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Lymphocyte Subsets in Mild COVID-19 Pediatric Patients

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What is already known about this topic?

- *Children experience mild COVID-19 disease. However, the reason for this phenomenon is not exactly apparent. Studies on non-immune and immune mechanisms are ongoing. It is now known that there is a negative correlation between disease severity and lymphocyte count, lymphocyte subsets, and particularly CD8+ T cell count in adult patients.*

What this study adds on this topic?

- *Lymphocyte count in pediatric COVID-19 patients with asymptomatic or mild disease is similar to that of healthy children. However, natural killer cells, T cell, and CD4+ T cell counts are increased.*

ABSTRACT

Objective: The reasons for a high prevalence of asymptomatic or mild coronavirus disease (COVID-19) and rare severe disease in children have been explained by non-immune and immune mechanisms. This study aimed to evaluate the immune system's response to severe acute respiratory syndrome coronavirus 2 by investigating lymphocyte subsets.

Materials and Methods: This study included 33 coronavirus disease positive children, of whom 12 had mild disease and 21 had an asymptomatic infection as the patient group and 26 age- and gender-matched healthy children as the control group. The demographic information, symptoms, physical examination findings, complete blood count, C-reactive protein (CRP), procalcitonin, and lymphocyte subsets were recorded in all subjects.

Results: Leukocyte, lymphocyte, monocyte count, and hemoglobin levels of our pediatric coronavirus disease patients were similar to the control group. Neutrophil was lower in the coronavirus disease cases compared to the control group. CRP and procalcitonin levels of asymptomatic cases were similar to the control group. B cell count, CD8+ T cell count, and CD4/CD8 ratio (dividing the CD4 cell count by the CD8 cell count) ratio were similar in the patient and control groups. Natural killer, T cell, and CD4+ T cell counts were significantly higher in the whole patient group compared to the control group.

Conclusion: One reason for mild severe acute respiratory syndrome coronavirus 2 infection in children may be an increase in some lymphocyte subsets such as natural killer cells, T cell, and CD4+ T cell. Understanding the answer to the question of why children develop more protective immunity to the virus could be an essential step for developing new treatments.

Keywords: SARS-CoV-2, COVID-19, lymphocyte subsets, children.

INTRODUCTION

In the final days of 2019, some pneumonia cases of unknown etiology were identified in Wuhan City, China. The causative agent was severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of the coronavirus family. The disease was then named by World Health Organization (WHO) the coronavirus disease 2019 (COVID-19).[1] Upon a rapid rise of COVID-19 cases worldwide, WHO declared a pandemic on March 11, 2020.[2] The virus' fast transmission, its high virulence, and severe course, especially in adults, have caused millions of people to be hospitalized and die worldwide.

Coronaviruses are enveloped, positive-sense, single-stranded RNA viruses. They are divided into 4 genera by their α, β, γ, and δ genomic structures. They can infect a wide variety of host species. α and β genera only infect mammals. Some human coronaviruses are responsible

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for upper respiratory tract infections. SARS-CoV-2 is a novel human coronavirus that belongs to the β coronavirus family.[3]

It is usually transmitted with respiratory droplets and via close contact with infected persons. Crowded places are the ideal environment for the spread of the disease. Given the number of children in the general population and the risk of exposure, children are an important source of the virus.[4] The virus enters the human body through respiratory mucosa and conjunctiva. The upper respiratory tract mucosa is the first viral replication site. The virus enters the cell using the angiotensin-converting enzyme 2 (ACE2) receptor, a functional receptor that is mainly expressed in the upper airways, lungs, heart, kidneys, intestines, and vascular endothelial cells.[5]

When viruses bind to host receptors (attachment), they enter host cells via endocytosis or membrane fusion (penetration). When the viral contents are released inside the host cells, the viral RNA enters the nucleus for replication. Viral mRNA is used to make viral proteins (biosynthesis). New viral particles are made (matured) and then released. Epithelial cells, alveolar macrophages, and dendritic cells are the 3 main components of innate immunity in the respiratory tract. Dendritic cells' and macrophages' duty as innate immune cells are to fight viruses until adaptive immunity is developed. T-cell responses are initiated by antigen presentation by dendritic cells and macrophages. These antigen-presenting cells move to the draining lymph nodes to present viral antigens to T cells. T helper and T cytotoxic cells play a pivotal role. T helper cells activate B cells to promote the production of virus-specific antibodies, while T cytotoxic cells kill virally infected cells.[3]

Although the SARS-CoV-2 virus mainly affects the respiratory system, it can also affect other organ systems. It may cause various clinical manifestations in adults, including an asymptomatic disease, mild upper respiratory tract infection, mild-to-severe pulmonary infection, and severe systemic inflammation characterized by acute respiratory distress syndrome and coagulation abnormalities. It is believed that viral load is taken, viral cell entry, the protective immune response, and the effects of an abnormally severe immune response, including the cytokine storm, play a role in the COVID-19's presentation.[5-6] Asymptomatic or mild disease in children causes the disease to become undiagnosed. Symptoms generally include fever, cough, sore throat, runny nose, myalgia, malaise, vomiting, diarrhea, and abdominal pain. The percentage of children requiring intensive care due to pneumonia is low. Severe disease has been reported in 1-5% of affected children. Death is extremely rare. The risk of severe disease is higher, especially in infants and patients with comorbidities. Multisystem inflammatory syndrome, a condition resembling the Kawasaki syndrome, which develops after acute infection and may have a severe presentation, has been defined in an increasing number of children.[5-7]

It is not entirely clear why children develop mild COVID-19 disease. It is believed that since children are more frequently exposed to seasonal coronaviruses and experience a greater number of viral infections and since some childhood vaccines keep their immune systems more active, they develop a more controlled and appropriate immune response against the virus. In addition, the expression of ACE2 receptors in the nasal epithelium and lower respiratory tract in children is different from that in adults.[2,4,8]

It is known that lymphopenia, and especially a decrease in T lymphocyte count in adults, is negatively associated with prognosis during COVID-19 disease.[1] Lymphocyte and its subsets may have an impact on the development of the protective immune system in children during COVID 19 disease. Herein, we discuss the numerical change of lymphocyte subsets and their effect on mild COVID-19 infection in children.

