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ARAŞTIRMA YAZISI / RESEARCH ARTICLE

ÇOCUKLARDA SARS-COV-2 ENFEKSİYONUNDA İMMÜNOGLOBULİNLER HASTALIK ŞİDDETİ VE HASTANEDE YATIŞ SÜRESİNE ETKİLİ MİYDİ ?

WAS IMMUNOGLOBULINS EFFECTIVE IN DISEASE SEVERITY AND LENGTH OF HOSPITAL STAY IN CHILDREN WITH THE INFECTION OF SARS-COV-2 ?

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ÖZET

AMAÇ: Hastaneye yatış gerektiren ciddi hastalığı olan çocuklarda başvuru sırasındaki immünoglobulin düzeylerinin hastalık şiddeti, SARS-CoV-2 RT-PCR testinin negatifleşmesine kadar geçen süre ve hastanede kalış süresi ile ilişkili olup olmadığını araştırdık.

GEREÇ VE YÖNTEM: Hastaneye yatırılan ve COVID-19 tedavisi gören kırk dört pediatrik hasta dahil edildi. Hastalar değerlendirme kolaylığı açısından hafif-orta (n=35) ve ağır hastalığı olanlar (n=9) olarak iki gruba ayrıldı. İmmünoglobulin düzeylerinin hastalık şiddeti, SARS-CoV-2 RT-PCR testi negatifleşmesine kadar geçen süre ve hastanede kalış süresi ile ilişkisi incelendi.

BULGULAR: Çalışma popülasyonunun ortanca (min-maks) yaşı 13 (1-18) olup, 25'i (%56,8) kız ve 19'u (%43,2) erkekten oluşmaktadır. Hafif-orta hastalığı olan çocukların %89.2'sinde (n=33) IgG seviyeleri normaldi ve %5.7'sinde (n=2) yükselmişti. Ağır hastalığı olan hastaların %44.4'ünde (n=4) IgG seviyeleri normaldi ve %55.6'sında (n=5) yükselmişti. IgG düzeyleri açısından gruplar arasında anlamlı fark bulundu (p=0,002). IgG düzeyinin hastanede kalış süresi ve SARS-CoV-2 RT-PCR testi negatifleşmesine kadar geçen süre ile ilişkisi incelendiğinde, SARS-CoV-2 RT-PCR test negatifleşmesine kadar geçen süre ile Ig G düzeyi arasında anlamlı bir ilişki gözlenmedi (p=0.096, z=1.667). Ancak Ig G düzeyi yüksek olan hastalarda hastanede kalış süresi anlamlı olarak daha uzundu (p=0.096, p=0.002).

SONUÇ: Normalden yüksek endojen IgG seviyeleri, COVID-19 nedeniyle hastaneye yatırılan çocuklarda ciddi hastalık gelişimi ve uzun süreli hastanede kalış süresi ile bağımsız olarak ilişkili olabilir.

ANAHTAR KELİMELER: COVID-19, Çocuk, İmmünoglobulinler.

ABSTRACT

OBJECTIVE: We investigated whether immunoglobulin levels on admission are associated with disease severity, time to negativization of SARS-CoV-2 RT-PCR test, and length of hospital stay in children with severe illness requiring hospitalization.

MATERIAL AND METHODS: Forty-four pediatric patients hospitalized and treated for COVID-19 were included. The patients were divided into two groups as those with mild-to-moderate (n=35) and those with severe disease (n=9) for ease of evaluation. The relationship of immunoglobulin levels with disease severity, time to SARS-CoV-2 RT-PCR test negativization and length of hospital stay was examined.

RESULTS: The study population had a median (min-max) age of 13 (1-18) years and consisted of 25 (56.8%) girls and 19 (43.2%) boys. IgG levels were normal in 89.2% (n=33) and elevated in 5.7% (n=2) of the children with mild-to-moderate disease. Among patients with severe disease, IgG levels were normal in 44.4% (n=4) and elevated in 55.6% (n=5). A significant difference was found between the groups in terms of IgG levels (p=0.002). When the relationship of IgG level with length of hospital stay and time to SARS-CoV-2 RT-PCR test negativization was investigated, no significant correlation was observed between time to SARS-CoV-2 RT-PCR test negativization and Ig G level (p=0.096, z=1.667). However, the length of hospital stay was significantly longer in patients with elevated IgG levels (p=0.096, p=0.002).

