Aims and scope

Annals of Child Neurology is an interdisciplinary peer-reviewed biomedical journal publishing articles in the fields of child neurology, pediatric neurosurgery, pediatric neuroradiology, child psychiatry, pediatric neuropsychology, developmental and behavior pediatrics, pediatric neuroscience, and developmental neurobiology. The aims of Annals of Child Neurology are to contribute to the advancements in the fields of pediatric neurology through the scientific reviews and interchange of all of pediatric neurology. It aims to reflect the latest clinical, translational, and basic research trends from worldwide valuable achievements. In addition, genome research, epidemiology, public education and clinical practice guidelines in each country are welcomed for publication.

Focusing on the needs of neurologic patients from birth to age 18 years, Annals of Child Neurology covers topics ranging from assessment of new and changing therapies and procedures; diagnosis, evaluation, and management of neurologic, neuropsychiatric, and neurodevelopmental disorders; and pathophysiology of central nervous system diseases.

Area of specific interest include the following: behavioral neurology & neuropsychiatry, clinical neurophysiology, epilepsy, headache medicine, neurocritical care, neurodevelopmental disabilities, neuropsychometrics, neuroimaging, neuromuscular medicine, neuroimmunology and inflammation, neuro-oncology, sleep medicine, vascular neurology, as well as other diseases affecting the developing nervous system.

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Clinical Characteristics and Effects of Steroid Therapy in Children with Acute Cerebellar Ataxia

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Purpose: Acute cerebellar ataxia (ACA) is characterized by unsteady gait and instability of the trunk, and is caused by secondary autoimmune responses to infection or vaccination in healthy children. Although its prognosis is usually very good, full symptom recovery generally takes 2 to 3 months. This study aimed to investigate clinical symptoms, neuroimaging findings, and laboratory findings in children with ACA, and to evaluate the effects of steroid therapy on ACA according to the method of administration (intravenous methylprednisolone vs. oral prednisolone).

Methods: We retrospectively analyzed nine patients diagnosed with ACA or acute cerebellitis (AC) who received steroid therapy.

Results: Nine children were included in this study (mean age, 3.71 ± 2.89 years). The mean duration between prodromal febrile illness and cerebellar symptoms was 9.63 ± 4.66 days. Ataxia (limb and/or truncal) was the most common cerebellar sign. Steroids were administered in two ways: methylprednisolone (20 to 30 mg/kg/day) was changed to an oral steroid (prednisolone, 1 mg/kg/day) after 2 to 3 days of administration; an oral steroid was used from the beginning of treatment. The cerebellar symptoms began to improve within 2 to 4 days of steroid therapy. All patients fully recovered without sequelae. The mean interval until full recovery of the cerebellar symptoms was 28.0 ± 19.3 days, and was not significantly different between patients who received an oral steroid after methylprednisolone pulse therapy and patients who only received an oral steroid (P > 0.05).

Conclusion: Regardless of the method of drug administration, steroid therapy helps to improve cerebellar symptoms in children with ACA/AC.

Keywords: Cerebellar ataxia; Cerebellar diseases; Steroids

Introduction

Acute cerebellar ataxia (ACA), which is a disease characterized by unsteady gait with a sudden onset, is caused by a secondary autoimmune response to infection or vaccination in healthy children; the term ACA is commonly used together with acute cerebellitis (AC), which refers to more severe cases [1-4].

Most ACA patients are young children who cannot describe their symptoms accurately, so it is often difficult to diagnose ACA. Furthermore, the exact frequency of the disease has never been investigated, as it occurs infrequently. However, ACA is known to be the most common cause of childhood ataxia, accounting for 30%
to 50% of childhood ataxia cases [5-7]. Garone et al. [8] reported that the number of patients with acute ataxia as the chief complaint was 0.021% of all patients who visited the emergency room, and 33.6% of patients with acute ataxia were diagnosed with postinfectious ACA, which was the most common cause of acute ataxia.

Postinfectious ACA associated with a viral infection usually does not require special treatment, and most patients fully recover without treatment after several weeks. Although steroid or intravenous immunoglobulin use has been reported in some severe cases, its effectiveness has not been clearly demonstrated [6,9,10].

The symptoms of ACA are alarming, because when a healthy child suddenly fails to balance and cannot walk well, his or her trunk is unstable during sitting, his or her pronunciation becomes inarticulate and the speed of articulation slows down, it causes the patient discomfort in everyday life, the child’s quality of life decreases, and the child’s parents become very embarrassed.

The aims of this study were to identify clinical symptoms, neuroimaging findings, and laboratory findings in children with ACA, and to evaluate the effects of steroid therapy on ACA according to the method of drug administration (intravenous methylprednisolone [MPD] vs. oral prednisolone).

Materials and Methods

We enrolled patients who received steroid therapy from January 2009 to December 2019 under the diagnosis of ACA or AC at the department of pediatric neurology of Seoul St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, and conducted a retrospective study.

The inclusion criteria for ACA/AC were: (1) healthy children without an underlying disease; (2) acute onset of cerebellar symptoms/signs (e.g., gait/truncal ataxia, nystagmus, or dysmetria); (3) children who had symptoms of infection within the last 3 weeks (febrile or non-febrile). Among the patients who matched the above criteria, those who had abnormal findings on brain magnetic resonance imaging (MRI) and/or changes in mental status were classified as having AC, while those who had normal mental status and normal brain MRI findings were classified as having ACA. Children with metabolic, endocrinological, or genetic problems or a history of trauma were excluded from this study.

All patients were admitted and underwent the following tests: complete blood count, blood chemistry, cerebrospinal fluid (CSF) study, and MRI of the brain. In some patients, the following additional tests were conducted: electroencephalography (EEG), respiratory polymerase chain reaction (PCR) through a nasopharyngeal swab, Mycoplasma immunoglobulin M (IgM) in blood/PCR through a throat swab, cytomegalovirus (CMV) IgM/G in blood, varicella zoster virus (VZV) IgM and PCR in blood, and glutamic acid decarboxylase (GAD) antibody test in blood.

After checking the results of these tests, steroid therapy was started on the date of diagnosis or the following day. Steroids were administered in two ways: (1) MPD (20 to 30 mg/kg/day) was changed to an oral steroid (prednisolone, 1 mg/kg/day) after 2 to 3 days of administration, or (2) an oral steroid (prednisolone, 1 mg/kg/day) was used from the start of treatment. Symptom improvement after steroid therapy was based on the patient’s clinical symptoms such as walking appearance and sitting posture, the performance of the finger to nose test and tandem gait, and the disappearance of truncal ataxia, dysarthria, nystagmus, and tremor.

In addition to demographic factors, we also identified early-onset symptoms (cerebellar or non-cerebellar symptoms), the interval between symptom onset and the patient’s visit to the hospital, a history of recent infection or vaccination, and the period until complete recovery based on follow-up observations.

1. Statistical analysis

The statistical analysis was done using Stata/IC version 15.1 (StataCorp LLC, College Station, TX, USA). The median test was applied to the data to confirm non-parametric equality and a P value of < 0.05 was regarded as indicating statistical significance.

2. Ethics statement

This study was approved by the Institutional Review Board of Seoul St. Mary’s Hospital (KC20WISI1033). The study was exempted from the requirement for informed consent due to its retrospective nature.