MATERIALS AND METHODS

Ethical Approval and Consent to Participate

This study was approved by Kayseri City Hospital clinical research ethics committee (April/2020; no.64). The study was conducted in accordance with the declaration of Helsinki. Informed consent was obtained from the parents of all patients and also from the adolescent patients themselves.

Subjects

This study enrolled a total of 33 pediatric patients who were hospitalized with COVID-19 at Kayseri City Hospital Pediatric Clinic between April 2020 and June 2020.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: detection of SARS-CoV-2 nucleic acids using real-time fluorescent polymerase chain reaction (PCR) in the throat and nasal swab sample in a pediatric patient with suspected COVID 19.

The exclusion criteria were as follows: having a neurological, allergic, immunological, cardiac, or other chronic disease and using medication due to chronic disease.

Clinical Classification of Disease Severity

The patients were either symptomatic or asymptomatic, and each patient had a history of contact with a COVID-19-positive person. Cases with positive COVID-19 PCR tests in the throat and nasal swab samples were accepted as COVID 19.

In Turkey, at the beginning of the pandemic, pediatric cases with the asymptomatic or mild disease had been hospitalized for contact isolation. Therefore, all of our patients were hospitalized. Symptomatic patients were administered azithromycin (10 mg/kg/day, first day; then, 5 mg/kg/day for 4 days) per oral route. The control group was composed of age- and gender-similar healthy children.

COVID 19 patients were classified as a follow-up. Asymptomatic: cases with no clinical symptoms and signs and normal chest imaging. Mild: patients with signs of acute upper respiratory tract infection (fever, fatigue, myalgia, cough, sore throat, runny nose, and sneezing) or digestive system symptoms (nausea, vomiting, abdominal pain, and diarrhea). Moderate: pneumonia without significant hypoxemia (frequent fever and cough) and chest computed tomography with lesions.[3]

General Laboratory Tests

The demographic information, symptoms, physical examination findings, C-reactive protein (CRP), procalcitonin, complete blood count, and lymphocyte subsets were recorded in all subjects.

Full blood count was performed with an automatic hematological analyzer.

Flow Cytometry Assay

Peripheral blood lymphocyte subsets were determined using flow cytometry. EDTA (Ethylene diamine tetra acetic acid)-anticoagulated peripheral blood (2 mL) was collected from patients with COVID-19 before initial treatment. All samples were tested within 6 hours of being obtained. CD3+/CD4+/CD8+ T-cell, CD19+ B-cell, and CD16+CD56+ natural killer (NK)-cell counts (cells/μL) were measured by multiple-color flow cytometry with a human monoclonal anti-CD3-fluorescein isothiocyanate, anti-CD4-phycoerythrin cyanine7, anti- CD8-allophycocyanin H7, anti-CD19-APC, and anti-CD16-56PE antibodies according to the manufacturer's instructions. The cells were analyzed and calculated using the clinical program of the FACS Lyric flow cytometer device.

Statistical Analysis

Study data were analyzed with The Statistical Package for Social Sciences version 25.0 software (IBM Corp.; Armonk, NY, USA). Descriptive statistics were presented as number (n), percentage (%), mean ± standard deviation ((x)' ± SD), median (M), and interquartile range. Normality of the distribution of numerical variables was tested with the Shapiro–Wilk normality test and Q–Q graphics. Mann–Whitney *U* test was used to compare non-normally distributed variables between the 2 groups. One-way analysis of variance (ANOVA) was used to compare

Table 1. The Demographic Characteristics, Complete Blood Count, CRP, Procalcitonin, and Lymphocyte Subsets of All, Symptomatic, and Asymptomatic Patients as well as the Control Group Were Shown

