Analysis of Inflammatory Markers and Electroencephalogram Findings in Pediatric Patients with COVID-19: A Single-Center Study in Korea

Sunho Lee, MD¹, Kyung-Ran Kim, MD², Chungmo Koo, MD³

¹Department of Pediatrics, CHA Ilsan Medical Center, CHA University, Goyang, Korea
²Department of Pediatrics, Gyeongsang National University Changwon Hospital, Changwon, Korea
³Department of Pediatrics, Dankook University College of Medicine, Cheonan, Korea

Purpose: The Omicron variant wave spread rapidly from February 2022 in South Korea following the initial management of the coronavirus disease 2019 (COVID-19) outbreak. This study examined electroencephalogram (EEG) findings and serological inflammatory markers in pediatric patients with COVID-19 (Omicron variant).

Methods: We retrospectively reviewed the medical records of 41 patients who presented at Gyeongsang National University Changwon Hospital between March and May 2022 and were diagnosed with COVID-19. All serological tests were performed within 24 hours of fever or seizure onset.

Results: The median patient age was 3.6 years (range, 0.08 to 14.00), and the average hospital stay was 3.7 days (range, 1.0 to 7.0). Interleukin-6 (IL-6) levels were elevated above the normal range in all patients (median, 43.18 pg/mL; range, 7.0 to 190.0) and were higher among those who experienced seizures. Of the 41 total patients, 17 (41.5%; mean age, 5.4 years) visited the clinic for seizure. Three patients experienced prolonged seizures (lasting longer than 30 minutes) and received intravenous lorazepam, while eight presented with complex febrile seizures. Nine patients underwent EEG, of whom five exhibited abnormal initial findings. Linear regression demonstrated correlations between prolonged seizure duration and both serum IL-6 level and blood lymphocyte count.

Conclusion: Numerous serological markers associated with the immune cascade were found to be elevated in children with COVID-19. Nevertheless, febrile seizures represent a relatively common neurological presentation among pediatric patients infected with Omicron variants. Consequently, COVID-19 infection exhibits both familiar and distinct characteristics regarding the mechanisms inducing seizures and fever in children.

Keywords: Pediatrics; Seizures; COVID-19; Cytokines; Theta rhythm

Introduction

Since the onset of the coronavirus disease 2019 (COVID-19) pandemic, the virus has undergone mutation, leading to a shift in clinical symptoms from primarily affecting the respiratory system to involving multiple organs. Neurological manifestations, known as...
“neuroCOVID,” have been noted in adults. Meanwhile, children, including those with no history of febrile seizures, have presented with seizures when infected with the Omicron variant [1-3]. Febrile seizures, which typically occur in children aged 6 months to 5 years, can arise during viral infection [4-6]. Seizures induced by fever associated with COVID-19 have been linked to status epilepticus or increased seizure frequency in patients with pre-existing epilepsy [7]. Several hypotheses have been introduced regarding the impact of COVID-19 on the brain, with evidence pointing to the role of inflammatory cytokines in causing coagulopathy [8,9]. Abnormalities in electroencephalograms (EEGs) have been detected, particularly in severe cases, with prognostic implications [10,11]. Marinelli et al. [12] observed attenuated EEG patterns in adult patients with severe COVID-19 and found elevated interleukin 6 (IL-6) levels in those who died from the virus. However, the relationship between serological markers and EEG findings in pediatric patients remains to be clarified. This study was performed to investigate this correlation and to establish baseline characteristics in such patients.

Materials and Methods

1. Study population and data collection
We conducted a retrospective review of the medical records of patients diagnosed with COVID-19 who attended Gyeongsang National University Changwon Hospital between March and May 2022. The study examined data from patients with COVID-19 who experienced seizures accompanied by fever. Febrile seizures are defined as seizures occurring with fever in children, typically between 6 months and 5 years old. Our study also included patients older than 5 years who presented with seizures and fever, which are classified as late-onset febrile seizures. None of the patients included in the study had a prior diagnosis of epilepsy or encephalopathy, nor did they exhibit clinical signs of encephalitis. We excluded patients over the age of 18 years, as well as those with incomplete data or who declined to provide laboratory measurements. To confirm the presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), we used real-time reverse-transcriptase polymerase chain reaction testing on nasopharyngeal swab specimens. EEG data were collected from patients who had fully recovered from their seizures. The inspection timing was within 1 day to 25 days after the fever started and resolved. The EEG recordings included phases of both wakefulness and sleep. All procedures were conducted in compliance with relevant guidelines and regulations, and informed consent was obtained from all participants and their legal guardians. The Institutional Review Board of Gyeongsang National University Changwon Hospital approved this research (IRB No: GNUCH 2022-04-022).

