

# Cytokine profile in Egyptian children and adolescents with COVID-19 pneumonia: A multicenter study

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## Abstract

**Background:** To date, the cytokine profile in children and adolescent with novel coronavirus disease 2019 (COVID-19) has not been reported.

**Objectives:** We investigated serum levels of a panel of key cytokines in children and adolescent with COVID-19 pneumonia with a primary focus on “cytokine storm” cytokines such as interleukin (IL)-1 $\beta$ , IL-6, IL-17, IL-2, IL-4, IL-10, interferon (IFN- $\gamma$ ), tumor necrosis factor (TNF)- $\alpha$ , and two chemokines interferon-inducible protein-10 (IP-10) and IL-8. We also studied whether these cytokines could be potential markers for illness severity in COVID-19 pneumonia.

**Methods:** Ninety-two symptomatic patients aged less than 18 years with confirmed COVID-19 pneumonia and 100 well-matched healthy controls were included in this multi-center study. For all patients, the presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in respiratory fluid specimens was detected by real-time reverse-transcriptase polymerase chain reaction. We measured serum concentrations of studied cytokines by using flow cytometry.

**Abbreviations:** ACE-2, angiotensin converting enzyme-2; ARDS, acute respiratory distress syndrome; AUC, the area under ROC curve; CHD, congenital heart disease; CI, confidence interval; COVID-19, coronavirus disease-2019; CRP, C-reactive protein; IFN- $\gamma$ , interferon; IL, interleukin; IP-10, interferon inducible protein-10; LDH, lactate dehydrogenase; LRTI, lower respiratory tract infection; OR, odds ratio; ROC, receiver Operating characteristic curve analysis; rRT-PCR, real-time reverse-transcriptase polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNF- $\alpha$ , tumor necrosis factor.

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**Results:** Patients with COVID-19 had significantly higher median IL-1 $\beta$ , IL-6, IL-8, IL-10, IL-17, TNF- $\alpha$ , and IP-10 serum levels than did control children (all  $p < 0.01$ ). Patients with severe COVID-19 pneumonia had significantly higher median IL-1 $\beta$ , IL-6, and IP-10 serum levels as compared with those with moderate COVID-19 pneumonia; all  $p < 0.01$ . ROC analysis revealed that three of the studied markers (IL-6, IL-1 $\beta$ , and IP-10) could predict severe COVID-19 pneumonia cases with the largest AUC for IL-6 of 0.893 (95% confidence interval: 0.84–0.98;  $p < 0.01$ ).

**Conclusion:** Our study shows that pediatric patients with COVID-19 pneumonia have markedly elevated serum IL-1 $\beta$ , IL-6, IL-8, IL-10, IL-17, TNF- $\alpha$ , and IP-10 levels at the initial phase of the illness indicating a cytokine storm following SARS-CoV-2 infection. Moreover, serum IL-6, IL-1 $\beta$ , and IP-10 concentrations were independent predictors for severe COVID-19 pneumonia.

#### KEYWORDS

coronavirus disease 2019 (COVID-19), pneumonia, cytokine profile, children, adolescents

## 1 | INTRODUCTION

The World Health Organization declared coronavirus disease-2019 (COVID-19) a global pandemic health emergency on January 30, 2020. Since then, more than 176 million confirmed cases and 3.8 million deaths have been reported worldwide.<sup>1</sup>

A novel enveloped positive-strand RNA coronavirus was identified and named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).<sup>2</sup> Children with COVID-19 may be asymptomatic or with mild clinical course compared with adults.<sup>3</sup>

In the latest Italian study, very low case-fatality rates have been described in children and adolescents.<sup>4</sup> However, one of the most serious complications of COVID-19 is the development of pneumonia followed by acute respiratory distress syndrome (ARDS), sepsis, and multiple organ failure.<sup>5</sup>

Cytokines are polypeptide signaling molecules that regulate many biological processes via cell surface receptors.<sup>6</sup> Key cytokines include pro-inflammatory cytokines e.g., (interleukin [IL]-1 $\beta$ , IL-6,

IL-17, interferon [IFN]- $\gamma$ , and tumor necrosis factor [TNF]- $\alpha$ ); and anti-inflammatory cytokines (e.g., IL-10) and those involved in adaptive immunity such as IL-2 and IL-4.<sup>7</sup> In severe cases of SARS-CoV-2 infection, excessive levels of inflammatory cytokines have been described as cytokine storm contributes to an uncontrolled inflammatory immune response and widespread tissue damage.<sup>8</sup> The aberrant release of pro-inflammatory cytokines leads to pulmonary cell apoptosis which damages the alveolar and microvascular epithelial cell barrier, leading to alveolar edema and hypoxia in SARS patients.<sup>9</sup>