Characteristics	All COVID-19 Cases (n = 33)	Symptomatic COVID-19 Patients (Mild Disease) (n = 12)	Asymptomatic COVID-19 Cases (n = 21)	Healthy Control (n = 26)
	114.7 ± 61.3	117.3 ± 59.9	113.3 ± 63.5	133.2 ± 67.4
¥Gender (male/female), N (%)	(16/17) (48.5/51.5)	(4/8) (33.3/66.7)	(12/9) (57.1/42.9)	(15/11) (57.7/42.3)
Blood count, $\times 10^9$/L				
‡Leucocytes Median (25-75p)	6.220 (5.195-7.390)	5.685 (4.682-6.882)	6.580 (5.895-8.005)	7.095 (5.772-8.282)
†Hemoglobin (g/dL)	13.2 ± 1.4	13.1 ± 0.7	13.2 ± 1.7	13.3 ± 1.4
†Hemotocrit (%)	38.5 ± 4.0	38.5 ± 2.6	38.6 ± 4.7	39.3 ± 3.4
†Mean corpuscular volume (fL)	77.9 ±4.3	77.1 ± 3.8	78.4 ± 4.7	80.1 ± 3.8
†Red blood cell distribution width (%)	12.7 ± 0.9	12.9 ± 0.6	12.6 ± 1.0	12.7 ± 0.9
‡Platelet	274 (228-310)	240 (200.5-293.5)* $P = .015$	279 (266.5-316)	298.5 (262.5-354)
ᵍC-reactive protein (mg/L)	1.40 (0.60-3.75)	2.50 (1.12-5.92)	1.40 (0.50-3.25)	
ᵍProcalcitonin (ng/dL)	0.05 ± 0.03	0.06 ± 0.04	0.04 ± 0.01	
Flow cytometry, $\times 10^6$/L				
ᵍNeutrophil	2975 (2232-3558)* $P = .001$	2438 (1958-3501)* $P = .004$	3069 (2481-3621)* $P = .036$	3971 (3396-4793)
‡Monocyte	506 (374-715)	567 (311-779)	485 (402-693)	574 (446-663)
‡Lymphocyte	2301 (1915-3177)	1996 (1630-2794)	2380 (2047-3551)	2091 (1787-2521)
‡NK cell	252 (147-327)* $P = .035$	202 (119-289)	282 (172-332)* $P = .028$	199 (100-266)
‡B cell	307 (206-580)	242 (184-441)	414 (214-664)	363 (282-521)
‡ T cell	1818 (1397-2098)* $P = .029$	1620 (1256-2076)	1838 (1433-2182)	1489 (1108-1778)
‡CD4 cell	923 (826-1288)* $P = .027$	960 (827-1255)	923 (824-1337)	841 (643-992)
‡CD8 cell	711 (483-951)	483 (428-839)	797 (559-984)	580 (405-758)
†CD4/CD8 ratio	1.70 ± 0.78	1.76 ± 0.61	1.66 ± 0.87	1.61 ± 0.82

*Significant difference compared to control group, $P < .05$.
Data are expressed as mean ± standard deviation, median (1st quarter/3rd quarter) and n (%).
†One-way analysis of variance, ¥Pearson chi-square test with exact method, ‡Kruskal–Wallis analysis, ᵍMann–Whitney *U* test
COVID-19, coronavirus disease; NK, natural killer cell; CRP, C-reactive protein.

normally distributed variables between 3 groups. Tukey honestly significant difference was used as the multiple comparison test for ANOVA. Kruskal–Wallis test was used to compare non-normally distributed variables between 3 groups. If the Kruskal–Wallis test indicated significant inter-group differences, the Dunn–Bonferroni test was used as the multiple comparison test. Categoric variables were compared by Pearson's chi-square test. If the latter indicated a significant difference, inter-group differences were sought with the two proportion Z test with Bonferroni correction. A *P*-value of less than .05 was considered statistically significant.

RESULTS

Clinical Characteristics of Patients with COVID-19

In total, 33 children with COVID-19 (12 symptomatic cases and 21 asymptomatic cases) and 26 healthy children enrolled as the control group had similar age and gender distribution. The symptomatic patients (n = 12) had symptoms of an acute upper respiratory tract infection, including fever, cough, myalgia, malaise, vomiting, and diarrhea.

Physical examination of the symptomatic patients revealed findings consistent with an upper respiratory tract infection. No patient had pneumonia. All symptomatic patients had mild disease. Twenty-one asymptomatic patients had a normal physical examination. All patients were discharged after 5 days with full recovery.

Complete Blood Count, Acute Phase Reactants, and Lymphocyte Subsets

The demographic characteristics, complete blood count, CRP, procalcitonin, and lymphocyte subsets of all symptomatic and asymptomatic patients and the control group were shown in Table 1. There was no significant difference between the groups with respect to leukocyte count and hemoglobin value. Platelet count was significantly lower in the symptomatic group compared to the control group. CRP and procalcitonin were not elevated in the patient groups. Neutrophil count was considerably lower in each patient group compared to the control group. Monocyte, lymphocyte, B cell, CD8+ T cell counts, and CD4/CD8 ratio were similar in the patient and control groups. NK cell, T cell, and CD4+ T cell counts were significantly higher in all patients compared to the control group.

DISCUSSION

Our study revealed that the whole pediatric patient group that was composed of asymptomatic and mild cases had a lower neutrophil count; higher NK cell, T cell, and CD4+ T cell counts; and similar leukocyte, lymphocyte, monocyte, CD8+ T cell counts, and CD4/CD8 ratio compared to the controls.

Lymphocytes have an essential role in defense against viruses. CD4+ T lymphocytes produce potent cytokines to further activate the immune system and help B lymphocytes produce antibodies. CD8+ T lymphocytes destroy virus-infected cells to reduce viral load and limit the viral spread.[8]

In adults, SARS-CoV-2 infection causes lymphopenia, depending on disease severity. Lymphopenia appears to be related to apoptosis and cell death during cytokine release. Studies on adults have reported that a more significant drop occurs in CD8+ T lymphocyte count. In contrast to adults with severe disease, children with mild disease have similar or increased T lymphocyte counts.[8,9]