CONCLUSIONS: Higher-than-normal endogenous IgG levels may be independently associated with the development of severe illness and prolonged hospital stay in children hospitalized for COVID-19.

KEYWORDS: COVID-19, Children, Immunoglobulins.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). While most of the infected children do not show any symptoms or experience mild symptoms, severe clinical manifestations may develop especially in children with an underlying chronic disease. Currently, no specific treatment with demonstrated effectiveness is available for COVID-19 (1). In general, the severity of an infection depends on the virulence of the pathogen involved and the immunological response of the host. Although an active immune response is required to eliminate pathogens, uncontrolled host immune responses can cause damage in healthy cells and tissues, consequently determining the disease outcome. There is ongoing debate on the pathophysiology of COVID-19 and high pathogenicity of the virus. In the adult population, cytokine release syndrome and lymphopenia have been identified as hallmarks of severe COVID-19, which indicate increased systemic inflammatory response (2). Immunoglobulins (Ig) produced by plasma cells act as a critical component of the overall immune response and are mainly involved in the recognition, neutralization, opsonization and direct lysis of the pathogens. They also have anti-inflammatory and immunomodulatory properties. There is little information about the changes in serum Ig levels in patients with COVID-19. To the best of our knowledge, the effects of Ig levels on the length of hospital stay and the time to negativization of SARS-CoV-2 polymerase chain reaction (PCR) test in COVID-19 patients are unknown (3 - 8). For these reasons, we sought to investigate whether Ig levels (IgG, IgM and IgA) measured shortly after admission affect the disease severity, length of hospital stay and time to SARS-CoV-2 PCR test negativization in pediatric patients with mild-to-moderate or severe COVID-19 requiring hospitalization.

MATERIAL AND METHODS

This single-center, retrospective, cross-sectional study included pediatric patients with mild-to-moderate or severe COVID-19 who were hospitalized and followed at the Pediatric Infectious Diseases clinic of a tertiary care hospital between 01.04.2020 and 01.03.2022 whose Ig levels were available. Initially, 246 hospitalized pediatric patients were recruited for the study. Subsequently, a total of 202 children with pre-existing immune deficiency syndromes and/or with no Ig measurements available were excluded. Ultimately, the study was conducted with 44 children. All children had been fully vaccinated in accordance with the national immunization schedule of the Republic of Turkey. For all participants, the diagnosis of COVID-19 was confirmed by detection of SARS-CoV-2 in combined oropharyngeal and nasopharyngeal swabs using real-time reverse transcription PCR (RT-PCR) test. The pediatric cases were divided into two groups as those with mild-to-moderate disease and those with severe disease to facilitate assessment. Patients with significant tachypnea on admission and/or at any time during hospitalization, those needing oxygen support >2 L/min and respiratory support with continuous positive airway pressure (CPAP), those who were intubated and/or required intensive care support were included in the severe disease group. Clinical classification of the study patients was performed according to the COVID-19 (SARS CoV-2 infection) guidelines issued by Republic of Turkey Ministry of Health General Directorate of Public Health updated on 06.01.2022 (9). The need for hospitalization was determined individually for each patient based on their clinical condition and in line with the recommendations set forth in the national COVID-19 Guidelines of Turkey, which were periodically updated in the early stages of the pandemic. Even patients with a mild clinical course were hospitalized because in the early phase of the pandemic, there were recommendations to isolate and follow these patients at the hospital due to greater uncertainties at that time.

Age, sex, time to RT-PCR test negativization and length of hospital stay were retrieved from patient files retrospectively. Among laboratory parameters, white blood cell (WBC) and platelet (PLT) counts, hemoglobin level, and lymphocyte and neutrophil counts were noted. IgG, IgM and IgA levels were evaluated

according to age and recorded as decreased, normal or increased. The Nelson Textbook of Pediatrics, 20th Edition was used as a guide to determine the reference ranges of Ig levels (10).

Ethical Committee

This study was approved by the Institutional Ethics Review Committee of University of Adıyaman Turkey, (decision no: 5-15, date: 24.05.2022).

