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RESEARCH ARTICLE

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Predictive value of cytokine/chemokine responses for the disease severity and management in children and adult cases with COVID-19

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Abstract

The disease course of children with coronavirus disease 2019 (COVID-19) seems milder as compared with adults, however, actual reason of the pathogenesis still remains unclear. There is a growing interest on possible relationship between pathogenicity or disease severity and biomarkers including cytokines or chemokines. We wondered whether these biomarkers could be used for the prediction of the prognosis of COVID-19 and improving our understanding on the variations between pediatric and adult cases with COVID-19. The acute phase serum levels of 25 cytokines and chemokines in the serum samples from 60 COVID-19 pediatric (n = 30) and adult cases (n = 30) including 20 severe or critically ill, 25 moderate and 15 mild patients and 30 healthy pediatric (n = 15) and adult (n = 15) volunteers were measured using commercially available fluorescent bead immunoassay and analyzed in combination with clinical data. Interferon gamma-induced protein 10 (IP-10) and macrophage inflammatory protein (MIP)−3β levels were significantly higher in patient cohort including pediatric and adult cases with COVID-19 when compared with all healthy volunteers ($p \leq .001$ in each) and whereas IP-10 levels were significantly higher in both pediatric and adult cases with severe disease course, MIP-3β were significantly lower in healthy controls. Additionally, IP-10 is an independent predictor for disease severity, particularly in children and interleukin-6 seems a relatively good predictor for disease severity in adults. IP-10 and MIP-3β seem good research candidates to understand severity of COVID-19 in both pediatric and adult population and to investigate possible pathophysiological mechanism of COVID-19.

KEYWORDS
coronavirus, critically ill patient, pediatrics, treatment

1 | INTRODUCTION

Despite its worldwide spread, childhood data on the disease caused by the virus, called 2019 novel coronavirus (2019-nCoV) (coronavirus disease 2019 [COVID-19]) are even more limited, with many uncertainties particularly regarding its clinical course as compared with adults. Although there are many different comorbid conditions and underlying diseases in children, unlike other viruses, COVID-19 causes less disease and progresses better in children in worldwide.[1–6] In fact, while many diseases in the childhood, particularly in children under 2 years of age tend to have a more severe and complicated course. However, serious complication besides fatal outcome of children with COVID-19 is also less than adults with COVID-19 and there is no explanation on where this difference might have arisen in children as compared with adults.[7–10]

The causal relationship between pathogenicity or disease severity and biomarkers is still unclear in many studies. To understand the milder course of children, which have an immature immune system, the need for antigenic stimulation and sequential changes in the functional capacity of lymphocytes should be reviewed. Additionally, excessive immune responses during the infection, called as cytokine storm, have been found to be associated with high cytokine levels and widespread tissue damage.[11] A couple of studies have shown that cytokine storm may have occurred in patients with COVID-19.[2,12] Interleukin-1B (IL-1B), IL-1RA, IL-7, IL-8, IL-9, IL-10, fibroblast growth factor, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, interferon γ (IFN-γ), interferon gamma-induced protein 10 (IP-10), monocyte chemoattractant protein (MCP-1), macrophage inflammatory protein (MIP)−1A, MIP-1B, platelet derived growth factor, tumor necrosis factor α (TNF-α) and vascular endothelial growth factor were studied in an adult study consist of 41 patients with COVID-19 and found to be higher in patients compared to healthy individuals. Moreover, the IL-2, IL-7, IL-10, GCSF, IP-10, MCP-1, MIP-1A, and TNF-α levels were higher in patients hospitalized in the intensive care unit (ICU).[2] Pierce et al.[13] investigated the reasons for the differences of clinical outcome in pediatric versus adult patients with COVID-19 and reported that pediatric patients with COVID-19 who had milder clinical course had lower IL-6, TNF-α, and IP-10 concentrations compared to adults with severe outcome. Conversely, the levels of IL-17A and IFN-γ were higher in patients aged under 24 years versus adults and this difference was found to be age-related, particularly for IL-17A. We wondered whether these biomarkers could be used in the diagnosis of COVID-19 or in predicting its prognosis. Therefore, in this study, it was planned to study cytokines and members of the chemokine family in children and adults diagnosed with COVID-19. Thus, we aimed to understand the differences between pediatric and adult patients and whether the prognosis can be predicted with the help of these biomarkers in addition to conventional methods.

2 | MATERIALS AND METHODS

Serum samples were taken from pediatric and adult cases with COVID-19 at the admission and the symptom days of pediatric and adult cases before admission was median 2 days. The diagnosis of the cases was confirmed via reverse transcriptase-polymerase chain reaction (PCR). The samples were collected from patients between April 10 and July 4, 2020 and stored at −80°C and, then, cytokine and chemokine levels were measured. Thirty age and gender-matched pediatric and adult healthy volunteers were enrolled as controls. Hacettepe University Ethics Board for Non-Interventional Studies reviewed and approved the study protocol (Decision no: 17.04.2020-GO 20/385). Data regarding the demographic and clinical characteristics of pediatric and adult patients were collected via patients' charts and laboratory databases of the hospitals. Based on the disease status, the patients were divided into four groups: Group 1, children with COVID-19; Group 2, adults with COVID-19; Group 3, healthy children; and Group 4, healthy adults at the admission.