Studies on COVID-19 and lymphocyte subsets in adults have indicated that they show a negative correlation, especially with disease severity and outcome. Deng Z et al[1] found significantly lower CD3+, CD4+, and CD8+ T cell counts in patients with severe COVID-19 disease compared to patients without severe COVID-19 disease. The authors suggested that these findings were related to disease severity, progression, and prognosis. Chen J et al[6] found a negative correlation between disease severity and CD3+, CD4 +, and CD8+ T lymphocyte counts. They interpreted that these findings may indicate that symptomatic patients experience some immunological disorders. Kazancıoğlu S et al[10] reported an increased granulocyte count and reduced lymphocyte, CD3+ T cell, CD4+ T cell, NK cell, and monocyte counts in patients with severe COVID-19 disease. Gan J et al[11] reported that the number of lymphocyte subsets was correlated to a favorable outcome in patients with COVID-19 pneumonia. Qin et al[12] reported increased levels of inflammatory cytokines, a higher leukocyte count, and lower lymphocyte and T cell counts in patients with severe infection. Jiang et al[13] reported that patients with COVID-19 had severely depleted CD3+ T, CD4+ T, CD8+ T cell counts, with the depletion in CD8+ T cells being more severe. In a systematic review, Li et al[14] reported that COVID-19 progression and mortality showed a significant negative correlation with lymphocyte count but not CD3+, CD4+, CD8+ T cell, B cell, and NK cell counts. Sun et al[15] found that CD8+ T cell count was lower in severe and critical diseases. They interpreted this finding as being an independent predictor of disease severity. Wang et al[16] reported that patients with severe COVID-19 had more severely reduced CD4+ T cell, CD8+ T cell, B cell, and NK cell counts. They also reported that CD8+ T cells tended to be an independent predictor of disease severity and treatment efficacy. Lymphopenia and hypercoagulopathy are now considered the signs of a poor prognosis in adult patients.[17]

Children experience an asymptomatic or mild disease characterized by fever, cough, and gastrointestinal symptoms.[18] Among inflammatory markers, CRP and procalcitonin are normal in a majority of patients. A procalcitonin level above 0.5 ng/mL indicates a bacterial co-infection.[19] Hepatic enzymes, muscle enzymes, and D-dimer may increase in severe or critical cases. Anemia and abnormal platelet count are rare. White blood cell count is normal in most cases. Leukopenia is the most common white blood cell abnormality. Our patients had a lower neutrophil count than healthy children. While platelet count was lower in our symptomatic patients, CRP and procalcitonin were similar to the control group.

Lymphopenia is rare in children than adults.[17,20] In a study, lymphopenia was found in only 3.5% of 71 pediatric COVID-19 cases.[21] In a review article, Henry BM et al[22] reported that, unlike adults, pediatric COVID-19 cases usually have inconsistent changes in leukocyte indices, suggesting that these parameters do not appear as reliable markers of disease severity. Li et al[23] categorized 125 pediatric COVID-19 cases as

either upper respiratory tract infection or pneumonia and found no significant difference between CD4+ T cell, CD8+ T cell, B cell, and NK cell counts and CD4+/CD8+ cell ratio. However, the percentage of regulatory (CD4+ CD25+) T cells was lower in the pneumonia cases. Li et al[24] compared 40 pediatric cases of COVID-19 pneumonia and 16 pediatric cases of respiratory syncytial virus pneumonia and reported a higher CD8+ T cell count in COVID-19 (+) patients. They suggested that an effective CD8+response may be related to mild to moderate symptoms in children with COVID-19 pneumonia. Lu et al[25] reported that a decrease in the initial number of T cells, T helper cells, and T cytotoxic cells is a valuable indicator for the severity of the disease in children with SARS-CoV-2 infection. They emphasized that the severe decrease in the number of T cells and T helper cells in pediatric patients with critical SARS-CoV-2 infection may be closely related to the cytokine storm caused by immune dysregulation.

There was no decrease in lymphocyte count in our COVID-19 pediatric patients. Moreover, some lymphocyte subsets such as NK cell, T cell, and CD4+ T cell counts were increased. This may be due to the fact that the majority of our patients were asymptomatic, and the remainders suffered a mild disease. Children may be developing a more protective immune response to the virus than adults. These limits viral spread in the body and prevents an excessive immune response from being developed, leading to absent or limited systemic inflammation.

CONCLUSION

Asymptomatic and mild COVID 19 pediatric patients had lower neutrophil counts, similar lymphocyte counts, and higher NK cell, T cell, and CD4 cell counts compared to the healthy children. One reason for mild SARS-CoV-2 infection in children may be an increase in some lymphocyte subsets such as NK cell, T cell, and CD4 cell. The absence of lymphopenia and no decrease in lymphocyte subsets in pediatric patients with COVID 19 seem to be related to mild illness. Understanding why children develop protective immunity to the virus and adults develop an extreme immune response could be an important step toward developing new treatments.

The limitations of our study include a small patient number and the lack of analysis of the change in study parameters with treatment. It is not possible to evaluate the immune system only with the lymphocyte subset. The absence of a serious disease group in the study is another limitation. An additional limitation is that the cytokine level has not been studied.

Ethics Committee Approval: This study was approved by Ethics committee of Kayseri City Hospital (Approval No: April/2020; no.64).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.A.; Design – M.A.,; Supervision – M.A., H.T.H.Ö.; Resources – M.A., D.B.İ., M.O.D, İ.Ç.; Materials – M.A., D.B.İ., G.B.,; Data Collection and/or Processing – M.A., D.B.İ., M.O.D., G.B.; Analysis and/or Interpretation – F.E., M.A.; Literature Search – M.A.,; Writing Manuscript – M.A.,; Critical Review – M.A., H.T.H.Ö., İ.Ç., F.E.

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Acute Respiratory Distress Syndrome Management in Pediatric Intensive Care Units in Turkey: A Prospective Survey

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What is already known on this topic?

- *The complexity of pediatric acute respiratory distress syndrome (pARDS) makes it particularly difficult to establish commonly accepted treatment practices in children. It is well-known that the management of pARDS may demonstrate differences even in the same pediatric intensive care unit. Therefore, the treatment and management of ARDS in compliance with international guidelines would be highly helpful.*

What this study adds on this topic?