Elevated IL-6 serum levels have been reported in adult patients with COVID-19 and were closely correlated to the severity of symptoms and the upcoming acute respiratory failure (ARF), thus may be of prognostic value in these patients.<sup>10,11</sup> Moreover, stimulated IL-1 $\beta$  in SARS-CoV-2 infection was proposed to mediate lung and systemic inflammation, fever, and fibrosis.<sup>12</sup>

Since cytokine storm is believed to be central to pulmonary immunopathology in SARS-CoV-2 infection, several studies have

focused on cytokine milieu and in particular IL-6 and IL-1 $\beta$  as potential blocking targets to calm the inflammatory storm.<sup>10–14</sup>

Given the sparse data on the cytokine profile in pediatric COVID-19, we investigated serum levels of a panel of key cytokines in children and adolescents with COVID-19 pneumonia with a primary focus on “cytokine storm” cytokines such as IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-10, IL-17, IFN- $\gamma$ , TNF- $\alpha$ , and two chemokines interferon-inducible protein-10 (IP-10) and IL-8. We also studied whether these cytokines could be potential markers for illness severity in COVID-19 pneumonia.

## 2 | METHODS

This was a prospective, multicenter study conducted from March 2020 to April 2021 at three pediatric hospitals in Egypt (AinShams University Hospitals, Benha University Hospitals, and Sohag University Hospital). The study protocol was approved by the Medical Ethics Committee of Ain-Shams, Benha, and Sohag University. Written informed consent was obtained for all subjects who conformed to the Declaration of Helsinki.

### 2.1 | Case definition

Ninety-two symptomatic patients aged less than 18 years diagnosed to have COVID-19 pneumonia were recruited in this study on hospital admission. Only patients with positive real-time reverse-transcription polymerase chain reaction (rRT-PCR) for SARS-CoV-2 and radiological confirmed pneumonia were accepted as cases according to the recently published criteria by Shen et al.<sup>15</sup> Patients' COVID-19 illness severity was categorized based on the latest classification by Chen et al.<sup>16</sup>

- I. Asymptomatic: Cases with a positive RT-PCR test for SARS-CoV-2 without any clinical or radiological findings;
- II. Mild: Cases with upper respiratory symptoms and a positive RT-PCR test for SARS-CoV-2 with normal respiratory system examination;
- III. Moderate pneumonia: cases with pneumonia (respiratory symptoms plus fever >38°C and age-specific tachypnea); not fulfilling the criteria of severe pneumonia;
- IV. Severe pneumonia: cases who develop dyspnea, hypoxia (arterial oxygen saturation < 92% under resting state), dehydration with feeding difficulty, impaired consciousness, coma, convulsions, elevated liver enzymes, coagulation dysfunction and those with features of bilateral or multilobar rapidly progressive infiltrates or pleural effusion on chest computed tomography scan; and
- V. Critical: cases admitted to pediatric intensive care unit [ICU] with ARF requiring mechanical ventilation (partial arterial oxygen pressure/fraction of inspired oxygen [PaO<sub>2</sub>/FiO<sub>2</sub>] ratio < 300 despite oxygen therapy), shock, or organ failure.

Patients were further divided into two groups: Group 1 included 68 patients who had a moderate clinical type and Group 2 in which

18 cases had severe COVID-19 pneumonia and six were critical cases. No asymptomatic or mild cases were seen. Patients were admitted 24–48 h from onset of fever and cough and did not receive any treatment before enrollment in this study. For all patients, Chest X-rays and computed tomography (CT) images at the time of diagnosis were evaluated by an experienced emergency radiologist (SFO) who was blinded to the patients' clinical examination and lab results. Typical CT features for COVID-19 were bilateral and multilobar ground-glass opacities mainly located in the lower lobes, consolidation with reverse halo sign, pleural thickening, pleural effusion, crazy paving pattern, interlobular septal thickening, and vacuolar sign.<sup>17,18</sup>

### 2.2 | Exclusion criteria

Patients with primary or acquired immunodeficiency; malignancy; congenital heart disease (CHD); autoimmune disorders; or any chronic debilitating disease were excluded.

### 2.3 | Control group

The control population comprised one hundred healthy children and adolescents, of matched age and gender; while attending for a routine checkup at outpatient clinics of the study hospitals (all were tested negative for SARS-CoV-2 by RT-PCR and without a previous history or diagnosis of lower respiratory tract infection).

### 2.4 | Laboratory investigations

For all subjects, nasal or pharyngeal swab, sputum sample, BALF, and/or tracheal aspirate were obtained. The presence of SARS-CoV-2 in respiratory fluid specimens was detected by rRT-PCR method using the forward primer 5'-ACTTCTTTTCTTGCTTCGTGGT-3'; reverse primer 5'-GCAGCAGTACGCACACAATC-3'; and the probe 5'CY5-CTAGTTACTAGCCATCCTTACTGC-3'BHQ1 as previously described.<sup>19</sup> Respiratory fluid specimens from patients were cultured and screened for common bacterial or viral respiratory pathogen using the BioFIRE® FilmARRAY® Pneumoniaplus Panel-Multiplex PCR system (bioMérieux).