2. Variables and analysis groups
The data extracted from electronic medical records comprised baseline demographic details such as age, sex, body weight, and medical history. Additionally, body weight was categorized into quartiles based on the 2017 Korean National Growth Chart [13]. Blood samples were collected upon admission and within 24 hours following the onset of fever or seizure. All serological tests were conducted within 2 hours of sample collection.

Several clinical hematological and chemical auto-analyzers were utilized to estimate serological markers as follows: the XN-9000 (Sysmex Co, Kobe, Japan) for the total white blood cell count and the proportions of neutrophils and lymphocytes; the Alifax Test 1 (Alifax Srl, Polverara, Italy) for erythrocyte sedimentation rate; the AU5800 (Beckman Coulter Inc., Brea, CA, USA) for C-reactive protein levels; and the STA-R MAX coagulometer (Stago, Paris, France) for D-dimer levels. Additionally, the ADVIA Centaur XPT immunoassay analyzer (Siemens Healthineers, Milano, Italy) was employed to measure serum procalcitonin and ferritin levels. Finally, an automated immunoassay using the Cobas 8000 device (Roche Diagnostics, Basel, Switzerland) was used to quantify IL-6 concentrations.

The EEG data were recorded using a NicoletOne EEG device (Natus Medical Inc., Middleton, WI, USA) in accordance with the International 10–20 system for a minimum duration of 30 minutes. Seizure descriptions employed terminology from the classification system of the International League Against Epilepsy [14]. Clinical neurophysiologists and medical college professors (SL and CK) independently reviewed the EEG tracings and reached a consensus on their interpretations. The revised terminology version [15] was utilized to interpret the EEG findings.

3. Statistical analysis
Statistical analyses were conducted using R version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria, 2021). Analysis of variance and the t-test, along with the Kruskal-Wallis test and the Mann-Whitney U test, were employed to examine the quantitative data of the participants. Furthermore, the chi-square and Fisher exact tests were utilized to compare groups categorized by seizure presentation and EEG findings. Finally, linear regression analysis was performed to assess the relationships between fever peak or seizure duration and inflammatory markers. A $P$ value of 0.05 was considered to indicate significance for all statistical calculations.
4. Availability of data and materials

The authors confirm that the data supporting the findings of this study are presented within the article and its supplementary materials. Anonymized data from this study are available upon reasonable request to Chungmo Koo, the corresponding author.

Results

We retrieved the medical chart data of 585 patients with confirmed COVID-19. We excluded a total of 544 patients aged 18 years and older, as well as those with missing data or who declined to provide laboratory measurements. Consequently, 41 patients with COVID-19 were included in the study. Among these individuals, we conducted further analysis of 17 patients who visited our clinics with seizures, as well as nine patients with available EEG results (Fig. 1). None of the study participants had a history of multisystem inflammatory syndrome in children, nor had they received treatment in intensive care units. All patients had unremarkable perinatal and birth histories, and their neurodevelopmental examinations were normal for their age.

The mean age of the patients was 3.6 years, with an average hospital stay of 3.7 days (range, 1.0 to 7.0) and a predominance of male patients. Among those with seizures, boys were also more prevalent than girls, and the mean age was higher compared to the non-seizure group. Patients with seizures experienced higher peak fever temperatures than those without seizures. Serum IL-6 concentrations were elevated in all enrolled patients, with the seizure group displaying levels about twice as high as those not exhibiting seizures. Additionally, D-dimer levels were higher in the seizure group, while both the proportion and counts of lymphocytes were lower compared to the non-seizure group. No significant difference was observed between groups in white blood cell or neutrophil counts. Similarly, no significant differences were noted in body weight proportions, although a higher percentage of patients in the seizure group were above the 75th percentile for body weight (Table 1).