Statistical Analysis

Statistical analyses were conducted to determine the impact of disease severity on laboratory parameters and the relationship of immunoglobulin levels with length of hospital stay and time to PCR negativization. Descriptive statistics were summarized as means and standard deviation (minimum-maximum) for continuous variables and numbers (n) and percentages (%) for categorical variables. The normality of data distribution was checked with Kolmogorov-Smirnov for continuous variables. The results were analyzed using independent samples t-test or Mann Whitney U test depending on the outcome of the normality test. Fisher's exact test and chi-square test were used for categorical variables. All statistical analyses were performed using SPSS software, version 23.0 (IBM Corp., Armonk, NY), and the level of significance was set at $p<0.05$.

RESULTS

The study sample had a median (min-max) age of 13 (1-18) years and consisted of 25 (56.8%) girls and 19 (43.2%) boys. 79.5% (n=35) of the participants had mild-to-moderate disease and 20.5% (n=9) had severe disease. Four patients with severe COVID-19 had additional chronic conditions, including Down's syndrome (n=2), atrial septal defect not causing heart failure and epilepsy (n=1 each). During follow-up, two patients required intensive care but all patients were discharged with full recovery after receiving appropriate treatment. The study groups did not differ significantly in terms of demographic characteristics, which are presented in **Table 1** by disease severity. The length of hospital stay [median (min-max)] was 6 (5-8) days in patients with mild-to-moderate disease and 11 (7-16) days in patients with severe disease, with significantly longer hospitalization observed for severe patients ($p<0.001$, $z=4.615$). The time to negativization of SARS-CoV-2 RT-PCR test [median (min-max)] was 6 (4-8) days in patients with mild-to-moderate disease and 6 (5-13) days for patients with severe disease, with severely ill patients showing significantly longer time to PCR negativization ($p=0.026$, $z=2.228$). Data on routine laboratory parameters and immunoglobulin values are summarized in Table 1. Severely ill patients were found to have a significantly lower lymphocyte count ($p=0.008$, $z=2.662$) and significantly higher prevalence of elevated IgG levels ($p=0.002$). Other laboratory findings were similar between the groups.

When the relation of IgG level with the length of hospital stay and time to SARS-CoV-2 RT-PCR test was examined, no significant association was found between time to SARS-CoV-2 RT-PCR test negativization [median (min –max), normal IgG group: 6 (4-8) days, elevated IgG group: 7 (5-13) days] and IgG level ($p=0.096$, $z=1.667$). However, the length of hospital stay [median (min –max), normal IgG group: 6 (5-12) days, elevated IgG group: 11 (6-16) days] was significantly longer in patients with elevated IgG levels ($p=0.096$, $p=0.002$).

Table 1: Demographic and laboratory data of the study groups by disease severity

Parameters (mean ± SD)	Mild-to-moderate disease (n=35)	Severe disease (n=9)	p
Age, years	11.69 ± 5.14	12.77 ± 4.89	0.365
Sex, n (%)			
• Female	21 (60%)	4 (44.4%)	0.401
• Male	14 (40%)	5 (55.6%)	
Hemoglobin (g/dL)	13.19 ± 1.27	14.13 ± 1.59	0.091
Platelet count (10^3/uL)	255.0 ± 70.0	212.0 ± 68.0	0.180
White blood cell count (10^3/uL)	6.877 ± 2.341	5.325 ± 2.780	0.120
Lymphocyte count (10^3/uL)	2.749 ± 1.965	1.521 ± 5.87	**0.008**
Polymorphonuclear leukocytes (10^3/uL)	3.347 ± 1.446	4.782 ± 4.013	0.141
IgA, n (%)			
• Normal	32 (91.4%)	9 (100%)	0.494
• Increased	3 (8.6%)	0 (0%)	
IgG, n (%)			
• Normal	33 (89.2%)	4 (44.4%)	0.002
• Increased	2 (5.7%)	5 (55.6%)	
IgM, n (%)			
• Normal	33 (94.3%)	8 (88.9%)	0.506
• Increased	2 (5.7%)	1 (11.1%)	

DISCUSSION

To the best of our knowledge, this is the first study examining the association of Ig (IgG, IgM and IgA) levels with the length of hospi-

tal stay and time to SARS-CoV-2 PCR negativization in pediatric patients. We found that the children with severe disease had higher IgG levels than children with mild-to-moderate disease and children with higher IgG levels had a longer duration of hospitalization. Higher IgG levels emerged as an independent predictor of prolonged hospitalization and the development of severe disease. However, IgM and IgA levels were within normal range in patients with mild-to-moderate disease and severely ill patients, and therefore, their relations with time to SARS-CoV-2 PCR were not analyzed. The aforementioned findings suggest that higher IgG levels can be used to predict disease severity and length of hospital stay.