Five ml of venous blood sample was withdrawn from each participant within 48 h after admission. Serum separated immediately by centrifuge and then, stored at -80°C until processing. Hematologic parameters and inflammatory biomarkers including CBC, D-Dimer, Lactate dehydrogenase (LDH), ferritin, and C-reactive protein (CRP) were analyzed in the clinical laboratory at each study center on admission. Leucopenia and/or lymphopenia were determined according to age groups.<sup>20</sup>

Chest tube inserted in the sixth intercostal space between the mid and posterior axillary line for cases with pleural effusion and/or empyema. An expert cardiothoracic surgeon (AIB) managed complicated cases.

## 2.5 | Cytokine assay

For all subjects, we measured serum concentrations of 10 cytokines (IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-8, IL-10, IL-17, IFN- $\gamma$ , TNF- $\alpha$ , and IP-10) by the Human Cytokine Multiplex<sup>®</sup> Bead Array Kit (Biosource Int.; Luminesx 100/200 reader) using flow cytometry as previously described.<sup>21</sup> The assay sensitivity were as follows: 15 pg/ml for IL-1B; 0.2 pg/ml for IL-2; 3 pg/ml for IL-6, IL-8; 5 pg/ml for IL-4, IL-10, IFN- $\gamma$ ; 10 pg/ml for IL-17; 3.7 pg/ml for TNF-  $\alpha$ , and 2.8 pg/ml for IP-10.

## 2.6 | Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics, version 23 for Windows. Categorical variables were compared by  $\chi^2$  test; meanwhile continuous parameters were compared using Student's *t*-test or analysis of variance test for normally distributed variables and Mann-Whitney U test for nonparametric variables. To evaluate the diagnostic value of studied cytokines as potential predictors for COVID-19 disease severity, Receiver Operating characteristic (ROC) curve analysis was used and the area under ROC curve (AUC) was estimated and interpreted as: AUC < 0.7 (poor accuracy); AUC = 0.7–0.8 (fair); AUC = 0.8–0.9 (good); AUC = 0.9–1.0 (excellent). Boxplots were drawn to describe serum cytokine concentrations. Correlation between paired data was analyzed by the Spearman correlation coefficient. A *p* value of less than 0.05 was considered statistically significant.

## 3 | RESULTS

The median age of patients diagnosed with COVID-19 pneumonia was 10.5 years (range: 8.6–17.8 years) and 58 (63%) of them were males. The healthy control subjects were well-matched for age 9.7 (8–18 years) and gender (65 [65%] were males; (*p* = 0.746, *p* = 0.382, respectively)); Table 1. Seventy-nine (86%) patients had a definitive contact history with a family member with COVID-19. Sixty-eight patients (74%) had moderate clinical type (Group 1) and 18 (19.5) cases had severe COVID-19 pneumonia and six (6.5%) were critical cases (Group 2). Six cases were admitted to pediatric ICU; five (5.4%) patients had ARF required mechanical ventilation; and one patient (1.08%) had septic shock. All patients survived.

Fever, dry cough, and polypnea were the most prevalent symptoms on admission, reported in all patients (100%). About one-fourth of patients (24 [26%]) developed dyspnea and 23% had nausea/vomiting. Other symptoms were recorded infrequently as listed in Table 1.

The blood counts of patients on admission showed decreased lymphocytes (29 [31.5%]), leucopenia (15 [16%]), and thrombocytopenia (18 [20%]); Table 1.

Abnormal laboratory findings in pediatric patients were also increased procalcitonin and D-dimer levels (median procalcitonin level 13 ng/ml [interquartile range 1.2–18.7] and (median D-dimer level

**TABLE 1** The demographic data, clinical features, radiographic, and laboratory characteristics of COVID-19 patients

	Patients (n = 92)	Control group (n = 100)	<i>p</i>
Age (years)	10.5 (8.6–17.8)	9.7 (8–18)	0.746
Gender male, <i>n</i> (%)	58 (63)	65 (65)	0.382
Family member with COVID-19	79 (86)	–	–
Fever >38°C	92 (100)	–	–
COVID-19 severity			
Moderate	68 (74)		
Severe	18 (19.5)		
Critical	6 (6.5)		
Dry cough	92 (100)	–	–
Dyspnea	24 (26)	–	–
Nausea/vomiting	21 (23)		
Sore throat	12 (13)	–	–
Headache	9 (10)	–	–
Diarrhea	15 (17)	–	–
Anosmia	8 (9)	–	–
Loss of taste	8 (9)	–	–
Fatigue	11 (12)	–	–
Hypoxemia	12 (13)	–	–
Cyanosis	7 (7.6)	–	–
ARF	5 (5.4)	–	–
Shock	1 (1.08)		
Duration of fever (in days)	5 (2–8)		
Duration of hospital stay (in days)	11 (9–20)	–	–
Chest CT scan			
Ground-glass opacities	92 (100)		
Pulmonary consolidation	92 (100)		
Laboratory findings			
CRP, (<8 mg/dl)	7.4 (0.98–25)		
Increased, <i>n</i> (%)	35 (38)		
Procalcitonin, (<0.5 ng/ml)	13 (1.2–18.7)		
Increased, <i>n</i> (%)	45 (49)		
D-Dimer, (<0.5 $\mu$ g/ml)	0.6 (0.35–3.4)		
Increased, <i>n</i> (%)	52 (56)		
Lactate dehydrogenase (LDH) U/L	239 (198–307)		
Serum ferritin, ref. (15–140) ng/ml	40 (23–385)		