The variables potentially associated with the infections included: age; sex; medical history; underlying diseases; use of medical devices (mechanical ventilation, extracorporeal membrane oxygenation [ECMO], etc.); laboratory findings; treatment modalities, such as antiviral, antimicrobial therapies, immunosuppressive and immunomodulatory treatments; ICU/pediatric ICU (PICU) admission; and outcome. Data regarding the demographic and clinical characteristics of pediatric and adult patients were collected via patients' charts, computerized administrative, pharmacy, and laboratory databases of the hospitals.

2.1 | Definitions

The severity of pediatric COVID-19 cases, based on the clinical characteristics and the results of laboratory examinations and radiologic imaging, as defined by Dong et al.,[8] as follows: (a) asymptomatic infection: cases with positive PCR despite the absence of the clinical or radiological findings; (b) mild disease: cases with the symptoms of the upper respiratory tract infections without pneumonia; (c) moderate disease: cases with pneumonia; (d) severe disease: cases with progressive respiratory disease, dyspnea, and central cyanosis; and (e) critically ill: cases who are presented with acute respiratory distress syndrome (ARDS) or respiratory failure, shock, and organ dysfunction.

The severity of adult cases was defined according to criteria of World Health Organization interim guidelines,[14] as follows: (i) mild disease: symptomatic cases without pneumonia; (ii) moderate disease: cases with the clinical signs of pneumonia, (iii) severe disease: clinical signs of severe pneumonia, (iv) critical disease: the diagnosis of ARDS, sepsis, septic shock or acute life-threatening organ dysfunction.

2.2 | Estimation of cytokine and chemokine levels in serum samples

The acute phase serum levels of cytokine and chemokine levels including IFN-γ, IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, IL-27, LL-37, IL-33, IP-10, MIP-1β, IL-1β, TNF-α, 6CKine, interferon-inducible T-cell alpha chemoattractant (I-TAC), chemokine (C-C motif) ligand 2 (CCL2; MCP-1), CCL3 (MIP-1α), MIP-3β, and macrophage-derived chemokine (MDC) (CCL22) were measured using commercially available fluorescent bead immunoassay, Human Inflammation 18-Plex Panel (AIMPLEX BIOSCIENCES) and Human Inflammatory Chemokine 7-Plex Panel (AIMPLEX BIOSCIENCES) according to the manufacturer's instructions. Briefly, fluorescent beads were suspended in serum samples or serially diluted standards. Those fluorescent beads are coated with cytokine or chemokine specific antibodies that are not biotinylated. Only the detection antibodies are biotinylated. After 1-h incubation, the beads were washed twice and incubated with streptavidin–phycoerythrin. For another 20 min and then the beads were washed twice again. After adding the reading buffer to all the samples, the samples were ready for flow cytometric analysis. We used Beckman Coulter Navios EX flow cytometry equipped with two lasers (488 and 633 nm) and 6 fluorescent detectors. Forward and side scatter voltages were adjusted by using assayed Standard tubes. FL2 detectors (575 nm) were used for bead quantitation, and an FL4 detector (675 nm) was used for bead differentiation. For each analysis, 5000 beads were collected. The concentrations were measured using Flow Cytomix Pro 2.3 software (Bender MedSystems).

2.3 | Statistical analysis

Categorical variables are presented as per frequencies and in percentages and were compared using the χ^2 test. Continuous variables are presented as median (min–max) and differences in continuous variables between the groups were tested by using the Mann– Whitney *U* test. Kruskal–Wallis test was used to compare the plasma cytokine or chemokine levels among the mild, moderate, severe, and critical groups. After Kruskal–Wallis test, the Dunn's test was used to determine the differences between the groups. Receiver-operating characteristic (ROC) curve and area under the ROC curve (AUC) of serum cytokine or chemokine levels was estimated for the patients with severe disease course or not. Moreover, a logistic regression model was established with IP-10, MIF 1β, and MIP-3β variables. The combined values (score) for the prediction of developing severe disease was calculated using binary logistic regression. ROC analysis was performed for these combined values. All statistical tests were calculated using SPSS 22.0 for Windows (IBM). *p* Value of less than .05 was considered statistically significant.

3 | RESULTS

Thirty pediatric cases (14 males, 16 female) with a median age 10.5 and 30 adult cases (14 males, 16 female) aged median 62.5 with COVID-19 were enrolled in the present study. The male percentages were 46.7% for both children and adults (Table 1). There were two pediatric cases and one adult patient with fatal outcome. Nine (30%) children had severe or critical disease and 21 (70%) children had mild or moderate disease. Eleven (36.7%) adult cases had severe or critical disease and 19 (63.3%) adult cases had mild or moderate disease. Patients were hospitalised, admitted to either inpatient wards and the ICU/PICU according to disease course. ICU admission and PICU admission accounted for 7 (23.3%) in adults and 4 (13.3%) in children, respectively (Table 1). Chronic pulmonary diseases and neurologic diseases in children and chronic pulmonary diseases and hypertension in adults were the leading underlying problems. ECMO support was needed for two paediatric patients and one of them had fulminant fatal myocarditis and the other patient was presented with Stevens-Johnson syndrome before the exposure of 2019-nCoV. The pediatric patients who died were aged 1 and 7 years; both had no any pre-existing comorbidities. The adult patient, who died at the age of 82 due to respiratory failure, had multiple comorbidities including type 2 diabetes, hypertension, benign prostate hyperplasia, and newly diagnosed atrial fibrillation.