Of the total patients, eight children experienced complex febrile seizures. Within this group, three children had focal seizures, two experienced seizures more than twice a day, and three exhibited prolonged seizures lasting over 30 minutes, for which they received intravenous lorazepam. Four patients had a family history of febrile seizures, three patients had previous febrile seizures, and all these patients presented simple types of febrile seizures. Seven patients underwent brain magnetic resonance imaging (MRI). Of these, two exhibited abnormal findings, namely choroidal fissure cyst and delayed myelination. The remaining MRI results were non-specific, with no evidence of encephalitis. All patients achieved complete control of seizure activity, maintained an alert mental status, and were discharged from the clinic without the need for anti-seizure medications.

Furthermore, patients with available EEG findings were categorized by the presence or absence of theta burst. The serum IL-6 level was approximately twice as high in the group with theta burst (84.2 pg/mL) compared to the group without such theta activity (42.6 pg/mL). However, this relationship was not statistically significant (Table 2).

Using the results of a linear regression model based on fever peak, we found that a high serum IL-6 level was correlated with a high fever peak ($\beta=0.01$; 95% confidence interval [CI], 0.004 to 0.016; $P=0.002$). However, we observed no significant correlation between fever peak and serum ferritin level ($\beta=-0.004$; 95% CI, $-0.006$ to 0.001; $P=0.014$) or total lymphocyte count ($\beta=-0.099$; 95% CI, $-0.192$ to $-0.006$; $P=0.038$). In the multivariate linear regression model examining the relationship between seizure duration and inflammatory markers, IL-6 levels were associated with prolonged seizure duration, although the association weakened after adjustment (crude: 0.175 [95% CI, $-0.032$ to 0.382; $P=0.09$]; adjusted: 0.131 [95% CI, $-0.024$ to 0.287; $P=0.087$]). Conversely, only lymphocyte counts were correlated with prolonged seizure duration.
Table 1. General characteristics of pediatric patients with COVID-19 infection

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Febrile seizure</th>
<th>All patients (n=41)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (n=24)</td>
<td>Yes (n=17)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>13 (54.2)</td>
<td>6 (35.3)</td>
<td>0.381</td>
</tr>
<tr>
<td>Male</td>
<td>11 (45.8)</td>
<td>11 (64.7)</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>2.4±4.0</td>
<td>5.4±3.9</td>
<td>0.002</td>
</tr>
<tr>
<td>Fever peak (°C)</td>
<td>38.5±0.8</td>
<td>39.1±0.5</td>
<td>0.019</td>
</tr>
<tr>
<td>Inflammatory markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>6.8±6.2</td>
<td>3.4±2.2</td>
<td>0.18</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>5.6±9.5</td>
<td>3.1±1.3</td>
<td>0.671</td>
</tr>
<tr>
<td>D-dimer (μg/mL)</td>
<td>0.6±0.4</td>
<td>2.9±9.7</td>
<td>0.024</td>
</tr>
<tr>
<td>Procalcitonin (ng/mL)</td>
<td>0.3±0.6</td>
<td>0.1±0.1</td>
<td>0.389</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>29.1±30.0</td>
<td>60.3±50.9</td>
<td>0.044</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>169.1±112.7</td>
<td>40.4±17.9</td>
<td>0.001</td>
</tr>
<tr>
<td>WBC (fL)</td>
<td>7,822.3±5,171.9</td>
<td>6,937.6±1,493.0</td>
<td>0.265</td>
</tr>
<tr>
<td>Neutrophil count (fL)</td>
<td>3,379.6±2,755.1</td>
<td>4,950.6±1,770.9</td>
<td>0.005</td>
</tr>
<tr>
<td>Lymphocyte count (fL)</td>
<td>3,275.1±2,959.9</td>
<td>1,059.8±929.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td></td>
<td></td>
<td>0.635</td>
</tr>
<tr>
<td>&gt;75</td>
<td>11 (45.8)</td>
<td>5 (29.4)</td>
<td></td>
</tr>
<tr>
<td>50–75</td>
<td>6 (25.0)</td>
<td>4 (23.5)</td>
<td></td>
</tr>
<tr>
<td>25–50</td>
<td>2 (8.3)</td>
<td>3 (17.6)</td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>5 (20.8)</td>
<td>5 (29.4)</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as number (%) or mean±standard error.
COVID-19, coronavirus disease 2019; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IL-6, interleukin 6; WBC, white blood cell.