(Continues)

TABLE 1 (Continued)

	Patients (n = 92)	Control group (n = 100)	p
White blood cell, ref. (4–10) × 10 <sup>9</sup> /L	7.5 (5.8–11.6)		
Leucopenia, n (%)	15 (16)		
Neutrophils, ref. (2.0–7.2) × 10 <sup>9</sup> /L	4.2 (2.34–7.68)		
Neutrophilia, n (%)	19 (21)		
Lymphocytes, ref. (1.1–3.2) × 10 <sup>9</sup> /L	2.23 (1.15–3.4)		
Lymphopenia, n (%)	29 (31.5)		
Platelets, ref. (140–440) × 10 <sup>9</sup> /L	167 (143–320)		
Thrombocytopenia, n (%)	18 (20)		
ALT (<50 U/L)	20 (12–78)		
Increased, n (%)	15 (16)		
AST (<60 U/L)	27 (16–95)		
Increased, n (%)	21 (23)		
Creatinine, <62 μmol/L	46 (29–78)		
Increased, n (%)	19 (21)		

Note: Values in parentheses are percentages or data are presented as mean ± SD or median (range); p value < 0.05 indicates a significant difference.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ARF, acute respiratory failure; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; CT, computed tomography; SD, standard deviation.

0.6 μg/ml [0.35–3.4]; Table 1. The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were increased in (16% and 23%) of patients with a median ALT and AST level of 20 U/L and 27 U/L, respectively; Table 1.

The demographic data, clinical features, radiographic, and laboratory characteristics of COVID-19 patients at admission are presented in Table 1.

Of note, severe cases had significantly higher mean D-dimer level (0.87 ± 1.3 μg/ml) as compared to moderate COVID-19 cases (0.24 ± 0.35 μg/ml,  $p = 0.003$ ; Table S1).

We first compared the levels of IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-17, TNF-α, IFN-γ, and IP-10 in serum samples from COVID-19 patients and healthy control group. Patients with COVID-19 had significantly higher median IL-1β, IL-6, IL-8, IL-10, IL-17, TNF-α, and IP-10 levels than did control children (all  $p < 0.01$ ); Table 2. Also, there was no significant difference in IL-2, IL-4, and IFN-γ levels between COVID-19 patients and the control group (all  $p > 0.05$ ); Table 2.

TABLE 2 Comparison of serum cytokines concentrations (pg/ml) in pediatric patients with COVID-19 and control group

	Patients COVID-19 (n = 92)	Control group (n = 100)	p
IL-1B	18 (9–57)	2.3 (0.25–4.15)	0.038
IL-6	32 (13–146)	8 (2–14.7)	0.004
IL-8	26 (21–95)	12 (3.6–24)	0.045
IL-17	21.5 (7.6–28.4)	8.7 (5.6–14)	0.013
TNF-α	5.7 (3.5–18)	1.8 (0.4–2.6)	0.036
IL-2	16 (10.8–20)	14 (10–19)	0.731
IL-4	7.5 (4.7–21)	6.4 (0.9–20)	0.426
IL-10	11.8 (5–24)	3.4 (2–3.8)	0.041
IFN-γ	4.6 (1.6–14)	3.9 (1.3–12)	0.342
IP-10	1175 (234–3125)	157(84–467)	0.026