The laboratory parameters and treatment options among this cohort are summarized in Table 1. The median white blood cell, lymphocyte, neutrophil and platelet count of adult cases (4400, 740, 2390, and 168×10^3, respectively) were different as compared with the children (8470, 1300, 4300, and $209 \times 10^3/\mu l$, respectively) ($p \le .001$, $p \le .001$, $p = .026$, and $p = .001$, respectively). Whereas the median CRP level (2.69 mg/dl) was significantly higher in adult patients ($p = .007$), the median PCT level (0.09 ng/ml) was significantly higher in pediatric patients ($p = .015$). Whereas various therapies to target the viral infection was used in 29 adult patients (96.7%), limited targeted therapies was preferred in children and only used in severe or critical cases with a ratio of 23.3% ($n = 7$). Anticoagulant therapies were also mainly used in adult patients (96.7%) and treatment differences between children and adults in terms of antivirals and anticoagulants was statistically significant ($p \le .001$ in each, respectively). Corticosteroids and intravenous immunoglobulin were mainly used in children.

MDC, MIP-1α, MIP-3β, and IL-17A were significantly higher in children with COVID-19 compared to adults with COVID-19 ($p = .001$, $p = .045$, $p = .003$, and $p = .031$, respectively). When the parameters of pediatric patients with COVID-19 and healthy children were compared, significantly higher serum levels of IP-10 and MIP-3β and lower levels of MIP-1β were detected in the former group ($p = .005$, $p \le .001$, and $p = .006$, respectively). Similarly, significantly higher serum levels of IP-10 and MIP-3β were detected in adult patients with COVID-19 as compared to healthy adults ($p \le .001$ and $p = .003$, respectively). Additionally, significantly higher IL-10, IL-6, IL-1α, and IL-27 levels were revealed in patients as compared with healthy volunteers in adult cohort ($p = .018$, $p \le .001$, $p = .015$, and $p = .02$, respectively) (Table 2). Furthermore, the median IP-10 and MIP-3β levels (441.41; min–max, 21.48–4115.40 and 1278.08; min–max, 17.76–3588.56, respectively) were significantly higher in patient cohort including pediatric and adult cases with COVID-19 when compared with all healthy volunteers (97.36; min–max, 2.32–889.38 and 632.20; min–max, 0–3235.35) ($p \le .001$ in each).

TABLE 1 Demographic and clinical characteristics of patients with COVID-19

Characteristics	Children with COVID-19 (*n* = 30)	Adults with COVID-19 (*n* = 30)	*p* Value
Age (years; median [min–max])	10.5 (0–17)	62.5 (48–77)	NA
Sex (*n*, %)			1.0
Male	14 (46.7%)	14 (46.7%)	
Female	16 (53.3%)	16 (53.3%)	
Underlying diseases (*n*, %)	7 (23.3%)	15 (50%)	**.032**[a]
Intensive care unit (ICU)/pediatric ICU (PICU) admission	4 (13.3%)	7 (23.3%)	.317
Mechanical ventilation	5 (16.7%)	10 (33.3%)	.136
Extracorporeal membrane oxygenation (ECMO)	2 (6.7%)	0 (0)	.492
Laboratory parameters (median [min–max])			
White blood cells (WBC) (/ml)	8470 (4000–11600)	4400 (2100–7700)	**<.001**[a]
Lymphocytes (/ml)	1300 (420–6100)	740 (200–2400)	**<.001**[a]
Neutrophils (/ml)	4300 (600–9200)	2390 (1000–7040)	**.026**[a]
Platelets (/ml)	209 × 103 (153–344 × 103)	168 × 103 (63–252 × 103)	**.001**[a]
C-reactive protein (CRP) (mg/dl)	1.16 (0.08–12.36)	2.69 (0.08–31.7)	**.007**[a]
Procalcitonin (PCT) (ng/ml)	0.09 (0.01–7.9)	0.03 (0.01–12.75)	**.015**[a]
D-dimer (mg/L)	1.0 (0.19–6.65)	0.38 (0.03–80.0)	.055
Ferritin	63.2 (4–1967)	124 (12.9–1648)	.705
Treatment (*n*, %)			
Antiviral	7 (23.3%)	29 (96.7%)	**<.001**[a]
Anticoagulant	1 (3.3%)	29 (96.7%)	**<.001**[a]
Corticosteroids	2 (6.7%)	0	.492
IVIG	4 (13.3%)	0	.112
Antibacterial	18 (60%)	23 (76.7%)	.165
Outcome (*n*, %)			
Death	2 (6.7%)	1 (3.3%)	1.0

Abbreviations: COVID-19, coronaviru disease 2019; IVIG, intravenous immunoglobulin.