Results

We enrolled nine patients (three males and six females). Of them, six and three patients were diagnosed with ACA and AC, respectively. The mean age of the nine children was 3.71 ± 2.89 years (ACA, 2.9 ± 1.55 years; AC, 5.33 ± 4.65 years), and most patients visited the hospital within 3 days after symptom onset (2.22 ± 1.56 days). Before the cerebellar symptoms, eight out of nine patients showed gastrointestinal symptoms or upper respiratory symptoms with fever, and the mean duration between the gastrointestinal or upper respiratory symptoms and the cerebellar symptoms was 9.63 ± 4.66 days.

Ataxia (limb and/or truncal) was the most common cerebellar sign, which was seen in all subjects, and other cerebellar symptoms included dysarthria (n = 2), dysmetria (n = 2), and nystagmus (n = 1).

To rule out other diseases (e.g., infectious or metabolic diseases),
a lumbar puncture was performed in all subjects. The CSF examinations in eight patients were normal. One patient (patient no. 9) showed an increase in CSF protein levels (136.4 mg/dL) and CSF pleocytosis (white blood cell, 570 cells/μL) with lymphocytic predominance, but both CSF culture (bacterial, fungal culture) and viral/mycobacterial studies (enterovirus real-time PCR [RT-PCR], herpes zoster virus type 1 and 2 PCR, Mycobacterium tuberculosis PCR) in the CSF were negative (Table 1).

A detailed history was taken and additional tests were performed (respiratory RT-PCR, Mycoplasma IgM or PCR, VZV PCR, CMV IgM/IgG, GAD antibody test) to determine the cause of ACA/AC. Only two patients (patients no. 2 and 7) showed abnormal results in the above tests (equivocal for Mycoplasma IgM and positive for CMV IgM, respectively). None of the patients had VZV infections or had been vaccinated within 3 months prior to symptoms, and none were PCR-positive for VZV (Table 1).

MRI was performed in all patients, and abnormal findings were found in three patients (patients no. 2, 3, and 9). All of them showed increased signal intensity in the cerebellar hemispheres (unilaterally or bilaterally), accompanied by diffuse cerebellar swelling on both T2-weighted and fluid attenuated inversion recovery sequences. EEG was obtained in four patients and the findings were normal in all of them (Table 1).

Steroid therapy was performed in two different ways. Six patients were treated with oral prednisolone after MPD pulse therapy. The other three patients received oral prednisolone from the start of treatment. Steroid therapy began on average 13.22 ± 5.31 days after the prodromal illness, but there was no statistically significant difference between the two groups (mean ± standard deviation [SD]: MPD pulse therapy + oral prednisolone, 10.33 ± 3.38 days; oral prednisolone only, 19.0 ± 3.0 days; P > 0.05). The mean duration of steroid therapy was 20.17 ± 8.13 days in patients who underwent MPD pulse therapy, whereas it was 16.67 ± 10.60 days in patients who received oral steroid therapy only; this difference was not statistically significant. The cerebellar symptoms began to improve within 2 to 4 days of steroid therapy (mean ± SD: 2.67 ± 0.87 days), and there was no statistically significant difference between patients who received pulse therapy and those treated with oral prednisolone only (mean ± SD: MPD pulse therapy + oral prednisolone, 2.83 ± 0.98 days; oral prednisolone only, 2.33 ± 0.58 days; P > 0.05) (Table 2).

The mean duration of follow-up was 7.1 ± 6.3 months, and the mean interval until complete recovery of the cerebellar symptoms was 28.0 ± 19.3 days. In patients who received MPD pulse therapy, complete recovery took 25.83 ± 20.61 days, while patients who received prednisolone only took 30.0 ± 21.0 days to fully recover. The interval until complete recovery was shorter in patients who received MPD pulse therapy than in patients who received oral prednisolone only, but the difference was not statistically significant (Table 2).

**Discussion**

ACA/AC patients feel uncomfortable in everyday life because they cannot balance their bodies, and their parents often worry that the cerebellar symptoms may persist. In addition, physicians who see patients with this disease may also feel embarrassed because no treatment has yet been proven to be successful. ACA/AC is known to be a self-limiting disease with a benign course, but there are several reports of neurological sequelae, which may develop in up to 5% to 33% of cases [10,11].

In our study, the mean age of patients affected by ACA/AC was 3.71 ± 2.89 years, which aligns with the age range described in previous reports (2 to 6 years) [5,11,12]. In our study, the mean age of patients with ACA was lower than that of patients with AC. This is consistent with previous studies showing that the age at the onset of AC is higher than that of ACA [4]. However, unlike previous reports describing a male predilection for this disease [6,11], the proportion of females was twice as high in this study.

The mean interval between the onset of cerebellar symptoms/signs and visiting our hospital was 2.22 ± 1.56 days. As such, most patients visited a tertiary hospital within 3 days after the symptoms developed, showing that their parents took their symptoms very seriously.

The causes of ACA include varicella-zoster, mumps, Epstein-Barr virus, herpes simplex virus, influenza virus, coxsackie virus, mycoplasma, hepatitis A virus, human parvovirus B19, and as well as chickenpox, which is the most common cause in children [7,11,12]. In this study, eight patients (88.9%) had upper respiratory or gastrointestinal symptoms with fever before developing cerebellar symptoms, reflecting a slightly higher proportion than reported in previous studies (about 75%) [11,12]. However, only two patients showed abnormal test results suggestive of the cause of ACA (CMV, Mycoplasma), and none showed evidence of VZV infections. For most of the patients, it was not possible to determine the cause of ACA; therefore, we presumed that our patients developed ACA related to non-specific viral infections.

The latency from prodromal illness until the development of cerebellar symptoms/signs has been reported to be 7 to 10 days on average [6,11,12], which is similar to that of our study (9.62 ± 4.66 days).

Ataxia was the most common cerebellar symptom in this study, and it developed in all patients. This finding is similar to those of previous studies that reported ataxia to be the most common...
<table>
<thead>
<tr>
<th>Case</th>
<th>Age at diagnosis (yr)</th>
<th>Sex</th>
<th>Past history</th>
<th>Duration from febrile illness to cerebellar symptoms (day)</th>
<th>First symptom</th>
<th>Disease duration before hospitalization (day)</th>
<th>Abnormal laboratory test</th>
<th>CSF study</th>
<th>Etiologic evaluation</th>
<th>EEG</th>
<th>Brain MRI</th>
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<tbody>
<tr>
<td>1</td>
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<td>F</td>
<td>Febrile illness (URI)</td>
<td>8</td>
<td>Ataxia (limb)</td>
<td>Dysarthria</td>
<td>Dysmetria</td>
<td>Tremor</td>
<td>2</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
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<td>F</td>
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<td>8</td>
<td>Ataxia (limb)</td>
<td>Dysarthria</td>
<td>Dysmetria</td>
<td>Diarrhea</td>
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<td>Elevated ESR (31 mm/hr)</td>
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<tr>
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<td>1.5</td>
<td>F</td>
<td>Non-febrile (URI)</td>
<td>-</td>
<td>Ataxia (truncal/limb)</td>
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<td>N</td>
<td>N</td>
<td>RT-PCR for respiratory virus: (-)</td>
<td>N</td>
<td>Abnormal</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>M</td>
<td>Febrile illness (GI)</td>
<td>4</td>
<td>Ataxia (limb)</td>
<td>Fever</td>
<td>Vomiting</td>
<td>Diarrhea</td>
<td>1</td>
<td>Elevated CRP (2.39 mg/dL)</td>
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</tr>
<tr>
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<td>2.5</td>
<td>M</td>
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<td>Ataxia (limb)</td>
<td>Dizziness</td>
<td>Vomiting</td>
<td></td>
<td>2</td>
<td>Leukocytosis (11,680/μL)</td>
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<tr>
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<td>Ataxia (limb)</td>
<td>Headache</td>
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<td></td>
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<td>Elevated ESR (37 mm/hr)</td>
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<tr>
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<td>Ataxia (limb)</td>
<td>Tremor</td>
<td></td>
<td></td>
<td>3</td>
<td>N</td>
<td>N</td>
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<tr>
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<td>F</td>
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<td>Ataxia (truncal) nystagmus</td>
<td>1</td>
<td>N</td>
<td>N</td>
<td>RT-PCR for respiratory virus: (-)</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>9</td>
<td>10.5</td>
<td>M</td>
<td>Febrile illness (URI)</td>
<td>8</td>
<td>Ataxia (limb/truncal)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Leukocytosis (WBC 14,460)</td>
<td>WBC 570 (lymphocytes 60%)</td>
</tr>
</tbody>
</table>