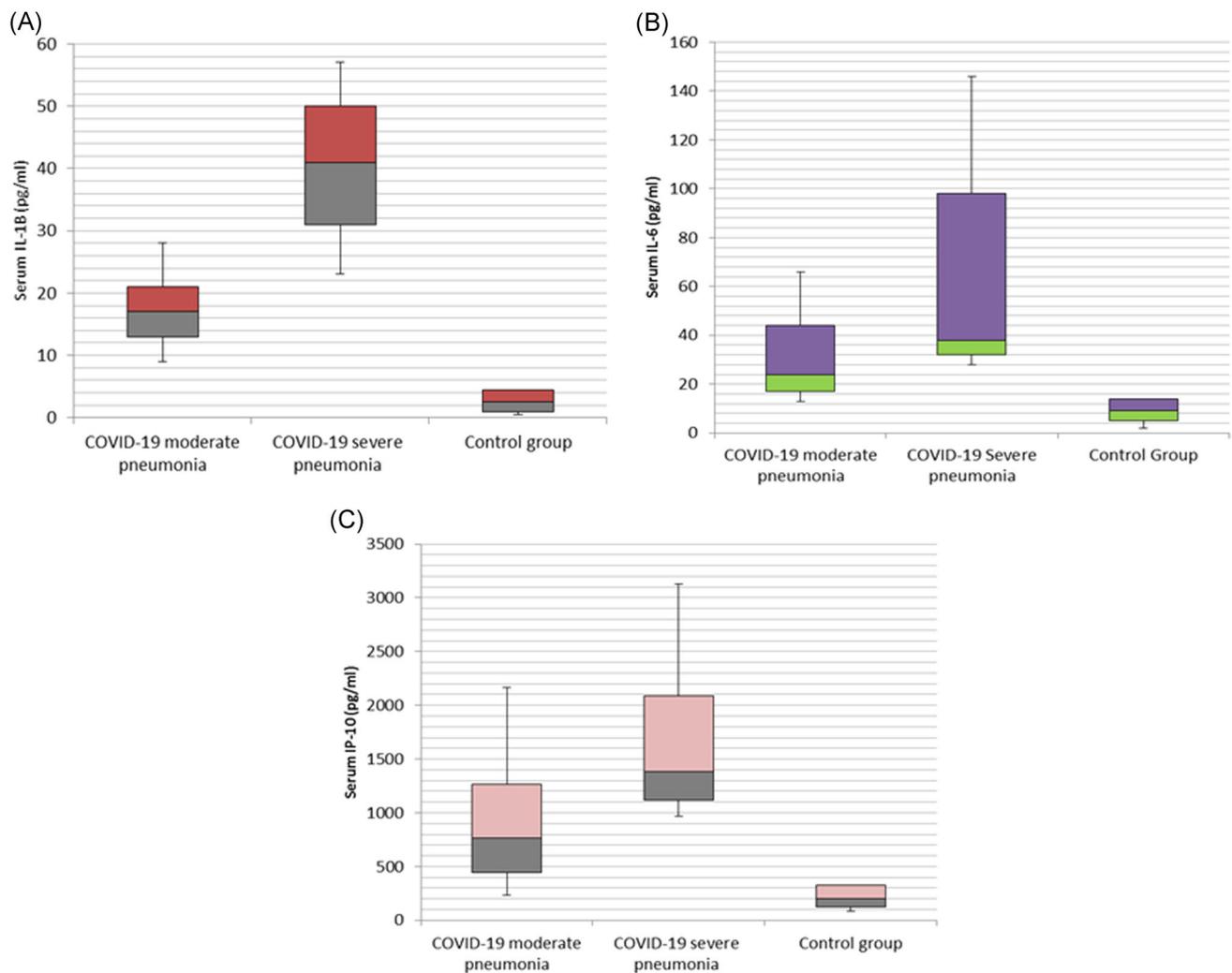
Note: Values in parentheses are data presented as median (range); p value < 0.05 indicates a significant difference.

Abbreviations: COVID-19, coronavirus disease-2019; IL, interleukin; IFN-γ, interferon-γ; IP-10, interferon-inducible protein-10; TNF-α, tumor necrosis factor-α.

We next analyzed cytokine profile of patients subgroups according to COVID-19 severity. Patients with severe COVID-19 pneumonia (Group 2) had significantly higher median IL-1β, IL-6, and IP-10 levels as compared with those with moderate COVID-19 pneumonia (Group 1) and control group; all  $p < 0.01$  (Figure 1A–C; respectively). Serum IL-1B, IL-6, and IP-10 levels were constantly increased across the severity of pneumonia (for serum IL-1β; median [range], moderate pneumonia 17 [9–28] pg/ml vs. severe pneumonia 41 [23–57] pg/ml; Figure 1A). For serum IL-6; moderate pneumonia 24 (13–65) pg/ml versus severe pneumonia 37 (28–146) pg/ml; Figure 1B. For serum IP-10; moderate pneumonia 765 (234–2170) pg/ml versus severe pneumonia 1385 (970–3125) pg/ml; Figure 1C; all  $p < 0.01$ . However, no significant difference was evident between severe and moderate groups as regards levels of IL-2, IL-4, IL-8, IL-10, IL-17, IFN-γ, and TNF-α; all  $p > 0.05$  (data not shown).

Of note, IL-6 and IP-10 serum concentrations were negatively correlated with lymphocyte count ( $r = -0.358$ ,  $p = 0.02$ ; and  $r = -0.374$ ,  $p = 0.004$ ; respectively and IP-10 concentration was also found to be negatively correlated with oxygen saturation ( $r = -0.415$ ,  $p = 0.01$ ). Other significant negative correlation was found between IL-1β and total WBC count ( $r = -0.317$ ,  $p = 0.02$ ).