[a]Statistically significant.

In addition to IP-10 and MIP-3β, I-TAC, IL-10, IL-6, and IL-1α levels were significantly different between combined COVID-19 patients and all healthy controls ($p = .007$, $p = .03$, $p = .05$, and $p = .04$, respectively).

When the parameters of pediatric patients with COVID-19 and healthy children were evaluated according to disease course, the IP10 and MIP-3β levels differed significantly between the groups ($p = .008$ and $p = .002$, respectively) (Table 3). The IP-10 levels were higher in pediatric patients with severe/critical disease course (757.5 ± 1212.7) compared to healthy controls (176.3 ± 245.7, $p = .004$). The MIP-3β levels were higher in patients with moderate disease course (2079.5 ± 609.1) compared to healthy controls (684.8 ± 784.6, $p = .001$). When compared to adult patients according to disease severity, the I-TAC, IP-10, MIP-3β, and IL-6 levels differed significantly between the groups ($p = .031$, $p \leq .001$, $p = .028$, and $p = .001$, respectively) (Table 4). The I-TAC levels were higher in adult patients with severe/critical disease course (23.7 ± 250) compared to healthy controls ($p = .027$). The IP-10 levels were lower in healthy controls (26.2 ± 116.5) compared to adult patients with moderate disease (629.9 ± 353.9, $p \leq .001$) and adult patients with severe disease course (655.8 ± 512.6, $p = .001$). The MIP-3β levels were higher in severe adult COVID-19 cases (1242.2 ± 589.6) compared to healthy controls (561.3 ± 773, $p = .047$). The IL-6 levels were higher in adult patients with severe disease (2399.9 ± 877.1) compared to healthy controls (1107.9 ± 760.8, $p = .001$).

We further analyzed whether these cytokines or chemokines could be used as predictors for the disease severity of COVID-19. These patients were divided into the severe group, which contained the severe and critically ill cases, and the non-severe group, which included mild and moderate cases. The ROC curve of cytokines which was determined as significant was calculated using the expression levels in both children and adult cases. The results of

TABLE 2 Cytokine or chemokine levels in patients and control groups

Cytokine/chemokine levels (pg/ml)	Group 1 (Children with COVID-19) (*n* = 30) Median (min–max)	Group 2 (Adults with COVID-19) (*n* = 30) Median (min–max)	Group 3 (Healthy children) (*n* = 15) Median (min–max)	Group 4 (Healthy adults) (*n* = 15) Median (min–max)	*p* Value
Chemokine levels					
I-TAC	118.7 (0–749.7)	0 (0–1382.4)	0 (0–615.3)	0 (0–0)	.071[a] .162[b] .005[c]
MDC	27686.7 (4900.3–102883.8)	16488.2 (2162.4–46068.7)	31668 (8923.3–73679.6)	17384.4 (0–38555.2)	**.001**[a] .523[b] .923[c]
MIP-1 α	1934.9 (0–21283.6)	11.5 (0–3997.4)	3201.5 (0–33177.7)	0.9 (0–6142.5)	**.045**[a] .202[b] .202[c]
IP-10	380.2 (158.4–4115.4)	525.2 (21.5–1635.1)	176.3 (10.1–889.4)	26.2 (2.3–386.6)	.183[a] **.005**[b] **.001**[c]
MIP-3 β	1504.2 (552.1–3588.6)	1051.9 (17.8–3159.9)	684.8 (0–3235.4)	561.3 (0–3017.2)	**.003**[a] **<.001**[b] **.003**[c]
MIP-1 β	697.9 (0–14573.1)	624.4 (68.0–2684.7)	2049.3 (170.8–12969.2)	587.4 (0–1650.2)	.579[a] **.006**[b] .42[c]
Cytokine Level					
IL-4	2086.6 (0–6803.2)	1668.6 (0–19131.5)	1653.9 (0–3729.2)	1545.1 (0–3184.9)	.146[a] .455[b] .346[c]
TNF-α	1283.9 (0–3760.7)	713.4 (0–3930.9)	1285.2 (0–2875.4)	435.6 (0–1520.5)	.125[a] .579[b] .183[c]
IL-17A	1794.3 (0–10495.1)	1335.3 (0–10721.5)	1525.5 (864.0–2561.9)	1235.1 (0–2059.7)	**.031**[a] .531[b] .621[c]
IL-13	52.2 (0–210.6)	43.4 (0–232.8)	75.8 (0–153.3)	0 (0–102.6)	.471[a] .149[b] .179[c]
MCP-1	0 (0–29634.3)	0 (0–6410.9)	0 (0–2127.9)	0 (0–0)	.512[a] .175[b] **.045**[c]
IL-1 β	100.2 (0–151.02)	93.4 (0–199.4)	111.6 (46.9–18636.3)	73.6 (0–121.6)	.191[a] .051[b] .123[c]
IL-10	3777.8 (0–10846.3)	2850.2 (0–23439.2)	3633.1 (1171.3–6114.7)	2361.9 (0–4100.5)	.056[a] .295[b] **.018**[c]
IL-6	1922.4 (0–56607.6)	2124.8 (1058.1–4358.2)	2103.5 (899.0–59838.3)	1107.9 (0–2357.9)	.416[a] .531[b] **<.001**[c]
IL-1 α	20.2 (0–185.2)	13.2 (0–412.3)	7.6 (0–92.8)	0 (0–28.1)	.685[a] .532[b] **.015**[c]