CSF reference range: WBC 0 to 5 cells/μL, protein 15 to 45 mg/dL, glucose >2/3 of blood glucose level.

ACA, acute cerebellar ataxia; AC, acute cerebellitis; CSF, cerebrospinal fluid; EEG, electroencephalogram; MRI, magnetic resonance imaging; URI, upper respiratory infection; N, normal; -, negative findings; RT-PCR, real-time polymerase chain reaction; GI, gastrointestinal infection; ESR, erythrocyte sedimentation rate; Ig, immunoglobulin; VZV, varicella zoster virus; CRP, C-reactive protein; CMV, cytomegalovirus; +, positive findings; GAD, glutamic acid decarboxylase; Ab, antibody; WBC, white blood cell.
symptom of ACA (70% to 100%) [6,10,11].

Extracerebellar symptoms (e.g., fever, vomiting, diarrhea, headache, and dizziness) were also present, and these symptoms were similar to those described in previous studies [9,10]. Symptoms such as seizure and altered mental status, which have been reported to occur in some severe cases [4], were not observed in our patients.

The findings of CSF examinations are usually normal in ACA [3], but abnormal findings have been reported in some patients, most commonly lymphodominant pleocytosis and increased protein levels [5,10-12]. In our study, most patients showed normal CSF findings, but one of them showed abnormal findings that were the same as reported in other previous studies. Although epileptiform discharge or slowing on EEG was observed in some previous cases [10,12], EEG frequently shows normal findings in ACA patients, and all the patients in our study had normal EEG findings. Three of our patients showed abnormal findings on MRI of the brain, which were the same as those previously reported in children with AC (i.e., hyperintensity in T2-weighted sequences) [4,10]. In previous studies, brain atrophy or diffuse cerebellar signal changes were observed in some patients on follow-up MRI of the brain [4,13], but all our patients showed improvement. These results suggest that abnormal findings on the initial MRI of the brain do not persist in all patients, and our patients generally showed improvements.

The prognosis of ACA is usually very good. Although a study reported full recovery of ACA within 24 days without treatment [12], it is generally recognized that full recovery of symptoms takes 2 to 3 months without treatment [3,11]. There is no consensus on the treatment for ACA, as some studies have reported that steroids were effective for ACA treatment [4], while others reported that they were not helpful [10]. We started using steroids on the day of ACA/AC diagnosis or the following day. All nine children began to show symptom improvement within 2 to 4 days of treatment, and all patients recovered fully within 30 days without neurological sequelae. Direct comparisons may be difficult, but this is considered to be somewhat faster than the generally known recovery time of ACA patients not treated with steroids (about 2 to 3 months).

These results suggest that using steroids may help to improve the symptoms of ACA/AC patients and promote recovery. ACA/AC is an autoimmune disease that develops after infection or immunization [3], and it is believed that the immunosuppressive and anti-inflammatory effects of steroids could contribute to a relatively rapid recovery [14]. Previous studies have not compared the speed of symptom improvement following steroid administration. In this study, we found no significant difference in the speed of symptom improvement in ACA/AC according to the method of steroid administration (intravenous vs. oral). Therefore, it is believed that proper steroid therapy in the early stages of the disease, regardless of whether an oral or intravenous steroid is administered, plays an important role in improving the quality of life by alleviating symptoms. As a result, if the patients’ overall condition is good and regular follow-up is possible, and there is no gradual deterioration of symptoms, outpatient oral steroid treatment can also be considered.

The principal limitations of this study are the small number of patients and the retrospective design. The fact that no objective indicators or assessment tools were used to evaluate whether patients’ symptoms improved, and clinicians relied on the improvement of clinical symptoms, is also a limitation of this study. Further prospective studies are needed on the effects of steroid therapy according to the severity of ACA/AC in a larger number of patients.

### Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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### Author contribution

Conceptualization: JYL, JUM, DHY, JYH, and IGL. Data curation:
References


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Risk Factors for Seizures after Hematopoietic Stem Cell Transplantation in Pediatric Hemato-Oncologic Patients: A Single Tertiary Center Study in the Republic of Korea

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Purpose: The aim of this study was to assess the incidence of seizures, clinical manifestations, and risk factors that could predict the occurrence of seizures after hematopoietic stem cell transplantation (HSCT) in children.

Methods: The study group consisted of 543 patients (311 males and 232 females) registered at the Catholic University of Korea's Seoul St. Mary's Hospital who received HSCT before the age of 18 from January 2009 to January 2019. Their medical records and test results were retrospectively reviewed.

Results: The incidence of seizure after HSCT was 6.6% and the average age of seizure patients was 8.33 ± 5.5 years. The use of calcineurin inhibitors combined with methotrexate as prophylaxis for graft versus host disease (GVHD) was a statistically significant risk factor for seizures (P=0.006). Pediatric patients with grade 2–4 acute GVHD (P=0.003) also showed a higher incidence of seizures than those with grade 0–1 acute GVHD after HSCT.

Conclusion: Our findings indicate that among pediatric patients who underwent HSCT, using calcineurin inhibitors with methotrexate as a conditioning regimen and a higher grade (≥2) of acute GVHD are risk factors of seizures.

Keywords: Neurologic manifestations; Seizures; Hematopoietic stem cell transplantation; Risk factors

Introduction

Hematopoietic stem cell transplantation (HSCT) is widely used as a complete cure for patients with reduced bone marrow function due to various malignancies (such as leukemia, malignant lymphoma, and solid neoplasms) and some nonmalignant conditions (such as severe aplastic anemia, sickle cell disease, thalassemia, immune disorders, certain metabolic disorders, and severe refractory autoimmune diseases) [1]. The HSCT procedure consists of infusing hematopoietic stem cells after a short course of high-dose chemotherapy that is frequently associated with total body irradiation (TBI) to suppress the host immune system in order to prevent graft rejection. HSCT often places patients at risk of life-threatening complications throughout the course of treatment and beyond. Despite significant improvements in peri-transplant treatment, neurological complications—especially those involving the central nervous system (CNS)—along with many other complications, such as graft rejection or graft failure, thrombocytopenia, metabol-
ic disturbances, chemotherapy/radiotherapy-induced toxicity, infection, and graft versus host disease (GVHD), remain contributors to morbidity and mortality during the post-HSCT period [2-4]. The total incidence of neurological complications after HSCT has been reported to range from 8% to 70% [5,6]. Seizure is the most common clinical manifestation of neurological complications [7,8], and younger people have a higher risk of seizures than adults after HSCT [9]. However, only a few studies have analyzed differences between patients with seizures and those without seizures and risk factors for seizures after HSCT, especially in the pediatric population. The aim of this study was to evaluate the frequency, clinical features, and risk factors of seizures to identify information that could be used to prevent seizures and improve the prognosis of pediatric patients after HSCT.

