, ACE2 expression, and pediatric susceptibility to SARS-CoV-2.

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AUTHORS' CONTRIBUTION

ZLM contributed to raw data analysis and manuscript drafting. ZN was responsible for patient enrollment and data collection. SM performed the statistical analysis and assisted in manuscript editing. MTHA, MV, and NHR conceptualized the study, supervised data acquisition and interpretation, and contributed to manuscript editing. All authors reviewed and approved the final version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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