TABLE 2 (Continued)

Cytokine/chemokine levels (pg/ml)	Group 1 (Children with COVID-19) (*n* = 30) Median (min–max)	Group 2 (Adults with COVID-19) (*n* = 30) Median (min–max)	Group 3 (Healthy children) (*n* = 15) Median (min–max)	Group 4 (Healthy adults) (*n* = 15) Median (min–max)	*p* Value
IL-27	475.6 (0–1757.3)	385.9 (0–10490.2)	340.3 (0–1204.6)	237.6 (0–1305.1)	.819[a] .691[b] **.024**[c]
IFN-γ	1422.8 (0–4422.2)	1391.9 (0–5444.3)	1286.3 (0–2980.1)	1065.9 (0–2115.8)	.912[a] .895[b] .295[c]

Note: Bold values are significant.

Abbreviations: IFN-γ, interferon γ; IL, interleukin; I-TAC, interferon-inducible T-cell alpha chemoattractant; MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; TNF-α, tumor necrosis factor α.

[a]Group 1 versus Group 2.

[b]Group 1 versus Group 3.

[c]Group 2 versus Group 4.

children showed that the AUC of the ROC curve was 0.77 for IP-10 ($p = .019$) (Figure 1A). Combination of IP-10, MIP-1β, and MIP-3β showed the AUC of 0.80 ($p = .009$) in children (Figure 1B). The results of adults showed that the AUC of the ROC curve was 0.70 for IL-6 ($p = .06$) (Figure 1C). Combination of the four cytokines or chemokines in adults showed the AUC of 0.78 ($p = .01$) (Figure 1D). All the *p* values for other cytokines were higher than .05.

4 | DISCUSSION

We found that IP-10 levels were significantly higher in both pediatric and adult cases with severe disease course and MIP-3β were significantly lower in healthy controls. Additionally, our data showed IP-10 is an independent predictor for disease severity, particularly in children. IP-10 and MIP-3β seem promising targets not only for

TABLE 3 Comparisons of the cytokine or chemokine levels of the children with COVID-19 according to the disease severity

Cytokine/chemokine levels (pg/ml)	Mild cases (*n* = 11) Median (min–max)	Moderate cases (*n* = 10) Median (min–max)	Severe/critical cases (*n* = 9) Median (min–max)	Controls (*n* = 15) Median (min–max)	*p* Value
Chemokines					
I-TAC	100.5 (0–749.7)	112.3 (0–366.3)	242.3 (0–413.8)	0 (0–615.3)	.548
MDC	28427.2 (14896–102883.8)	31198.7 (13728–74342.3)	20370.4 (4900.3–71733.8)	31668 (8923.3–73679.6)	.546
MIP-1 α	1821.1 (2.1–3435.1)	1658.3 (0–3435.1)	2252.2 (5.9–21283.6)	3201.5 (0–33177.7)	.297
IP-10	292.2 (158.4–1169.6)	281.4 (167.3–526.9)	757.5 (158.4–4115.4)	176.3 (10.1–889.4)	**.008**[a]
MIP-3 β	1393.9 (785–3399.7)	2079.5 (909.7–2758.5)	1305.1 (552.1–3588.6)	684.8 (0–3235.4)	**.002**[a]
MIP-1 β	644.9 (0–1593.8)	921.9 (0–1276.1)	702.1 (48.9–14573.1)	2049.3 (170.8–12969.2)	**.039**[a]
Cytokines					
IL-4	2199.9 (0–6803.2)	2049.5 (0–5909.4)	1973.3 (0–3536.3)	1653.9 (0–3729)	.866
TNF-α	1305.9 (0–3760.7)	1294.7 (0–2814.6)	1126.9 (0–2093.7)	1285.2 (0–2875.4)	.833
IL-17A	1816.3 (0–10495.1)	1794.3 (0–4613.2)	1696.4 (0–2286.9)	1525.5 (864–2561-95)	.739
IL-13	53.3 (0–148.1)	55.9 (0–210.6)	0 (0–134.5)	75.8 (0–153.4)	.329
MCP-1	0 (0–3391.3)	0 (0–2669.2)	0 (0–29634.3)	0 (0–2127.9)	.215
IL-1 β	105.3 (0–131.4)	102.6 (0–151)	99.5 (50.1–130.7)	111.6 (46.9–18636.3)	.222
IL-10	2845.9 (0–7648.4)	4467.2 (0–15733.6)	5141.5 (1989.5–108461.4)	3633.1 (1171.3–6114.7)	.136
IL-6	1912.8 (0–3158.3)	1771 (0–6173.2)	1932 (463.2–56607.6)	2103.5 (899–59838.3)	.833
IL-1 α	18.9 (0–185.2)	14.9 (0–82.7)	21.5 (0–42.2)	7.6 (0–92.8)	.94
IL-27	231.6 (0–1270.5)	617.5 (0–1757.4)	479.9 (0–1123.8)	340.3 (0–1204.6)	.607
IFN-γ	1448.6 (0–3463.3)	1603.9 (0–4422.2)	1165.1 (0–2091.3)	1286.3 (0–2980.1)	.749

Abbreviations: IFN-γ, interferon γ; IL, interleukin; I-TAC, interferon-inducible T-cell alpha chemoattractant; MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; TNF-α, tumor necrosis factor α.