Materials and Methods

1. Patients
The study group consisted of 543 patients (311 males and 232 females) registered at the Catholic University of Korea’s Seoul St. Mary’s Hospital who received allogeneic (506 patients) or autologous (37 patients) HSCT before the age of 18 as treatment for malignancies or other non-malignant hematologic diseases from January 2009 to January 2019.

We retrospectively reviewed the records of all patients who underwent at least one neurological diagnostic test, including brain magnetic resonance imaging (MRI) and electroencephalography (EEG), after HSCT. We also reviewed records of demographic characteristics, including patients’ age, sex, underlying disease, type of HSCT, conditioning regimen (including TBI, busulfan administration, or both), and grade of acute GVHD. The diagnosis of acute GVHD was based on the Glucksberg Seattle criteria [10]. Patients were divided into those younger than 5 years and older than 5 years based on the age at the time of HSCT. This study group was monitored for at least 6 months. Among the 36 patients who experienced seizures, seven patients with a preexisting history of seizures before HSCT were excluded from this study.

2. Type of HSCT
HSCT procedures can be classified into two types: (1) allogeneic HSCT, which uses hematopoietic stem cells harvested from a donor; and (2) autologous HSCT, which uses hematopoietic stem cells harvested from the patient’s bone marrow or peripheral blood.

3. Conditioning regimen
All patients received pre-transplantation conditioning treatment based on the underlying disease according to the transplant protocol. In this study, we divided the type of conditioning regimen as follows, regardless of overall intensity: TBI-based, busulfan-based, TBI and busulfan-based, and others.

4. Prophylaxis for GVHD
As prophylaxis for GVHD, calcineurin inhibitors (cyclosporine, tacrolimus) with or without methotrexate (MTX), antithymoglobulin, and a steroid were administered according to the institutional protocol.

5. Clinical profiles of seizures
The diagnosis of seizures was based on clinical manifestations and neurological/physical examination findings, laboratory findings, EEG findings, and brain MRI conducted at the time of the seizures. Seizure types were classified according to the 2017 International League against Epilepsy criteria. The timing of seizures was classified into three categories according to three phases of immune status, since the timing of neurological complications is similar to the timing of complications in other organs associated with the three phases of patient’s immune status after HSCT: (1) the pre-engraftment period (< 30 days post-HSCT); (2) the early post-engraftment period (30 to 100 days post-HSCT); and (3) the later post-engraftment period (> 100 days post-HSCT) [11]. Abnormalities on EEG included background abnormalities and abnormal epileptiform discharges. Epileptiform discharges were divided into focal and generalized depending on the region of the epileptic focus. The outcome of seizures included freedom from seizures, development of epilepsy, and death. Freedom from seizures was defined as a sustained seizure-free status for at least 12 months.

6. Statistical methods
Patients were classified into those with seizures and those without seizures. SSPS software version 24.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Normally distributed variables are presented as mean ± standard deviation. Univariate analysis was carried out to compare independent variables as risk factors for seizure after HSCT using the chi-square test. Statistically significant variables (P < 0.05) in the univariate analysis were included in the multivariate logistic regression analysis.

Results

1. Characteristics of patients after HSCT
A total of 543 patients underwent HSCT, including 370 patients (69.3%) who had malignant diseases such as leukemia, lymphoma, and solid neoplasms and 173 patients (32.4%) who had non-ma-
lignant hematologic diseases (Table 1). A total of 506 patients received allogeneic HSCT and 37 received autologous HSCT. The average age of the patients was 8.88 ± 5.5 years (range, 5 months to 18 years). There were 162 (29.8%) patients who were younger than 5 years and 381 (70.2%) patients who were older than 5 years (Table 1).

There were 138 patients (25.4%) who received TBI-based conditioning regimens for HSCT, 210 patients (38.7%) who received busulfan-based regimens, 45 patients (8.3%) who received both TBI- and busulfan-based regimens, and 150 patients (27.6%) who received other regimens. For GVHD prophylaxis, 311 patients (57.3%) received calcineurin inhibitors, 62 patients (11.4%) received MTX, 138 patients (25.4%) received calcineurin inhibitors combined with MTX, and 32 patients (5.9%) received other immunosuppressants (Table 1).

### Table 1: Comparison of characteristics between patients with and without seizures after hematopoietic stem cell transplantation

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>With seizures (n = 36, 6.6%)</th>
<th>Without seizures (n = 507, 93.3%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>8.33 ± 5.54</td>
<td>9.33 ± 5.62</td>
</tr>
<tr>
<td>0–5</td>
<td>9 (25.0)</td>
<td>153 (30.1)</td>
</tr>
<tr>
<td>&gt; 5</td>
<td>27 (75.0)</td>
<td>354 (69.9)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20 (55.0)</td>
<td>291 (57.4)</td>
</tr>
<tr>
<td>Female</td>
<td>16 (45.0)</td>
<td>216 (42.6)</td>
</tr>
<tr>
<td>Conditioning regimen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBI-based</td>
<td>12 (33.3)</td>
<td>126 (24.9)</td>
</tr>
<tr>
<td>Busulfan-based</td>
<td>13 (36.1)</td>
<td>197 (38.9)</td>
</tr>
<tr>
<td>TBI+Busulfan-based</td>
<td>2 (5.6)</td>
<td>43 (8.5)</td>
</tr>
<tr>
<td>Others</td>
<td>9 (25.0)</td>
<td>141 (27.8)</td>
</tr>
<tr>
<td>GVHD prophylaxis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcineurin inhibitors</td>
<td>17 (47.2)</td>
<td>294 (57.9)</td>
</tr>
<tr>
<td>MTX</td>
<td>4 (11.1)</td>
<td>58 (11.5)</td>
</tr>
<tr>
<td>Calcineurin inhibitors</td>
<td>13 (36.1)</td>
<td>125 (24.6)</td>
</tr>
<tr>
<td>+MTX</td>
<td>2 (5.6)</td>
<td>30 (6.0)</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of HSCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autologous</td>
<td>2 (5.6)</td>
<td>35 (7.0)</td>
</tr>
<tr>
<td>Allogeneic</td>
<td>34 (94.4)</td>
<td>472 (93.0)</td>
</tr>
<tr>
<td>aGVHD grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–1</td>
<td>19 (52.8)</td>
<td>365 (71.9)</td>
</tr>
<tr>
<td>2–4</td>
<td>17 (47.2)</td>
<td>142 (28.1)</td>
</tr>
<tr>
<td>Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignant</td>
<td>24 (66.7)</td>
<td>346 (68.2)</td>
</tr>
<tr>
<td>Non-malignant</td>
<td>12 (33.3)</td>
<td>161 (31.8)</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation or number (%).