Table 2. Comparison of serological markers in patients according to theta bursts on EEG

<table>
<thead>
<tr>
<th>Variable</th>
<th>Theta burst in EEG</th>
<th>Patients who underwent EEG (n=9)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (n=4)</td>
<td>Yes (n=5)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>0.444</td>
</tr>
<tr>
<td>Female</td>
<td>2 (40.0)</td>
<td>2 (22.2)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3 (60.0)</td>
<td>7 (77.8)</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>5.6±4.5</td>
<td>6.6±3.4</td>
<td>0.684</td>
</tr>
<tr>
<td>Fever peak (°C)</td>
<td>38.8±0.4</td>
<td>39.0±0.7</td>
<td>0.611</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>3.5±3.0</td>
<td>2.0±0.0</td>
<td>0.371</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>5.0±2.5</td>
<td>2.4±2.2</td>
<td>0.139</td>
</tr>
<tr>
<td>D-dimer (μg/mL)</td>
<td>10.0±18.6</td>
<td>0.3±0.1</td>
<td>0.125</td>
</tr>
<tr>
<td>Procalcitonin (ng/mL)</td>
<td>0.2±0.1</td>
<td>0.1±0.0</td>
<td>0.344</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>42.6±18.4</td>
<td>84.2±80.9</td>
<td>0.433</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>46.6±21.0</td>
<td>33.7±5.3</td>
<td>0.279</td>
</tr>
<tr>
<td>WBC (fL)</td>
<td>7,405.0±1,686.9</td>
<td>5,962.0±1,296.6</td>
<td>0.189</td>
</tr>
<tr>
<td>Neutrophil count (fL)</td>
<td>5,437.5±2,242.3</td>
<td>4,332.0±1,628.3</td>
<td>0.418</td>
</tr>
<tr>
<td>Lymphocyte count (fL)</td>
<td>1,255.0±1,439.7</td>
<td>1,025.1±959.0</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Values are presented as number (%) or mean±standard error.
EEG, electroencephalogram; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IL-6, interleukin 6; WBC, white blood cell.

duration (crude: 10.951 [95% CI, 3.490 to 18.411; P=0.007]; adjusted: 17.783 [95% CI, 5.224 to 30.341; P=0.011]).

**Discussion**

In this study, we analyzed inflammatory markers and EEG findings associated with COVID-19, observing several changes in serological markers among patients with the disease. Notably, a significant correlation was observed between increased serum IL-6 levels and peak body temperature. C-reactive protein and IL-6 levels have been shown to be significantly elevated in patients who died from COVID-19 [16]. Furthermore, a dramatic rise in IL-6 has been as-
associated with COVID-19—related severe pneumonia and bilateral interstitial lung disease [17]. However, previous research has also suggested that specific cytokines, including interferon-α/β/γ, tumor necrosis factor-α, and various interleukins (IL-1α/β, -2, -6, -12, -13, and -18), are implicated in the antiviral response, with IL-6 playing a pivotal role in the survival and proliferation of CD4 T cells [18,19]. Therefore, in light of our findings and a review of the literature, IL-6 can be considered a key immunological mediator involved in the COVID-19 immune response.