[a]Statistically significant.

TABLE 4 Comparisons of the cytokine or chemokine levels of the adult cases with COVID-19 according to the disease severity

Cytokine/chemokine levels (pg/ml)	Mild cases (*n* = 4) Median (min–max)	Moderate cases (*n* = 15) Median (min–max)	Severe/critical cases (*n* = 11) Median (min–max)	Controls (*n* = 15) Median (min–max)	*p* Value
Chemokines					
I-TAC	0 (0–1382.4)	0 (0–466.4)	23.7 (0–741.4)	0 (0–0)	**.031**[a]
MDC	17709.9 (11687.2–29591.7)	15800.6 (8630.8–46068.7)	11168 (2162.4–23385.9)	17384.4 (0–38555.2)	.662
MIP-1 α	1876.9 (0–3997.4)	6.8 (0–2793.1)	1454.2 (0–3423.5)	0.9 (0–6142.5)	.467
IP-10	121.9 (24.4–298.3)	629.9 (22–1229.1)	655.8 (21.5–1635.1)	26.2 (2.3–386.6)	**<.001**[a]
MIP-3 β	792.1 (621.2–3157.1)	922.3 (17.8–3159.9)	1242.2 (18.5–1796.6)	561.3 (0–3017.2)	**.028**[a]
MIP-1 β	839.9 (68–2684.7)	531.3 (153.9–1207.2)	899.4 (231.8–2167.4)	587.4 (0–1650.2)	.464
Cytokines					
IL-4	2305.8 (0–19131.5)	1746.4 (0–2742.4)	1291.6 (0–2556.9)	1545.1 (0–3184.9)	.42
TNF-α	451.2 (0–3930.9)	435.3 (0–2374.8)	888.9 (0–1932.5)	435.6 (0–1520.5)	.473
IL-17A	1284.6 (0–10721.5)	1564.8 (0–2409.1)	1235.2 (0–2264.9)	1235.1 (0–2059.7)	.585
IL-13	54.4 (0–232.8)	46.4 (0–135.5)	37.2 (0–109.2)	0 (0–102.6)	.501
MCP-1	0 (0–6410.9)	0 (0–2466.9)	0 (0–3062.3)	0 (0–0)	.091
IL-1 β	104.8 (82.9–199.4)	93.4 (0–119.1)	83.6 (0–125.6)	73.6 (0–121.6)	.125
IL-10	2848.3 (0–23439.2)	3066.9 (0–7647.8)	2682.1 (1173.1–7075.5)	2361.9 (0–4100.5)	.095
IL-6	1854 (1058.1–3715.7)	2061.1 (1183.9–3011.2)	2399.9 (1802.6–4358.2)	1107.9 (0–2357.9)	**.001**[a]
IL-1 α	21.1 (0–412.3)	13.8 (0–52.7)	12.6 (0–37.8)	0 (0–28.1)	.099
IL-27	857.5 (143.1–10490.2)	386.1 (251.9–788.5)	368.9 (0–788.5)	237.6 (0–1305.1)	.099
IFN-γ	704.1 (480.6–5444.3)	1460.8 (579.8–-2620.4)	1413.9 (0–2184.7)	1065.9 (0–2115.8)	.345

Abbreviations: IFN-γ, interferon γ; IL, interleukin; I-TAC, interferon-inducible T-cell alpha chemoattractant; MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; TNF-α, tumor necrosis factor α.

[a]Statistically significant.

investigating possible pathophysiological mechanism of COVID-19 but also for managing the specific strategy of treatment in patients with COVID-19, consistently with the literature.[15–17] Although cytokines are central drivers and controllers of immune-mediated virus elimination, the milder clinical course of children could not be explained by the cytokine network alone. Increasing evidence points to the equally important role played by the family of chemokines in modulating adaptive immune response.