TBI, total body irradiation; GVHD, graft versus host disease; MTX, methotrexate; HSCT, hematopoietic stem cell transplantation; aGVHD, acute graft versus host disease.

*Conditioning regimen without busulfan and total body irradiation; †Calcineurin inhibitors included cyclosporine and tacrolimus; ‡Steroid±antithymoglobulin administered as a prophylaxis.

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Sepsis, cytomegalovirus meningitis, and metabolic imbalances including hyponatremia (<135 mM), hypokalemia (<3.5 mM), and abnormal liver function were observed in patients who showed normal brain MRI. The most frequent abnormal brain MRI finding was posterior reversible encephalopathy syndrome (PRES; n = 13, 50.0%), followed by CNS infection (n = 5, 19.2%). Intracranial hemorrhage and cortical ischemia were present in four patients and one patient, respectively. Malignant brain lesions reflecting CNS involvement, including relapse or metastasis of original malignancies, were observed in three patients, and were also confirmed by a cerebrospinal fluid test. Post-ictal EEG was performed in 32 patients, of whom 26 (81.3%) had abnormal EEG results. Seventeen patients (65.4%) showed background abnormalities with diffuse slow waves, and nine patients (34.6%) had epileptiform discharges, including eight patients (30.8%) who showed focal epileptiform discharges and one patient (3.8%) who showed generalized epileptiform discharges.

Twenty-six of the seizure patients became seizure-free (72.2%) without requiring antiepileptic drugs, whereas six patients developed epilepsy and four patients died during follow-up.

3. Statistical analysis of seizures and risk factors
To analyze risk factors for developing seizures in HSCT patients, patients’ age at the time of seizures, sex, conditioning regimen, prophylaxis for GVHD, type of HSCT (allogeneic or autologous), grade of acute GVHD, and underlying disease were considered. The results of the univariate and multivariate analyses of significant risk factors are shown in Table 3.

1) Prophylaxis for GVHD
Of 449 patients who received a calcineurin inhibitor with or without other regimens as prophylaxis for GVHD, 30 (6.7%) developed seizures. In the univariate analysis, patients with calcineurin inhibitors alone were found to be more likely to have seizures (P = 0.046) than those who received GVHD prophylaxis without calcineurin inhibitors alone, whereas multivariate analysis showed no significant difference in the risk of developing seizures between the two groups. However, patients using calcineurin inhibitors combined with MTX showed a higher risk of developing seizures than patients with other prophylaxis regimens (P = 0.006).

2) Grade of acute GVHD
Among patients with acute GVHD, 19 (4.9%) out of 384 patients with grade 0–1 and 17 (10.7%) out of 159 patients with grade 2–4 developed seizures. Those with grade 2–4 acute GVHD had a higher risk of developing seizures than those with grade 0–1 acute GVHD (P = 0.010). In the multivariate analysis, grade 2–4 acute GVHD remained a significant risk factor for seizures (P = 0.003).

Discussion
Neurological complications associated with HSCT are important causes of morbidity and mortality in both children and adults. Most neurological complications are caused by the original disease or relapse, development of a secondary tumor, treatment-induced neurotoxicity including chemotherapy and TBI, CNS infection, cerebrovascular disease, and metabolic encephalopathy [6,9,12,13]. In our study, the incidence of seizures in children after HSCT was 6.6%, which is slightly lower than the incidence that we reported in the past (13.8%) [14]. This might be attributable to the improved treatment environment including prompt diagnosis and advanced prophylaxis/treatment of HSCT, including strictly controlled blood pressure and dose reduction or switching between...
calcineurin inhibitors to reduce drug-induced neurotoxicity. According to other studies, the incidence of seizures among HSCT patients varies from 1.6% to 15.4% \[9,12\]. This variability might be due to differences in patients, transplantation characteristics, duration of follow-up, and trial design.

Previous studies have identified high doses of TBI and chemotherapy (including calcineurin inhibitors, busulfan, and MTX) as risk factors for seizures after HSCT \[15,16\]. Calcineurin inhibitors (cyclosporine and tacrolimus) are commonly used immunosuppressants. They work by inhibiting T-lymphocyte activation and proliferation, but might increase the permeability of the blood-brain barrier (BBB), resulting in neurotoxicity \[17\]. In vitro and in vivo studies have reported that vasoconstrictive effects via activation of sympathetic outflow by calcineurin inhibitors might also increase the permeability of the BBB \[17,18\]. Comorbid conditions, including hypertension, infection, and metabolic disorders such as hypomagnesemia, might further exacerbate the permeability of the BBB \[19,20\]. Furthermore, calcineurin inhibitors may

### Table 3. Univariate and multivariate analysis of risk factors for seizures after hematopoietic stem cell transplantation

<table>
<thead>
<tr>
<th>Variable</th>
<th>No.</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Age, mean (yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–5</td>
<td>162</td>
<td>2.4 (1.16–5.84)</td>
<td>0.517</td>
</tr>
<tr>
<td>&gt;5</td>
<td>381</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>311</td>
<td>1.2 (0.61–4.32)</td>
<td>0.732</td>
</tr>
<tr>
<td>Female</td>
<td>232</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBI alone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>138</td>
<td>2.1 (0.95–5.27)</td>
<td>0.337</td>
</tr>
<tr>
<td>No</td>
<td>405</td>
<td></td>
<td></td>
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<tr>
<td>Busulfan alone</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>210</td>
<td>3.7 (1.44–9.41)</td>
<td>0.594</td>
</tr>
<tr>
<td>No</td>
<td>333</td>
<td></td>
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<tr>
<td>TBI+Busulfan</td>
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<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>45</td>
<td>1.5 (0.671–4.22)</td>
<td>0.411</td>
</tr>
<tr>
<td>No</td>
<td>498</td>
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<tr>
<td>Calcineurin inhibitor(^a) alone</td>
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<td></td>
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<tr>
<td>Yes</td>
<td>311</td>
<td>4.5 (1.81–11.39)</td>
<td>0.046</td>
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<td>232</td>
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<td>MTX alone</td>
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<td></td>
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<td>Yes</td>
<td>62</td>
<td>2.2 (1.05–6.14)</td>
<td>0.541</td>
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<tr>
<td>No</td>
<td>481</td>
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<tr>
<td>Calcineurin inhibitor(^a)+MTX</td>
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<td></td>
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<tr>
<td>Yes</td>
<td>138</td>
<td>3.4 (0.79–7.23)</td>
<td>0.033</td>
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<td>No</td>
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<td>Type of HSCT</td>
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<td></td>
</tr>
<tr>
<td>Autologous</td>
<td>37</td>
<td>1.4 (0.58–4.71)</td>
<td>0.521</td>
</tr>
<tr>
<td>Allogeneic</td>
<td>506</td>
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<td></td>
</tr>
<tr>
<td>aGVHD grade</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0–1</td>
<td>384</td>
<td>2.1 (1.17–4.87)</td>
<td>0.010</td>
</tr>
<tr>
<td>2–4</td>
<td>159</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignant</td>
<td>370</td>
<td>1.2 (0.76–3.21)</td>
<td>0.848</td>
</tr>
<tr>
<td>Non-malignant(^b)</td>
<td>173</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval; TBI, total body irradiation; MTX, methotrexate; HSCT, hematopoietic stem cell transplantation; aGVHD, acute graft versus host disease.