Additionally, our findings revealed a markedly higher level of cytokines in the group experiencing seizures, with serum IL-6 level displaying a significant correlation with seizure duration. While the exact mechanism by which COVID-19 may trigger seizures remains unconfirmed, several studies have proposed that inflammatory cytokines are crucial neuromodulators that become elevated following epileptic events, both in vivo and in vitro [8,20-22]. Specifically, the increased levels of circulating cytokines seen in patients with COVID-19 can increase the permeability of the blood-brain barrier [23,24] and activate additional inflammatory mediators, triggering a cascade of immune responses [25]. Moreover, inflammatory cytokines may stimulate activity in the developing brain, aligning with the evidence-based theory of epileptic events [26]. Animal studies of febrile seizures have demonstrated elevated cytokine levels in the hippocampus [27]. Our findings indicate that cytokines associated with COVID-19 could represent a crucial marker for understanding the mechanisms underlying fever-induced seizures in children. Additionally, our results suggested a relationship between increased total lymphocyte count and the duration of seizure. This correlation is particularly interesting in that it mirrors the early immune response to viral infection observed in studies of immunocompromised patients. For instance, a moderate rise in lymphocyte count is evident in the initial phase of human immunodeficiency virus infection, followed by a gradual decline [28]. Traditional febrile seizures in children are associated with a variety of viral infections that cause high fever [29]. Significant lymphocytopenia is indicative of an “immunological burst” involving circulating cytokines, which can influence the severity of viral infections, including COVID-19 [30]. However, our findings reveal an unexpected pattern, as lymphocytosis was significantly associated with prolonged seizures in patients with COVID-19. Thus, extended seizures induced by fever in the context of COVID-19 may be linked to lymphocytosis during the early stages of infection [31], offering a different perspective from the mechanisms associated with high fever in other viral infections.

Our findings indicate that patients exhibiting theta burst activity on EEG had IL-6 levels approximately twice as high as those without such activity. Theta burst activity is noteworthy, as it can signify focal abnormalities in brain function and is indicative of prolonged seizures in otherwise healthy children with febrile seizures [32,33]. Furthermore, the presence of theta burst activity is linked to an increased risk of recurrent febrile seizures and adverse outcomes in childhood epilepsy [34,35]. This leads us to consider the potential relationship between theta rhythm and seizure triggered by fever associated with COVID-19 in children. It is plausible that COVID-19 infection may provoke theta burst activity in the brain. Supporting this hypothesis, anosmia was a prominent and widely examined neurological symptom during the initial wave of the COVID-19 pandemic [36]. Autopsy findings suggest a direct pathway for SARS-CoV-2 entry, in which the virus interacts with angiotensin-converting enzyme 2 receptors in the olfactory mucosa [37]. Although the olfactory cortex is situated near the frontal lobe, it has anatomical connections with the hippocampus [38], a primary site of the generation of theta rhythms [39]. In vitro studies using a mouse model injected with SARS-CoV-2 RNA have demonstrated viral aggregation in the hippocampus [40]. Consequently, we propose that the mechanism underlying fever-induced seizures associated with COVID-19 may involve the neuronal invasion route through the hippocampal network.

This study had several limitations. First, the sample size was small, with relatively few patients enrolled. Second, the research was based on retrospective observational data obtained over a short period that did not include laboratory data, such as cerebrospinal fluid analysis. Lastly, the evaluation of developmental milestones relied on questionnaires completed by parents, and the study did not consider other prospective neurological outcomes, such as underlying susceptibility to epilepsy or encephalopathy. Despite these limitations, our research provides current insights into the relationship between serological markers and specific EEG features in healthy pediatric patients. Further follow-up studies should, therefore, be conducted to monitor the neurological outcomes of the enrolled patients, thereby gaining a deeper understanding of latent neurological complications. Additionally, larger populations and extended longitudinal studies are needed to investigate the long-term neurocognitive effects and the pathophysiology of seizures triggered by COVID-19–related fever. Such studies should include postmortem examinations and experimental animal models, along with cerebrospinal fluid analysis or brain biopsy.

**Conflicts of interest**

No potential conflict of interest relevant to this article was reported.
ORCID
Sunho Lee, https://orcid.org/0000-0002-9334-1917
Kyung-Ran Kim, https://orcid.org/0000-0003-2557-3000
Chungmo Koo, https://orcid.org/0000-0003-1434-6988

Author contribution
Conceptualization: SL. Data curation: SL and KRK. Formal analysis: SL and CK. Funding acquisition: SL and CK. Methodology: SL. Project administration: SL and CK. Visualization: SL. Writing - original draft: SL. Writing - review & editing: SL.

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References