- *Current mechanical ventilation and non-ventilation treatment strategies in pARDS in Turkey are largely compliant with international practices.*
- *We found that steroid and surfactant use are higher in most of the participating pediatric intensive care units (PICUs) compared to those reported in international studies.*
- *Finally, the study revealed that the use of cuffed endotracheal tubes was more common in closed model PICUs.*

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ABSTRACT

Objective: This study aimed to explore the compliance of management strategies for pediatric acute respiratory distress syndrome in pediatric intensive care units in Turkey with current guidelines.

Materials and Methods: This is a cross-sectional, prospective survey study. We delivered the survey, consisting of questions on topics in the relevant literature on acute respiratory distress syndrome management in children (1 month–18 years), to the heads/staff of the 100 units via email or phone.

Results: In total, 51 (51%) out of 100 targeted pediatric intensive care units responded to the survey. We found out that 17 (33%) units comply with no acute respiratory distress syndrome guideline, while 65% frequently utilize cuffed endotracheal tubes. The majority of the units (86%) achieve their mechanical ventilation targets with the help of pressure control modes. Besides, steroid and surfactant use are present in 47% and 45% of the units, respectively, while 16% and 38% of the units use inhaled nitric oxide and high-frequency oscillatory ventilation, respectively.

Conclusion: Lung-protective ventilation strategies preventing ventilator-associated lung injury are explicit in all responding units. The present survey revealed that current mechanical ventilation and non-ventilation treatment strategies in pediatric ARDS in Turkey are relatively uniform and largely consistent with international practices.

Keywords: Lung injury, mechanical ventilation, non-invasive support, pediatric acute respiratory distress syndrome, pediatrics, pediatric intensive care, ventilatory strategies

INTRODUCTION

Pediatric acute respiratory distress syndrome (pARDS) is one of the most life-threatening conditions, such as acute pulmonary inflammation, alveolar edema, and hypoxemia, often leading to respiratory failure.[1] Thus, pARDS requires following up-to-date guidelines and expert management.

A wide range of treatment strategies has been introduced since the very first diagnosis of lung injury. (High □ low tidal volume (TV), low □ high positive end-expiratory pressure (PEEP), and lung-protective strategies, etc.) Fortunately, these practices were found to reduce mortality in adults, and adult experiences have been taken as a basis for children.[2-5] Yet, it is well-known that pARDS management may demonstrate differences even in the same pediatric intensive care unit.[6] Therefore, the treatment and management of ARDS in compliance with international guidelines would be highly helpful. On the other hand, the complexity of pARDS makes it particularly difficult to establish commonly accepted treatment practices in children. Although there are similarities in the pathophysiology of ARDS in adults and children,

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pediatric-specific comorbidities, differences in clinical practice, and contrasts with adult outcomes clearly indicate the need for a definition of ARDS for pediatric patients.[7] Compared with previous definitions, the Pediatric Acute Lung Injury Consensus Conference (PALICC) criteria for pARDS identified more pARDS cases, and patients had lower rates of complications, severe ARDS, and overall mortality.[8,9]

The incidence of ARDS in children younger than 16 years is less than 3 cases per 100 000.[10] Yet, clinical trials on pARDS also require the long-term commitment of multiple centers to conduct a randomized, controlled trial (RCT) of acceptable quality. Therefore, it is of utmost importance to increase our knowledge on pARDS with mortality rates of 8%-35% and improve its treatment.[10-12]

Ultimately, the present study aimed to investigate the management practices for pediatric ARDS in pediatric intensive care units in Turkey and determine their compliance with international practices.

MATERIALS AND METHODS

Design and Population: Upon the ethical approval of Selçuk University, School of Medicine, Clinical Research Ethics Committee (Approval no: 2018-107), we requested a list of licensed levels 2 and 3 pediatric intensive care units from the Turkish Ministry of Health. As of April 2018, the list included a total of 111 units, among which some did not admit patients, and only 4 provided postoperative service to pediatric cardiac surgery patients. Hence, we delivered the survey to the heads/staff of the remaining 100 units via email or phone. The survey consisted of items on the issues in the relevant literature.[5,6] We performed all stages of the study in accordance with the revised version of the World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects 2013.

Definition of pARDS: Unlike previous ARDS definitions, the definition in the PALICC simplifies the radiological criteria and recommends the use of pulse oximetry measurements when routine arterial blood gas measurement is not practiced and PaO_2 is not available.[13] It also includes the use of oxygenation index and oxygenation saturation index instead of PaO_2/FiO_2 (P/F ratio) to classify ARDS severity. Furthermore, the PALICC definition establishes specific criteria — missing in the previous ones — to define ARDS in children with chronic lung and cyanotic heart diseases. In addition, children with lung injuries that are unique to the perinatal period are excluded, although the PALICC criteria do not identify an upper age limit .[13]

Development and Content of the Survey: The survey inquires about the use of different ventilation modes, pressure and volume settings, inhaled nitric oxide (iNO), prone positioning, high-frequency oscillatory ventilation (HFOV), extracorporeal membrane oxygenation (ECMO), and other ancillary methods. Acute lung injury (ALI), including acute respiratory distress syndrome (ARDS), is a complex syndrome with high morbidity and mortality caused by various pathological injuries, including pulmonary and extrapulmonary conditions, in critically ill patients.[14] Since ALI was excluded from the Berlin Definition in 2012, pARDS comprised both ALI and ARDS in the present study based on the PALICC criteria.[11] Patients of 1 month to 18 years are treated in pediatric intensive care units in Turkey; thus, we specified the questions in the survey regarding the management of pARDS in patients in this age range.