\(^a\)Calcineurin inhibitors included cyclosporine and tacrolimus; \(^b\)Non-malignant conditions included severe aplastic anemia, sickle cell disease, thalassemia, and immune disorders.

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modulate neuronal excitation and neuronal inhibition (known to play an important role in seizures) by altering gamma-aminobutyric acid (GABA) and N-methyl-D-aspartic acid (NMDA)–mediated responses via calcineurin [21]. The incidence of seizures caused by calcineurin inhibitors is generally less than 1%, although a previous study reported an incidence as high as 14.8% [22]. Although our results showed that the use of calcineurin inhibitors alone was not a statistically significant risk factor for seizure development, the fact that calcineurin inhibitor use combined with MTX did show statistical significance implicates calcineurin inhibitors as a risk factor for the development of seizures. A previous study also showed that calcineurin inhibitors, especially cyclosporine, combined with MTX in the regimen for prophylaxis of GVHD were a risk factor for seizures after HSCT [23], suggesting that cumulative exposure to immunosuppressants might exacerbate drug-induced neurotoxicity and susceptibility to CNS infection, thereby increasing the incidence of seizures.

Busulfan is frequently used in conditioning regimens for HSCT. It has therapeutic effects on the CNS, and it crosses the BBB freely [24]. However, it may cause neurotoxicity and result in seizures. The incidence of seizures in those who have received busulfan after HSCT ranges from 10% to 40% if anticonvulsants are not given [25]. In our study, 15 (5.9%) out of 255 patients who were administered busulfan in the conditioning regimen had seizures. However, none of them had seizures during the pre-treatment period for HSCT, possibly due to co-administration of phenytoin, which might have prevented busulfan-induced seizures.

During the pre-engraftment period (days 0–30), the patient’s immune system is completely suppressed. Due to high-dose chemotherapy with or without TBI as a result of the conditioning regimen for HSCT, the patient is vulnerable to severe infections [23]. In the early post-engraftment period (days 30–100), the patient’s immune system starts to recover. Acute GVHD may develop in this period. By 100 days after HSCT, the patient’s immune system continues to recover gradually, moving towards full recovery. Chronic GVHD may develop [11, 26]. Our results showed that, by a narrow margin, the emergence of seizures mostly occurred after 100 days of HSCT. In the post-transplantation period, especially at 3 months after HSCT and beyond, an increased concentration of immunosuppressants (e.g., cyclosporine and corticosteroids) administered as prophylaxis/treatment for chronic GVHD can result in neurological complications [15], which may lead to the development of seizures.

We found that 83.3% (n = 26) of all seizure patients had abnormalities on brain MRI, with PRES (n = 13, 50.0%) being the most frequent abnormal finding. PRES has been reported to be one of the most common encephalopathies that contribute to the occurrence of seizures after transplantation [27, 28]. The etiologies of PRES include hypertensive encephalopathy and treatment-related leukoencephalopathy, associated with both cranial irradiation and chemotherapy. Among 36 patients who experienced seizures in our study, 23 received cyclosporine to prevent and treat GVHD, and 17 of them showed a high blood concentration of cyclosporine (> 250 ng/mL). Although the pathophysiology of PRES is not fully understood [29] cyclosporine-associated PRES has been related to high levels of cyclosporine [27]. In addition, 29 patients with seizures received corticosteroids for GVHD treatment, which is relevant because corticosteroids can alter cyclosporine levels, potentially leading to the development of neurological complications [30]. Twenty-four patients showed increased blood pressure during seizures, whereas 12 patients had stable vital signs. The patients with hypertension were also being treated with immunosuppressants (cyclosporine and corticosteroids), which are known to cause high blood pressure [19, 29].

PRES can affect the white matter, particularly the subcortical white matter [31]. Although PRES is reversible and generally has a good prognosis, it can lead to permanent neurological deficits and an increased risk of mortality [23, 32]. Furthermore, PRES seems to be associated with epilepsy diagnosis as a long-term consequence [33]. In our study, more than half of the patients (72.2%) who experienced seizures were seizure-free, and most of them only had a single seizure, whereas only a minority of patients needed prolonged antiepileptic drug therapy. Most patients had a favorable prognosis; however, in patients with some conditions (especially CNS infection and intracranial hemorrhage), recurrent seizures occurred.

In a prior study based on serial EEG examinations, more than 80% of children who experienced seizures after HSCT showed diffuse slow waves of background activity [16]. Other studies have found focal and generalized slow activity consistent with encephalopathy based on post-ictal EEG obtained in the first 48 hours after the first seizures in HSCT patients [34]. In our data, 81.3% of patients who developed seizures had abnormalities on post-ictal EEG and more than half of them showed slow background activity, suggesting cerebral dysfunction. This shows that EEG might be a helpful tool for detecting abnormalities in brain function (e.g., encephalopathy) in HSCT patients, especially in children with seizures.

In addition to the use of calcineurin inhibitors with MTX for GVHD prophylaxis, another independent risk factor identified by multivariate statistical analysis for developing seizures in this study was high-grade (> grade 2) acute GVHD, similar to the findings reported in other studies [9, 34–36]. As a possible explanation, prolonged and increased doses of immunosuppression to control se-
vere GVHD might have resulted in high susceptibility to CNS infection and drug-induced neurotoxicity, thereby contributing to seizures [37]. Although acute GVHD greater than grade 2 has been reported to cause neurological complications [16,35,36], little is known about its CNS involvement. Some animal studies support the likelihood of the brain being targeted for GVHD development due to increased expression of immune mediators in the brain parenchyma and cerebral vessels during GVHD [38]. Some clinical trials have also revealed the possibility of CNS involvement in GVHD by presenting inflammation in the brain without evidence of apparent infection [39].

In summary, in pediatric patients receiving HSCT for any reason, especially in patients receiving calcineurin inhibitors with MTX for GVHD prophylaxis and those with a higher grade (≥2) of acute GVHD with a high risk of seizures, close observation and intensive care seem to be necessary to improve clinical outcomes.

The limitations of our research include its retrospective design and the small number of subjects. Thus, more studies are needed in the future to confirm our findings.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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In Goo Lee, https://orcid.org/0000-0001-8678-4050

Author contribution

Conceptualization: JUM, JYL, JWL, NGC, BC, and IGL. Data curation: JUM. Formal analysis: JUM, JWL, NGC, BC, and IGL. Methodology: JUM. Writing-original draft: JUM. Writing-review & editing: JUM, JWL, and IGL.

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Purpose: Hypothalamic hamartoma (HH), a rare congenital disorder, can cause intractable epilepsy and requires optimal surgical treatment. This study analyzed the clinical characteristics of HH and evaluated seizure outcomes and the safety of gamma-knife radiosurgery (GKS) in HH-related epilepsy to propose an optimal surgical treatment.

Methods: We reviewed the medical records of 18 patients with HH treated at Samsung Medical Center (1997 to 2018), and analyzed their presenting symptoms, brain magnetic resonance imaging (MRI) findings, treatment, and response.

Results: The median diagnostic age was 3.2 years. The first presenting symptom was a seizure in six (33.3%), precocious puberty in five (27.8%), both symptoms in six (33.3%), and no symptoms in one patient who was diagnosed incidentally on brain MRI. All mixed and intrahypothalamic types except one had seizures (n=12), while all five parahypothalamic types presented only precocious puberty. Eleven patients showed intractable epilepsy with medications and underwent surgical treatment (most commonly GKS). Eight patients underwent GKS, and two of them received repeated GKS for recurrent seizures. Six patients showed improved seizure control with GKS as the last treatment. Among them, two patients became seizure-free, and one patient had a decreased frequency of seizures after a single GKS. There was no adverse effect related to GKS.