ROC analysis revealed that three of the studied markers (IL-6, IL-1β, and IP-10) could differentiate severe and critical COVID-19 pneumonia with the largest AUC for IL-6 of 0.893 (95% confidence interval [CI]: 0.84–0.98;  $p < 0.01$ ) and optimum cut-off expression value was 31.7 pg/ml with a sensitivity of 85% and specificity of 78%. The AUC of IL-1β was 0.845 (95% CI: 0.82–0.94;  $p < 0.01$ ) and at a cutoff value of 43.6 pg/ml, IL-1β provided sensitivity and specificity of 83.4% and 76%, respectively; Figure 2A.



**FIGURE 1** (A) Serum IL-1 $\beta$  level among COVID-19 patients with moderate and severe pneumonia and control group. (B) Serum IL-6 level among COVID-19 patients with moderate and severe pneumonia and control group. (C) Serum IP-10 level among COVID-19 patients with moderate and severe pneumonia and control group. COVID-19, coronavirus disease-2019; IL, interleukin; IP-10, interferon-inducible protein-10 [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

IP-10 can also predict severe to critical COVID-19 pneumonia with AUC of 0.824 (95% CI: 0.73–0.88;  $p < 0.01$ ) and best cutoff value was 1376 pg/ml with a sensitivity of 81.6% and specificity of 75.6%; Figure 2A. Combinations of the three cytokines; IL-6, IL-1 $\beta$ , and IP-10 showed the highest AUC of 0.934, followed by the combination of IL-6 and IL-1 $\beta$  (AUC; 0.918) as well as the combination of IL-6 and IP-10 (AUC; 0.902); Figure 2B.

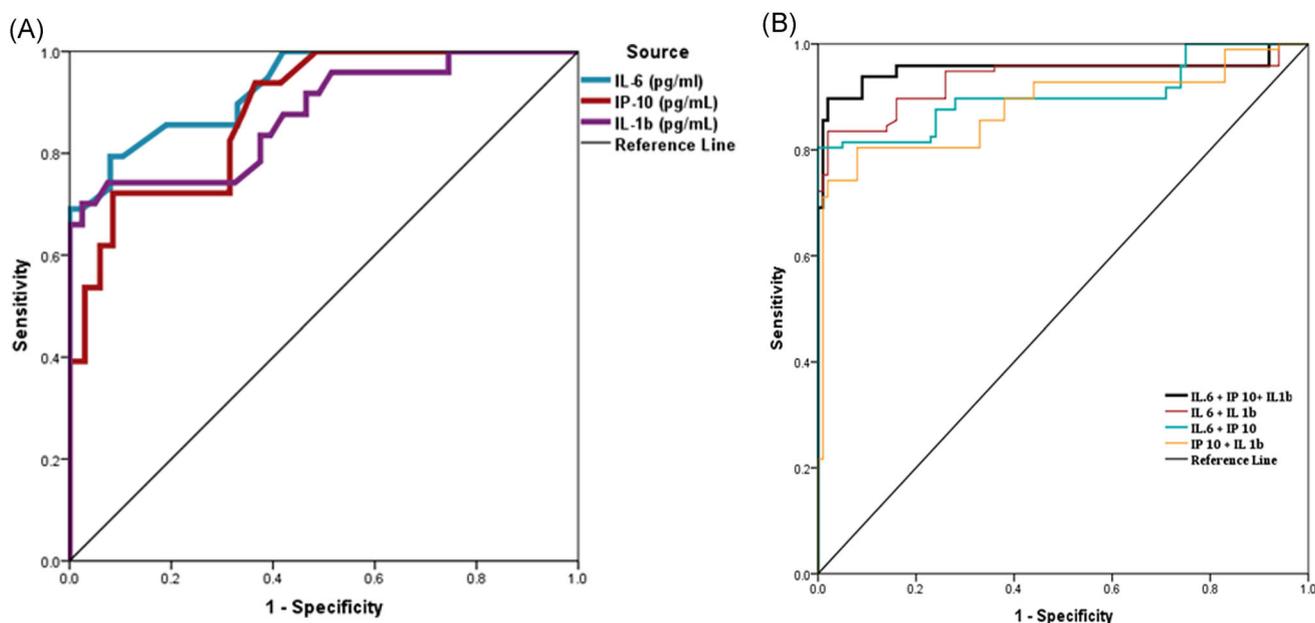
## 4 | DISCUSSION

A subset of pediatric patients progress into severe COVID-19 clinical presentation that reflects an aggressive inflammatory cascade induced by an aberrant immune response and may be associated with immuno-thrombosis.<sup>22,23</sup> Since cytokines not only provide the regulatory signals that amplify, and resolve the immune response but also have complex crosstalk with the coagulation system, recent

studies have focused on cytokine milieu and in particular cytokine storm as a major driver of illness severity in COVID-19.<sup>10–14,23–25</sup>