If the patients hospitalized in an intensive care unit are managed by the faculty members of the department who hospitalizes the patients and if the pediatric intensive care specialist acts only as an administrative manager, this is defined as an open unit. In the closed unit, all patient follow-up and administrative work are carried out by a pediatric intensive care specialist. When necessary, consultation is requested from the department that hospitalized the patient.

Statistical Analysis

We presented categorical data in absolute numbers and percentages. We performed the analyses on The Statistical Package for Social Sciences version 21.0 software (IBM Corp.; Armonk, NY, USA).

RESULTS

In the present study, 51 PICUs responded to our call among 111 in the list above. Twenty (40%) of the units operate within university (state) hospitals, 18 (36%) are within training and research hospitals, and 12 (24%) admit patients within state hospitals.

The average number of pARDS cases presenting to these centers range from 1 to 5 patients per month at the rate of 94.2%. Seventeen (33%) of the PICUs reported not complying with any guideline recommendations in the standard treatment of pARDS patients. A pediatric intensivist is responsible for inpatient cases in 22 (43%) PICUs, while a general pediatric specialist is in charge of the remaining units (57%).

We found that cuffed endotracheal tubes (ETT) are primarily used in all closed model PICUs (33; 64.7%), whereas it is not the case for 4 of 18 (7.8%) where PICUs are managed as an open model.

Pressure controlled/pressure support (PC/PS) is the most common ventilation mode reported by 37 (72.5%) PICUs, while 7 (13.7%) utilize volume-controlled (VC) ventilation. Only 1 unit (2%) regularly uses neurally adjusted ventilatory assist mode, whereas it is not the case for the remaining 50 (98%).

While inhaled NO (iNO) and HFOV are present in 8 (16%) and 19 (38%) PICUs, respectively, ECMO is only available in 18 (35%) (Table 1). Nineteen (38%) PICUs generally utilize HFOV as the

Table 1. The Use of Ancillary Treatment Strategies

Intervention	n	%
ECMO	18	35
HFOV	19	38
iNO	8	16
NAVA	1	2
Prone position	38	75
Steroids	24	47
Surfactant	23	45
Sedation/analgesia	50	100

ECMO, extracorporeal membrane oxygenation; HFOV, high-frequency oscillation ventilation; iNO, inhaled nitric oxide; NAVA, neurally adjusted ventilator assist.

Table 2. Mechanical Ventilation Parameters

PEEP		Tidal Volume		Peak Pressure	
Maximum Value (cmH_2O)	PICU, n (%)	Maximum Value (cmH_2O)	PICU, n (%)	Maximum Value (cmH_2O)	PICU, n (%)
<10	35 (69)	<4	4 (8)	<30	4 (8)
10-15	9 (17)	4-6	33 (64)	30-35	42 (82)
16-20	4 (8)	7-8	12 (24)	>35	5 (10)
21-25	1 (2)	9-10	1 (2)		
>25	2 (4)	>10	1 (2)		

PEEP, positive end-expiratory pressure; PICU, pediatric intensive care unit.

first alternative when conventional ventilation is insufficient. Besides, while 24 (47%) PICUs use steroids, 23 (45%) utilize surfactants. In 38 (75%) PICUs, prone positioning is preferred in the treatment of pARDS patients.

Maximum PEEP, TV, and peak pressure values prior to the change of treatment strategies are shown in Table 2. Seven (14%) intensive care units (ICUs) use a maximum PEEP value of >15 cmH_2O in all children regardless of age, while 35 (69%) prefer it of <10 cmH_2O during treatments. Two ICUs responded to the related survey question as "No Limit."

Forty-two (82.4%) ICUs use the same maximum peak pressure value in all children regardless of age, while 5 (9.8%) prefer higher maximum values in children. The most common maximum peak pressure value reported by 42 (82.4%) PICUs ranges from 30 to 35 cmH_2O. Five (17%) ICUs use a maximum peak pressure value < 30 cmH_2O, and 1 reporting "No Limit" sets the maximum value of >35 cmH_2O.

Tidal volume values in ventilation are kept between 4 and 6 mL/kg in 33 (65%) PICUs, >8 mL/kg in 2 (4%) PICUs, and <4 mL/kg in 4 (8%) PICUs. Besides, 12 (25%) PICUs keep it between 6 and 8 mL/kg at all times. In the present study, we determined that permissive hypercapnia and hypoxemia are used in 78.4% of the PICUs.

DISCUSSION

Although it is not an absolute method or consensus in pARDS management worldwide, even within the same units, the present study suggested that current management strategies in pARDS in PICUs in Turkey are relatively uniform and largely consistent with international practices. We found out that 33% of the PICUs do not refer to any guidelines for ventilation. Our findings also showed that the predominant ventilation mode is PC/PS, that the target TV value generally ranges between 4 and 8 mL/kg, and that the most frequently reported maximum peak pressure value is between 30 and 35 cmH_2O. Yet, we discovered that steroid and surfactant use are higher compared to international practices. Finally, the use of cuffed ETTs is more common in closed model PICUs.

The study by Santschi et al.[1] also known as the Pediatric Acute Lung Injury Mechanical Ventilation (PALIVE) study covering 59 PICUs in 12 countries in North America and Europe, aimed to determine the management strategies in pARDS. The recommendations published in 2017 aimed to adopt a consistency in the use of mechanical ventilation in children and can now be proposed as a standard-of-care applicable in routine clinical practices and ARDS research.[15]

In terms of mechanical ventilation in the management of pARDS cases, it was previously reported that 43% of the units use PC mode, 28.2% utilize pressure-regulated VC mode, and 26.6% prefer VC mode . A study in Italy reported that PC is used in 44% and pressure-regulated VC is preferred in 37% of the units.[16] In Brazil, while no unit uses VC, only 1 unit prefers volume-targeted pressure control mode and all other PICUs utilize pressure modes.[17] In Nordic countries, PC/PS ventilation mode is prevalently adopted in PICUs (89%).[6] Although the relevant literature does not offer satisfactory evidence to recommend 1 ventilation mode over another,[15,18] our study revealed that PC/PS is the most common ventilation mode reported by 37 (72.5%) of the PICUs in Turkey.