Chemokines regulate the trafficking of leukocytes via stimulating adaptive immunity by recruiting and activating lymphocytes at the infection site and modulating T-helper type-1 (Th1) or Th2 response.[18] Significantly higher MDC, MIP-1α, and MIP-3β levels were observed in children with COVID-19 as compared to adult cases with COVID-19 in the present study. Therefore, we speculated whether the mentioned members of chemokine family might be the responsible of the milder disease course in children. There is growing interest to examine the ability of chemokines, including MIP-3β in the field of virus specific immunity. After antigen uptake, CCR7 expression is strongly upregulated in dendritic cells (DCs) and mature DCs migrate to lymph nodes in response to MIP-3β, a ligand of CCR7.[17] Mature DCs present antigen to lymphocytes, thereby generating specific immunity.[19,20] These findings might possibly be attributed to the pivotal role of mature DCs, which control the type of immunity by activating $CD4^+$ T-helper cells in disease process of the cases with COVID-19. Moreover, CD4 and CD8 cells have a couple of actions in immune responses such as immune regulation, secretion of cytokines, and virus-specific antibody production, which is definitely needed in patients with COVID-19.[21] Adjuvanticity of chemokines including MIP-1α and MIP-3α have been recognized and significant immuno-adjuvant activity of MIP-1α, which also acts as a chemo-attractant to inflammatory cells such as immature DCs, macrophages, and monocytes has been studied by many groups.[17] Moreover, IFN-γ production that is essential for acquiring Th1 immunity is enhanced by the MIP-1α stimulation. Furthermore, the crucial role of MIP-1α in mediating virus-induced inflammation was demonstrated in animal models in decades ago.[22,23] MIP-1α, and MIP-3β, which are of great importance for modulating the efficacy and polarization of antigen-specific immunity. Although, the role of MDC in the regulation of TH2-related immune responses is well established, the role of MDC in the development of the lung inflammation response to viral diseases is unknown. However, previous reports suggested that high level of MDC was detected in cigarette smoke-induced pulmonary inflammation and lung inflammation after hemorrhage and resuscitation.[24,25] Therefore, our findings let us consider the possible role of MDC in the pathogenesis of virus-induced lung damage. Consistently with the literature,[13] the concentration of IL-17A was higher in children than in adults in our study. IL-17A-driven cytokine storm may possibly cause the arterial

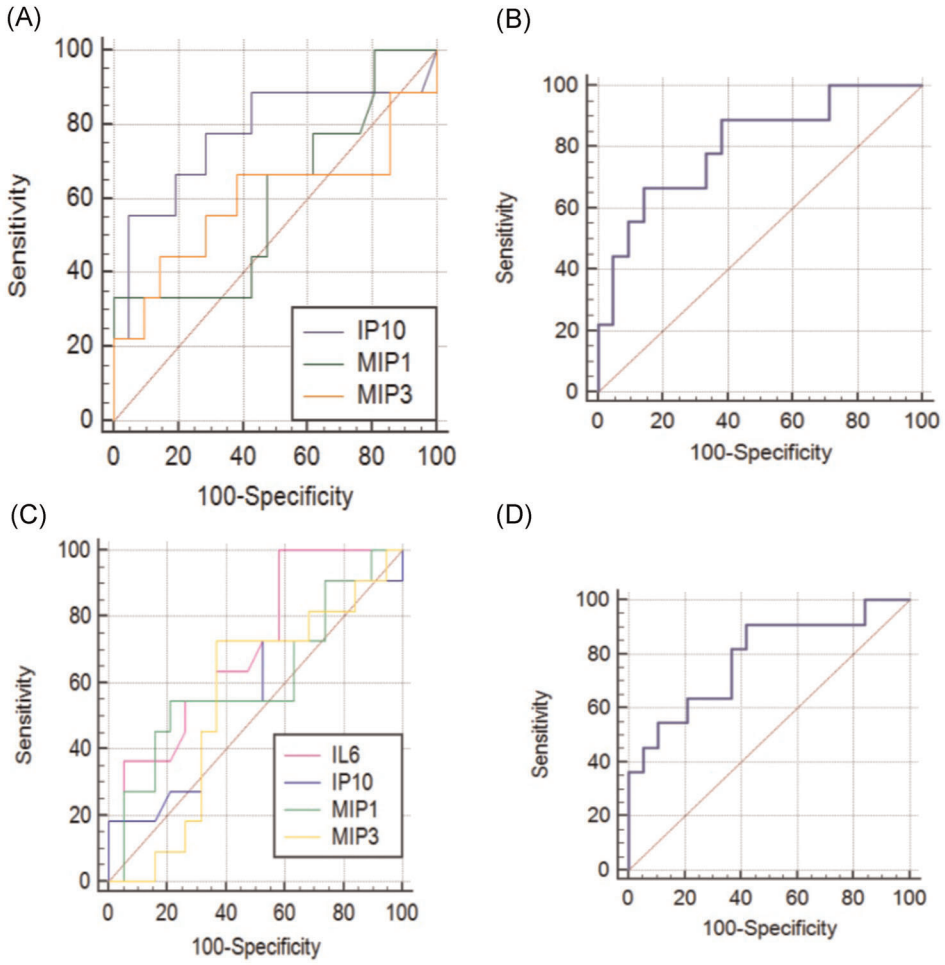

FIGURE 1 The ROC curve of plasma cytokine/chemokine levels on admission for patients with severe disease course or not. (A) The AUC of the ROC curve for IP-10, MIP-1β, and MIP-3β was calculated in children (B) The ROC curves of combination of IP-10, MIP-1β, and MIP-3β in children. (C) The AUC of the ROC curve for IL-6, IP-10, MIP-1β, and MIP-3β was calculated in adults. (D) The ROC curves of combination of IL-6, IP-10, MIP-1β, and MIP-3β in adults. AUC, area under tjhe ROC curve; IL, interleukin; MIP, macrophage inflammatory protein; ROC, receiver-operating characteristic