Conclusion: Intractable epilepsy was the most common indication for surgical treatment of pediatric HH. GKS was effective in controlling seizures in 75% of HH patients without any adverse effects. Repeated GKS could also be considered as a safe option for intractable and disabling seizures.

Keywords: Radiosurgery; Hypothalamic hamartomas; Drug resistant epilepsy; Puberty, precocious

Introduction

Hypothalamic hamartoma (HH) is a rare congenital malformation of the ventral hypothalamus. The estimated incidence of HH ranges from 1 in 50,000 to 1 in 100,000, and that of HH with epilepsy ranges from 1 in 200,000 to 1 in 625,000, according to a population-based study [1]. HH mainly presents with seizures, typically gelastic seizure (GS), and central precocious puberty. Clinical symptoms are also known to be associated with the location. HH connecting to the posterior hypothalamus in the region of the
mammillary bodies is associated with epilepsy [2], while HH in the anterior hypothalamus and in the region of the tuber cinereum is associated with central precocious puberty [3]. Furthermore, neurobehavioral comorbidities, such as cognitive impairment and psychiatric symptoms, are frequently present in patients with HH [4-6].

Numerous studies have established the cellular and structural mechanisms of epileptogenic pathogenesis in HH. Implanted intracranial electrodes in HH lesions record seizures associated with HH [2]. HH shows normal cell shapes; however, the cells have an abnormal organization, with variable neuronal size and density, and present pacemaker-like firing activity or excitatory projection-type neuronal phenotypes [7]. Therefore, a surgical treatment that disconnects the abnormal pacemaker from the normal brain tissue is considered in cases of HH with intractable epilepsy.

As HH with epilepsy usually tends to be intractable to anti-seizure medication (ASM), surgical approaches, including resection or ablation of the hamartoma, may be a suitable treatment option. However, the location and anatomical relationship of the HH limit surgical approaches, and the options vary depending on the patient’s age. Surgical treatments for HH are mainly divided into the following: invasive methods, such as lesion resection with open craniotomy; minimally invasive methods, such as laser interstitial thermal therapy (LiTT) [8], stereotactic thermocoagulation (stereotactic radiofrequency thermocoagulation [SRT], or radiofrequency ablation [RFA]); and noninvasive methods, such as gamma-knife radiosurgery (GKS) [9,10]. Although direct lesionectomy had better surgical outcomes than other methods, it showed a high complication rate and had restrictions on accessing the lesion [11]. Minimally invasive and noninvasive methods have been suggested to reduce these limitations, but there are also considerations such as patient’s age and size of HH [12,13].

Despite advances in surgical techniques and outcomes [14], there are difficulties in studying HH, such as the low incidence rate, variable prognosis according to HH location and size, and different experiential guidelines across various countries [15]. Therefore, further HH research is needed to suggest a guideline for surgical treatment and prognosis. Here, we delineated the clinical manifestation of HH and treatment of HH-related epilepsy and presented the effects and advantages of GKS as a preferred surgical treatment.

Materials and Methods

This study included pediatric patients who were diagnosed with HH between 1997 and 2018 at Samsung Medical Center. We retrospectively reviewed their medical records and analyzed the clinical manifestations, brain magnetic resonance imaging (MRI) findings, treatments for each symptom, and responses to those treatments. We classified the HH type according to the classification of Kameyama et al. [16]. The intrahypothalamic type was defined as being located in the third ventricle, while the parahypothalamic type extended inferiorly to the interpoduncular fossa. The mixed hypothalamic type included components of both the intrahypothalamic and parahypothalamic types. Furthermore, we analyzed treatment response based on both Engel’s classification and the International League Against Epilepsy (ILAE) [17].

We examined the clinical manifestations of precocious puberty, including breast budding, vaginal bleeding, increased testis volume, and pubic hair in the pediatric clinic and confirmed precocious puberty through laboratory tests of sex hormone (basal and stimulation tests) in patients with clinical suspicion.

I. Ethics statement

This study was approved by the Institutional Review Board of the Samsung Seoul Hospital (IRB File No. 2019-10-036). Informed consent was waived by the board.

Results

1. Clinical manifestations

Eighteen patients were diagnosed with HH at a median age of 3.2 years (range, 0.08 to 10.5) and were followed up for a median of 7.35 years (range, 0.4 to 18.6). The presenting clinical manifestation was a seizure in 12 patients (66.7%) and precocious puberty in 11 patients (61.1%). Six patients (33.3%) presented with both seizure and precocious puberty, and one patient was incidentally diagnosed with HH during the evaluation of an arachnoid cyst that had been detected on fetal sonography (Supplementary Fig. 1).

The most common type of seizure was GS, which was observed in 11 patients (91.7%), while one patient (8.3%) presented with focal seizures without GS. Seizures presented at a median age of 1.58 years (range, 0.08 to 6), and precocious puberty was diagnosed at a median age of 5 years (range, 0.67 to 10.5) (Table 1). We conducted neuropsychological tests only for patients who were suspected of having an intellectual disability or other psychological symptoms. Four patients (patients 2, 3, 7, and 9) underwent testing, and all were revealed to have mild-to-moderate intellectual disability. Additionally, patients 3 and 9 were diagnosed with attention deficit hyperactivity disorder and paranoid personality disorder, respectively (Table 1).
1) Relationship between clinical symptoms and radiological classification

Three patients (16.7%) were classified as having intrahypothalamic HH, five patients (27.8%) as having parahypothalamic HH, and 10 patients (55.6%) as having mixed hypothalamic HH. One patient with the intrahypothalamic type presented with seizures only and without precocious puberty, while none of the patients with the parahypothalamic type experienced seizures. All patients with the mixed hypothalamic type experienced seizures, and six (60%) had both seizures and precocious puberty. Patient 8, who was incidentally diagnosed via post-natal brain MRI for a large arachnoid cyst, has the parahypothalamic type and did not demonstrate any symptoms until the age of 14 years. Fig. 1 shows the brain MRIs at the initial diagnosis at 1 month of age (Fig. 1A) and follow-up at 2 months (Fig. 1B) and 9 years (Fig. 1C) after endoscopic fenestration.

2. Treatment modalities of intractable epilepsy

Among 18 patients with HH, 12 patients initially presented with seizures. Three patients were not followed up after presenting with a seizure, patient 16 was seizure-free for 3 months on a single ASM, and patients 13 and 14 were scheduled to undergo SRT at another hospital but were lost to follow-up (Fig. 2). The remaining nine patients did not respond to multiple ASMs (median, 2; range, 0 to 4) over a median period of 0.17 years (range, 0 to 3.25) (Table 2). Eight patients underwent GKS, and one patient (patient 17) underwent only SRT at another hospital in Japan. Eight patients underwent GKS at a median age of 6.7 years (range, 4.2 to 13.2), which was based on the initial treatment of GKS. After the initial GKS, patients 2 and 4 could discontinue ASMs and became seizure-free for 11 and 4 years, respectively. Patient 9 demonstrated improved seizure control for 3 years, with decreased ASMs (Table 2).