In the present study, we found that 45 (90%) of the ICUs use target TV between 4 and 8 mL/kg, while only 2 (4%) use it >8 mL/kg. These findings are consistent with the mean TV value (8.3 ± 3.3 mL/kg) that was reported in the PALIVE study. In the literature, target TV use was reported to be 8.0 Ml/kg in Australian and New Zealand PICUs, 8.1 mL/kg in a Canadian group,[10] and 7.1 ± 1.5 mL/kg in a Finnish group. In Nordic countries, 67% of the PICUs use target TV of 6-8mL/kg.[5,6] Santschi et al also indicated that most pediatric intensivists use TV in the range of 5-8 mL/kg.[1] Such findings also overlap the Paediatric Mechanical Ventilation Consensus Conference (PEMVECC) recommendations for targeting physiological TV and avoiding TV >10 mL/kg at ideal body weight.[15]

Adult-oriented guidelines for mechanical ventilation strategies in ARDS recommend maintaining plateau pressure (Pplat) ≤30 cmH_2O.[19] In the absence of transpulmonary pressure measurements, PEMVECC strongly recommends limiting Pplat ≤ 28 cmH_2O and ≤29-32 cmH_2O in the presence of restrictive lung disease.[15] In the present survey, we discovered that the maximum acceptable peak inspiratory pressure (PIP) prior to a change to ventilation strategy is in the range of 30-35 cmH_2O in 42 (82.4%) of the PICUs, similar to the results in the study by Santschi et al.[1] In this regard, both Pplat and PIP can be used as a high-pressure limit, but measuring Pplat may be difficult when using uncuffed ETT.

The present study demonstrated that 35 (69%) PICUs apply a maximum PEEP of 10 cmH_2O and 9 (17%) adopt it in the range of 10-15 cmH_2O. There are only 3 (6%) PICUs applying PEEP > 20 cmH_2O. The same maximum PEEP values were also reported by Santschi et al in Nordic countries and Brazil.[1,6,17] Although PEEP is recommended for respiratory support to prevent alveolar collapse, there are no universal recommendations for PEEP values in pediatric ARDS management.[15] Besides, the current research trend focuses on "higher PEEP and lower TV (peak pressure)," as Khemani and Newth argued.[11,13,17-21]

The use of cuffed ETTs reduces the risk of bronchoaspiration by providing better tracheal sealing and the risk of stridor following extubation . It also diminishes the need to replace the tube due to air leakage, provides more reliable measurements of lung capacity and volume, optimizes the use of capnography, and

finally, prevents an increase in morbidity in children with prolonged mechanical ventilation.[22,23] The PALIVE study reported the use of cuffed ETT in 62.9% of patients.[24] In our research, we found that 47 (96%) centers use cuffed ETT. Considering those not using cuffed ETT, we discovered that they operate as open model intensive care units. Clinics other than PICUs undertake the inpatient management as open model PICUs, which, unfortunately, suggests that the management is not compliant with the current guidelines.

The PALIVE study indicated that iNO is used for 12.7% of children with ARDS.[24] In Nordic countries, almost every unit has access to iNO.[6] Although some studies reported limited benefits of iNO for mechanical ventilation time or survival,[25] iNO can be used as rescue therapy in severe respiratory failure, which may improve oxygenation.[36] In our study, we determined that there is an iNO option in only 8 units (6%), but the success of the adopted method is not questioned over ventilation and assistive techniques.

High-frequency oscillatory ventilation is often used when conventional ventilation fails. Low TVs with HFOV are theoretically the ideal lung-protective ventilation approach to be used in ARDS. The safety and efficacy of HFOV were previously questioned following The Oscillation for Acute Respiratory Distress Syndrome (ARDS) Treated Early (OSCILLATE) trial study in adult ARDS and a retrospective observational pediatric study.[27] A recent study in Australia found survival to discharge to be 75% for the entire study group receiving HFOV and 2-year survival to be 62% for the entire cohort.[28] Another study reported that the HFOV approach is applicable in pediatric patients and does not impair gas exchange or hemodynamics regardless of age or pARDS severity.[29] When it comes to our study, we found that 19 (38%) units use HFOV, but its success is still contradictory in these units. Moreover, we have insufficient evidence to conclude that HFOV reduces mortality or long-term morbidity in pARDS.[15]

In the present study, steroid use in pARDS was reported by 24 (47%) of the ICUs, which was significantly higher than that reported in the PALIVE study[24] but considerably lower than in the Nordic countries.[6] The abnormal inflammation that occurs in ARDS has sparked interest in the use of steroids as anti-inflammatory therapy.[30] Yet, a systematic review of adult ARDS studies reveals mixed results.[31] Two meta-analyses involving studies of different doses of corticosteroids in adults showed that corticosteroids use probably worsens outcomes.[32] However, another meta-analysis reviewing the use of only low-dose corticosteroids (methylprednisolone: 0.5-2.5 mg/kg per day) reported improved morbidity and mortality outcomes in ARDS without increased adverse reactions.31 A meta-analysis by Meduri et al[33] published in 2018 provided moderate to high levels of evidence that low to moderate doses of prolonged glucocorticoid therapy in adult ARDS are safe and reduce mechanical ventilation, ICU and overall length of stay, and mortality. Nevertheless, the most promising results were in studies with a relatively early start of therapies (<3 days during early ARDS or 14 days in late ARDS) using low to medium doses (equivalent to methylprednisolone of 1-2 mg/kg per day), which are gradually reduced over time (12 or more days). While it may be prudent to recommend the routine use of corticosteroids in pARDS, it is a treatment that requires further study to determine the correct patient population, the time of administration, and the dosage regimen. Given the lack of clear evidence in pediatrics,[34] PALICC does not recommend using corticosteroids as routine therapy in pARDS until the prospective results of further research with specific populations.[35]