To date, the cytokine profile in children and adolescents infected with SARS-CoV-2 has not been reported. Of the 10 cytokines assessed in this study, significantly higher serum IL-1 $\beta$ , IL-6, IL-8, IL-10, IL-17, TNF- $\alpha$ , and IP-10 levels were found in patients with COVID-19 pneumonia as compared to the healthy control group suggesting that a cytokine storm may occur following SARS-CoV-2 infection as previously reported in adult studies.<sup>2,18,24,25</sup>

In keeping with a recent meta-analysis,<sup>26</sup> the most prevalent symptoms in our cohort were fever, dry cough, and polypnea, followed by shortness of breath and nausea/vomiting. Amongst the most common abnormal laboratory findings were lymphopenia, increased level of procalcitonin and CRP as well as increased liver enzymes. In addition, severe cases demonstrated significantly elevated D-dimer levels as compared with moderate COVID-19 cases, although this finding should be further investigated as an indicator of disease severity in future pediatric studies.



**FIGURE 2** (A) Performance of ROC curves for predicting severe COVID-19 pneumonia. (B) ROC curves showing performance of a different combinations of serum cytokines for diagnosis of severe COVID-19 pneumonia. COVID-19, coronavirus disease-2019; ROC, receiver operating characteristic [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

When we next compared the cytokine profile of studied patients subgrouped according to disease severity, we found that among studied cytokines only IL-1 $\beta$ , IL-6, and IP-10 showed significant differences between moderate and severe groups denoting increased expression of the three markers along with COVID-19 severity. Moreover, we found significant negative correlations between serum IL-6, IL-1 $\beta$ , and IP-10 levels and other laboratory/clinical parameters (lymphocyte count, total WBC count, and oxygen saturation); respectively in COVID-19 pneumonia patients.

These results are concordant with numerous studies which demonstrated that higher levels of early response pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ); results in what has been described as “cytokine storm,” were consistently associated with more severe COVID-19 disease and worse outcome.<sup>2,9,25–28</sup>

Ng et al.<sup>27</sup> investigated the cytokine profile of eight Chinese children with SARS-CoV-2 infection. The authors reported a predominant upregulation of circulating IL-1 $\beta$  levels at the initial phase of the illness, while other key pro-inflammatory cytokines such as TNF- $\alpha$  and IL-6 showed only mildly elevated levels. They added that the absence of overtly elevated TNF- $\alpha$  and IL-6 levels, could predict a favorable outcome after SARS-CoV-2 infection.<sup>27</sup> Sun et al.<sup>28</sup> reported an increased expression of IL-6, IL-10, and IFN- $\gamma$  and decreased blood CD16+ and CD56+ lymphocytes in pediatric patients with severe COVID-19. By contrast, Soraya and Ulhaq<sup>29</sup> evaluated data from seven pediatric studies and found that IL-6 levels of pediatric COVID-19 cases tend to be within the normal range. However, it was important to note that their COVID-19 series had relatively mild symptoms, and none required ICU care or mechanical ventilation. Qian et al.<sup>30</sup> reported that serum levels of pro-inflammatory cytokines including TNF- $\alpha$ , IL-2, IL-4, and IL-6 were not

different between moderate and severe subgroups of children with COVID-19 pneumonia. The authors added that CD4 + T cells, CD8 + T cells, and natural killer cells were not reduced, even in severe COVID-19 cases. They concluded that SARS-CoV-2 may not trigger a “cytokine storm” in children which partly explain the better outcome even in the presence of pneumonia.<sup>30</sup>

Earlier studies detected that binding of the SARS-CoV spike (S) protein to ACE2 receptors results in production of pro-inflammatory cytokines; in particular IL-1 $\beta$  and IL-6.<sup>31</sup> IL-1 $\beta$ , the master pro-inflammatory cytokine, stimulates acute-phase signaling and secondary cytokine production. It also increases the trafficking of immune cells to the site of primary infection and epithelial cell activation.<sup>32</sup> In animal models, IL-1 $\beta$  was secreted from macrophages following infection with a neurotropic strain of coronavirus.<sup>33</sup>

Another pleiotropic cytokine is IL-6. The main activators of IL-6 expression are IL-1 $\beta$  and TNF- $\alpha$ . It promotes activation of T helper 17 (TH17) cells, modulate antigen-dependent B-cell differentiation, and regulate the acute phase response, as well as insulin resistance, neuropsychological behavior, and neuroendocrine system.<sup>34</sup> Interestingly, serum SARS-CoV-2 nucleic acid (RNAemia) is closely correlated with extremely high IL-6 serum levels in critically ill patients.<sup>11</sup>

Furthermore, our ROC analysis confirmed that IL-6, IL-1 $\beta$ , and IP-10 were independent predictors for severe COVID-19 pneumonia with the largest AUC for IL-6 of 0.893 at a cutoff value of 31.7 pg/ml with high sensitivity and specificity.