inflammation in patients with Kawasaki disease, which involves the medium-sized arteries.[26] Damaged microcirculatory function due to diffuse endothelial inflammation, called COVID-19-endotheliitis, in different vascular beds might be responsible many clinical undesirable outcomes in patients with COVID-19, as well.[27] Perhaps, IL-17A-mediated inflammation should be considered in severe or critically ill pediatric patients with COVID-19 and IL-17A blocking agents, such as secukinumab might be a reasonable therapeutic option in such kind of patients. From another perspective, IL-17A associated immune response might play an age-related role for mitigation of the cytokine storm and might be one of the reasons of rapid resolution of viral infection.[13] These results indicate that there is a particular cytokine or chemokine profile in children with COVID-19, which differs from inflammation in adults with COVID-19 and that might have possible role to change in response to immunomodulatory therapies.

Uncontrolled proinflammatory responses, which is called as cytokine storm, induce an important immunopathology and is one of the main reasons of the disease severity during the viral infections.[28] Accordingly, not only the causative microorganisms but also the pathogen induced cytokine storm should be considered during the treatment course.[16] Severe disease outcome including death caused by cytokine storm could be downregulated by corticosteroids.[29] Although high dose corticosteroids have been shown to be associated with an increase in mortality and longer viral shedding in flu cases due to H7N9, use of corticosteroids at low-to-moderate was found to be associated with reduced mortality in pneumonia caused by viruses such as influenza and 2019-nCoV.[30,31] In a couple of studies, proper use of corticosteroids shortens the duration of hospitalization and reduce the need for mechanical ventilation without causing secondary infection and other complications, as well.[32–34] Because of this emerging situation in worldwide, Chinese Thoracic Society developed an expert consensus statement about the use of corticosteroids in patients with COVID-19 and recommended short course of corticosteroids at low to moderate dose in critically ill patients.[31] In the study period, although corticosteroids were used for only two pediatric

cases with critical disease course, we prudently started to evaluate the use of them in selected patients with severe disease course.

IL-6 and IL-1α seem to be the crucial therapeutic targets in patients with COVID-19 in this study. Accordingly, IL-6, although is not significant, seems to be a good predictor for disease severity, particularly in adult cases. There are several ongoing studies with the different anti-cytokine therapies including IL-1 and IL-6 blockade in severe cases.[35] It was reported in observational studies on tocilizumab that it decreases fever, systemic inflammation and associates with decreased risk of intubation and mortality.[36–41] Cavalli et al.[42] reported that 72% of the cases with COVID-19 who received anakinra experienced clinical improvement and showed significantly higher survival rate as compared with the historical control group and patients treated with anakinra had a significantly lower risk of death and need of mechanical ventilation than the controls in another study.[43] A couple of studies about sepsis, it was also reported that short-term, high dose anakinra treatment in patients with severe sepsis did not increase the bacterial superinfection risk, which is a great concern in patients with COVID-19 as well.[44,45] Nevertheless, the use of clinical judgement is crucial for the management of the cases because of variations of the background antivirals or immunomodulators for accurate interpretation of these results across both patients and studies.

Some several limitations of this study should be noted. First, the sample sizes were relatively small. The frequencies of patients with fatal outcome and the disease severity seem comparable between children and adults in this cohort, which is not consistent with most published studies; because our pediatric centers were tertiary care referral centers with high quality PICU facility in the capital of the Turkey. Therefore, our result cannot be generalized and need to be confirmed in larger cohort to reveal the actual disease nature. Second, detailed analyses of lymphocyte subgroups could not be performed simultaneously at the beginning of the pandemic period. However, we believe that our data will guide the physicians and ameliorate our understanding on the possible physio-pathological pathway of COVID-19.

As a conclusion, we compared the differences of cytokine/chemokine profiles between pediatric and adult cases with COVID-19, particularly according to disease severity and found that IP-10 and MIP-3β might be the significant predictors for the disease as well as the severity. Therefore, our findings let us understand roughly the mechanisms of the disease course of COVID-19 and help to figure out possible new-aged treatment modalities to cope with cytokine storm in COVID-19, such as global targeting of the inflammation or neutralizing a single key inflammatory mediator. Because the activation of a viral antigen-specific Th1 immune response is vital for COVID-19 protection, chemokines, such as MIP-1α, and MIP-3β seem likely promising immuno-adjuvant candidates for further vaccine studies.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Dr. Ozsurekci and Dr. Ceyhan conceptualized and designed the study, collected data, drafted the initial manuscript, and reviewed and revised the manuscript.Dr. Ozsurekci, Dr. Aykac, Dr. Yayla, Dr. Er, Dr. Halacli, Dr. Oygar, Dr. Gurlevik, Dr. Topeli, Dr. Cengiz, and Dr. Akova carried out the initial analyses, collected data, and reviewed and revised the manuscript. Dr. Arasli, Dr. Ozsurekci, and Dr. Alp performed the laboratory investigations. Dr. Karakaya performed statistical analyses. Dr. Ozsurekci, Dr. Ceyhan, Dr. Topeli, and Dr. Akova critically reviewed the manuscript for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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