Routine surfactant therapy is not recommended in pARDS[13] but can be used in primary severe ARDS (Meconium aspiration syndrome and viral or bacterial lung infections).[36] In our study, surfactants are used in 23 (45%) of the participating ICUs. The PALIVE study reported that only 4.2% of children receive surfactants.[24] In Nordic countries, they are used in 39% of the units.[6] However, the efficacy of exogenous surfactant treatment in children and adolescents with ARDS remains controversial. On the one hand, a multicenter, randomized, blind study showed improved oxygenation and reduced mortality in surfactant therapy.[37] On the other hand, another study suggested that the benefits of surfactants are uncertain, and they cannot be recommended for routine use in pARDS .

Numerous meta-analyses in adults provided conflicting results regarding the effect of the prone position. Two recent meta-analyses reported a significant reduction in ARDS-related mortality when prone positioning and lung-protective ventilation were combined.[38,39] Also, in one of these meta-analyses, the proning patients with severe ARDS study reported that prone positioning resulted in a 50% reduction in mortality in adult patients with severe ARDS.[38] In Nordic countries, 89% of pediatric intensive care units use the prone position, which was found to be 75% in our study. Contrary to many other management strategies in ARDS, a multicenter RCT evaluating prone positioning in pediatrics showed prone positioning to be safe[40] but found no difference in mechanical ventilation, mortality, or duration of other health outcomes. Curley reported that prone positioning in children with ALI improved oxygenation but did not significantly reduce ventilator-free days.[12,40] The ongoing PRO-Spect study aims to determine the effectiveness of prone positioning in severe PARDS more precisely.[41] The PALICC guidelines recommend prone positioning to be considered an option in severe cases of PARDS but do not recommend its use as a routine treatment in pARDS, given the available pediatric data.[13]

Sedation and analgesia are used to provide synchronization with mechanical ventilation and facilitate tolerance in patients undergoing invasive mechanical ventilation. They also help in optimizing the work of breathing, oxygen delivery, and consumption.[42] In our study, we noticed that all units use sedation and analgesia in pARDS management. Besides, muscle relaxants can be preferred in cases where sedation is not sufficient for effective mechanical ventilation.[13,42] While sedation and analgesia are used in pARDS management in all units enrolled in the present study, only 37 (72.5%) adopt muscle relaxants. In Finland, sedation is used in 90% of mechanically ventilated children, but no data were presented on the use of muscle relaxants.[5]

The success of ECMO in infantile RDS has led to the use of the technique in children and adults.[43] In pARDS, ECMO increases systemic oxygen delivery thereby allowing damaged lungs to rest and heal. However, ECMO bears serious risks and requires

substantial resources, skills, and expertise.[44,45] Unfortunately, despite robust evidence in neonates and potential benefits in adults, clinical trial evidence for the use of ECMO in pARDS is lacking.[43,46,47] The present study revealed that 18 (35%) units have access to ECMO, which is adopted as a contingent option in suitable patients.

The present study is an up-to-date review of treatment strategies used in PICUs for pARDS in Turkey. We aimed to reach every PICU that can treat children with ARDS in Turkey. However, despite being licensed by the Ministry of Health, there are many centers that are not operative and do not admit patients due to the absence of a physician in charge. Thus, with a 51% response rate, it seems reasonable to assume that the findings of the present study provide a satisfying picture of the current practices for the management of pARDS in Turkey. Our survey aimed to investigate the characteristics of participating PICUs, specifically regarding the guidelines they comply with, among other details. However, we could not extract the patients' treatment data, including those who received steroids, surfactants, HFOV, ECMO, or iNO, and when they received them. Therefore, we also assume that there may be other differences between units that were not revealed through the survey of the present study.

Limitations and Further Study Recommendations

Further studies may attempt to focus on some issues in pARDS management—the current definition of pARDS and pARDS treatments in international guidelines can be revisited. Also, future studies may not only identify the total number of PICUs using the above-mentioned international recommendations but also scrutinize the context of different implementation models. The lack of detailed treatment questions in the survey questions caused the study to be limited .Finally, further research may engage in comparing the detailed percentages by country.

CONCLUSION

Overall, lung-protective ventilation strategies preventing ventilator-associated lung injury are explicit in almost all responding units. Steroid and surfactant use are higher in most of the PICUs surveyed compared to those reported in international studies. Our survey has shown that current mechanical ventilation and non-ventilation treatment strategies in pARDS in Turkey are relatively uniform and largely compliant with international practices. Providing relevant training to physicians serving in the diagnosis and treatment process of pediatric intensive care patients in line with current guidelines will further promote such compliance and may contribute to the prognosis of pARDS cases.

Ethics Committee Approval: This study was approved by Ethics committee of Selçuk University, (Approval No: 2018/107).

Informed Consent: Since the research was a survey study, no personal information was shared about the patients, so it was not necessary to fill out an informed consent form.

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