Since the early reports on COVID-19, several prognostic factors have been increasingly cited in the literature including hematologic, cardiac, renal, inflammatory and coagulation biomarkers as well as demographic characteristics and comorbidities (11). In line with previous reports, in our study, lymphopenia and the presence of comorbidities were correlated with the development of severe COVID-19 manifestations. Contrastingly, relationships of sex and neutrophilia with severe disease as reported in the literature, could not be demonstrated in our study. As a matter of fact, only a limited number of studies have focused on serum Ig levels in relation to the severity of COVID-19 and clinical outcome. However, these studies have reported conflicting results. In a study by Qin et al., decrease was found only in IgM levels in patients with severe COVID-19, with no differences in other Ig levels (12). Marcos-Jiménez et al. reported that IgG levels on admission were not different between patients with severe COVID-19 and normal healthy individuals but when the disease severity was considered, IgG levels were lower in severely ill patients (4). Hasan Ali et al. showed increased IgA levels in patients with severe COVID-19 compared to those with mild and moderate disease but there was no marked difference in IgG levels (13). Zhao et al. investigated the correlation between mortality and Ig levels in COVID-19 and demonstrated elevated IgG, IgA and IgE concentrations in non-survivors versus survivors, with no difference in IgM levels (8). It is noteworthy that all studies were conducted in adult population and studies examining Ig levels in pediatric population are lacking.

Our study included children with mild-to-moderate disease or severe disease as well as children whose condition worsened and required intensive care despite being classified as severely ill. Although the current study had a retrospective design, we are aware that many uncertainties around COVID-19 management at the beginning of the pandemic have prompted clinicians to tread carefully. In an attempt to identify parameters that can predict disease prognosis, Ig levels were measured within 48 hours of admission in a portion of hospitalized children. Early assessment of immunoglobulins ensured that Ig levels remained unchanged by other pathogens causing coinfection or drugs administered. Patients with immunodeficiencies and/or IgG deficiency diagnosed with COVID-19 were shown to a more severe disease course and a higher mortality rate (14). In contrast, in our study, Ig levels were not lower than normal in any of the patients in both groups. This may be due to the exclusion of children with immunodeficiencies from the current study. There may be other mechanisms that could explain this finding, which are currently unknown.

Innate and adaptive immune responses vary depending on the severity of COVID-19 manifestations and are correlated with clinical outcome. More specifically, the severe form of COVID-19 has been linked to a dysfunctional innate immune response coupled with aberrant adaptive immunity including inadequate type I interferon response. In adaptive immunity, differential T-cell and B-cell responses have been found in patients with severe disease compared to those with mild disease (15 - 18). Irrespective of the mechanisms, IgG is a key component of humoral immunity and its presence is crucial for host defense against pathogens. IgG is the most abundant Ig and mediates many beneficial immunologic functions (3).

In contrast to adult studies, elevated IgG levels were observed in severely ill children in our study. Additionally, it was shown with the present study that children with increased IgG levels

developed clinically severe illness and required prolonged hospitalization but were discharged with full recovery. This may be the reason why children are often less affected by SARS-CoV-2 infection as a result of some unknown mechanisms mediated by immunoglobulins.

Some limitations should be noted for this study. First, the study had a retrospective design and the sample size was relatively small. Secondly, this was a single-center study. Further studies with large numbers of cases are warranted to demonstrate the generalizability of our findings. Finally, Ig measurements were obtained only once (on admission) for each patient. Serial Ig analyses can better describe the course of IgG over time.

Higher-than-normal endogenous IgG levels may be associated with the development of severe illness and prolonged hospital stay in pediatric patients hospitalized for COVID-19.

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