The AUC of IL-1 $\beta$  and IP-10 that was used to predict the severity of COVID-19 were 0.845 and 0.824, respectively. The optimum cut point of IL-1 $\beta$  and IP-10 were 43.6 pg/ml and 1376 pg/ml, respectively. In addition, combinations of the three cytokines; IL-6, IL-1 $\beta$ , and IP-10 showed the highest AUC of 0.934, followed by the

combination of IL-6 and IL-1B (AUC; 0.918) as well as the combination of IL-6 and IP-10 (AUC; 0.902).

In accordance with our findings, Grifoni et al.<sup>35</sup> reported that serum IL-6 level over 25 pg/ml was an independent risk factor for severe COVID-19 and hospital mortality in an Italian cohort of 77 adult patients. The AUC for IL-6 as a predictor of severe COVID-19 was 0.75, while it was 0.80 for IL-6 as a predictor of the combined endpoint. The authors concluded that IL-6 levels at hospital admission seem to be the best prognosticator for negative outcomes in COVID-19.<sup>35</sup> Similar study by Gao et al.<sup>36</sup> identified an optimal threshold of 24.3 pg/ml of IL-6 combined with D-Dimer for early prediction of severe COVID-19 cases in a Chinese cohort of 43 cases. A recent meta-analysis involved nine studies confirmed that mean serum level of IL-6 was more than three-fold higher in complicated COVID-19 cases and was also associated with in-hospital mortality risk.<sup>37</sup> In another German cohort, IL-6 levels of more than 80 pg/ml were associated with a 22 times more need for mechanical ventilation.<sup>38</sup>

Our findings also resonate with Yang et al.<sup>39</sup> who reported elevated serum IP-10 levels in moderate and severe cases of COVID-19 that was inversely correlated with the PaO<sub>2</sub>/FiO<sub>2</sub> ratio. The authors concluded that IP-10 expression was strongly associated with disease severity and fatal outcome, alongside the expression of IL-1Ra and MCP-3 as the combination of IP-10, MCP-3, and IL-1Ra showed the biggest AUC of their ROC calculations.<sup>39</sup> Similar studies associated high levels of IP-10 with a greater viral load and more lung damage in adult patients with COVID-19.<sup>2,40</sup> IP-10 (CXCL10) is a major chemokine secreted mainly by neutrophils, endothelial cells, dendritic cells, astrocytes, fibroblasts, and hepatocytes. Upon binding to chemokine receptor 3 (CXCR3), it activates and recruits leukocytes including T cells, NK cells, and monocytes to inflamed tissues. Therefore, IP-10-CXCR3 signaling pathway could perpetuate inflammation and tissue damage<sup>41</sup> and enhances the development of neutrophil-mediated fulminant lung injury of viral origin, supporting a critical role of this biomolecule in ARDS pathogenesis.<sup>42</sup>

Together with our findings, it is plausible to speculate that the three cytokines; IL-6, IL-1 $\beta$ , and IP-10 serum levels could be potential biomarkers for early detection of disease progression and severity in pediatric patients with COVID-19 pneumonia.

In SARS-CoV-2 experimental models, inhibition of nuclear factor kappa-B; a key transcription factor of IL-6, increased animal survival after a marked reduction in IL-6 levels.<sup>43</sup> IL-6 receptor monoclonal antibody (tocilizumab) has been recently used in a Chinese clinical trial (No. ChinCTR2000029765)<sup>44</sup> and it has been incorporated into Chinese guidelines for COVID-19 management. Therefore, identification of key mediators driving the cytokine storm such as IL-1 $\beta$ , IL-6, and IP-10 will guide the design of future clinical trials for cytokine blockade strategies that may prevent lung immunopathology, potentially enabling a marked reduction in COVID-19 mortality.

Some limitations may be considered in this study. First, the small sample size as well as the absence of mild cases among our COVID-19 pediatric cohort. Second, we have investigated only 10 cytokines associated with the cytokine storm; an exploratory analysis of

additional cytokines and chemokines should be addressed in future studies. Third, serum cytokines were assessed only at admission although serial measurements could better clarify kinetics profile and its role in disease severity and outcome.

## 5 | CONCLUSION

Our study shows that pediatric patients with COVID-19 pneumonia have markedly elevated serum IL-1 $\beta$ , IL-6, IL-8, IL-10, IL-17, TNF- $\alpha$ , and IP-10 levels at the initial phase of the illness indicating a cytokine storm following SARS-CoV-2 infection. Moreover, serum IL-6, IL-1 $\beta$ , and IP-10 concentrations were independent predictors for severe and critical COVID-19 pneumonia.

Finally, knowing the cytokine profile could help clinicians identifying potential targets in the cytokine milieu and inform the selection of candidate immunotherapy for robust evaluation against defined COVID-19 outcomes in future clinical trials setting.

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## CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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## DATA AVAILABILITY STATEMENT

Author elects to not share data due to privacy and ethical.

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#### SUPPORTING INFORMATION

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