Patient 1, who had undergone subtotal resection at the age of 18 months, underwent GKS at the age of 13 years. She no longer had focal and generalized seizures after GKS.
However, her GS did not decrease significantly despite the GKS, and she continued with ASMs during the 6 years until her last follow-up.

Four patients (patients 3, 7, 11, and 12) showed no improvement in seizure frequency after the initial GKS (last step of Fig. 2). Patient 7 underwent endoscopic disconnection (ED) after GKS.
Table 2. Radiologic characteristics and seizure outcomes in patients who underwent surgery for hypothalamic hamartoma

<table>
<thead>
<tr>
<th>Case</th>
<th>HH on brain MRI</th>
<th>Operation (age, yr)</th>
<th>FU after last operation (yr)</th>
<th>Radiation dose (Gy)</th>
<th>Size change of HH after operation (maximum diameter, mm)</th>
<th>Seizure outcomes after operation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sizea (mm)</td>
<td>Typeb</td>
<td></td>
<td></td>
<td></td>
<td>Type of seizure: reduction of frequency</td>
</tr>
<tr>
<td>1</td>
<td>20 M</td>
<td>STR (1.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>FSIA, GTC: seizure-free</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GKS (13.2)</td>
<td>6</td>
<td>15</td>
<td>No change</td>
<td>FSIA: seizure-free</td>
</tr>
<tr>
<td>2</td>
<td>27 M</td>
<td>GKS (7)</td>
<td>11</td>
<td>12</td>
<td>Minimal decrease (17→after 4 years: 10)</td>
<td>FSIA, GTC: seizure-free</td>
</tr>
<tr>
<td>3</td>
<td>18 M</td>
<td>GKS (6.5)</td>
<td>-</td>
<td>17</td>
<td>Minimal decrease (17→after 4 years: 10)</td>
<td>FSIA, GTC: seizure-free</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RFA (15.1)</td>
<td>-</td>
<td>-</td>
<td>&gt;50% decrease (7)</td>
<td>FSIA, GTC: seizure-free</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GKS (17.1)</td>
<td>18</td>
<td>-</td>
<td>No change</td>
<td>FSIA, GTC: seizure-free</td>
</tr>
<tr>
<td>4</td>
<td>18 M</td>
<td>GKS (4.8)</td>
<td>4</td>
<td>17</td>
<td>Minimal decrease (16)</td>
<td>FSIA: seizure-free</td>
</tr>
<tr>
<td>7</td>
<td>10 M</td>
<td>GKS (6)</td>
<td>17</td>
<td>-</td>
<td>No change</td>
<td>FSIA: seizure-free</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ED (7.4)</td>
<td>8</td>
<td>-</td>
<td>&gt;50% decrease (10)</td>
<td>FSIA, GT: newly appeared</td>
</tr>
<tr>
<td>9</td>
<td>12 I</td>
<td>GKS (15.5)</td>
<td>3</td>
<td>17</td>
<td>Minimal decrease (11.5→after 4 years: 8)</td>
<td>FSIA: seizure-free</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GKS (6.9)</td>
<td>-</td>
<td>18</td>
<td>Minimal decrease (9)</td>
<td>FSIA: seizure-free</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GKS (9.7)</td>
<td>2</td>
<td>17</td>
<td>Minimal decrease (7)</td>
<td>FSIA: seizure-free</td>
</tr>
<tr>
<td>12</td>
<td>9 M</td>
<td>GKS (5.1)</td>
<td>-</td>
<td>18</td>
<td>No change</td>
<td>FSIA: seizure-free</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SRT (7.1)</td>
<td>0.3</td>
<td>-</td>
<td>-</td>
<td>FSIA: seizure-free</td>
</tr>
<tr>
<td>17</td>
<td>9 M</td>
<td>SRT (3.2)</td>
<td>2</td>
<td>2</td>
<td>&gt;50% decrease (not visible)</td>
<td>FSIA: seizure-free</td>
</tr>
</tbody>
</table>

HH, hypothalamic hamartoma; MRI, magnetic resonance imaging; FU, follow-up; ASM, anti-seizure medication; ILAE, International League Against Epilepsy; M, mixed hypothalamic; STR, subtotal resection; GKS, gamma-knife radiosurgery; GS, gelastic seizure; FSIA, focal seizure with impaired awareness; GTC, generalized tonic clonic seizure; RFA, radiofrequency ablation; ED, endoscopic disconnection; GT, generalized tonic seizure; I, intrahypothalamic; SRT, stereotactic radiofrequency thermocoagulation.

aThe size of the HH was measured using the long axis on brain MRI; bHH types according to Kameyama et al. [16]; cSeizure outcome at the last follow-up; dNo initial coronal image of brain MRI.

and his GS was reduced by over 50%; however, generalized tonic and focal seizures with impaired awareness newly appeared. Patient 12 had no improvement in GS frequency and recently underwent SRT at another hospital 2 years after the initial GKS.

1) Repeated GKS

Two patients (patients 3 and 11) underwent repeated GKS after the first GKS. They presented no deleterious adverse effects and had a 50% or more reduction in seizure frequency. Patient 11, who had uncontrolled seizures after the first GKS, was treated with a second GKS, became seizure-free for 2 years until the last follow-up, and was able to reduce the number of ASMs from 3 to 2. Patient 3 underwent GKS at the age of 6.5 years and had no seizures for 3 years. He showed recurrence of seizures at the age of 8.6 years and underwent RFA as the second surgical option (Table 2). He had a seizure-free period for 2 years with ASM after RFA; however, he had seizures again. He was then treated with a second GKS, following which a seizure-free state was maintained for 1 year. Fig. 3 shows the brain MRI of two patients with repeated GKS before and after surgery.

3. Overall surgical outcome in epilepsy treatment

Based on Engel’s classification, four of the eight patients who underwent GKS were categorized as IA and two patients as ID after GKS as the last treatment. Based on the ILAE, four of the eight patients with GKS were categorized as class 1 and two patients as class 4 after GKS as the last treatment. Patient 12 showed a seizure outcome of IB/class 4, and the last surgical treatment was SRT. Patient 7, who underwent ED after GKS, was classified as IVC and class 6 (Table 2). Patient 17, who had a single SRT, was classified as IB and class 4.

Discussion

This study evaluated the surgical options and treatment results of 18 pediatric patients with HH. Seizure, which is an early predominant symptom, was intractable to medical treatment in 91.7% of the patients, and this was the leading cause of surgical treatment. In this study, GKS was the most common surgical treatment, and eight patients underwent GKS. Among these patients, six (n = 6/8) became seizure-free or had a >50% reduction in seizure frequency;
without any significant complications. Two patients underwent GKS twice and showed good seizure control without any complications associated with the procedure.

Epilepsy in patients with HH is mostly intractable to medical treatment [18,19]. In our study, 11 of 12 patients (91.7%) had uncontrolled seizures on ASMs, except for one patient (patient 16) who had been followed up for only 4 months. All of these patients underwent surgical treatment, and 88.9% showed controlled seizures. Therefore, surgical resection or disconnection has been considered a promising cure for HH-associated epilepsy [20]. In the past, classical resection with craniotomy was performed, but due to the risks related to open surgery, it has been replaced by less invasive methods, including the transcortical approach, brachytherapy, ED, LiTt, RFA, and GKS [21-30]. Table 3 shows seizure outcomes and adverse effects of these surgical approaches, all of which have shown as favorable outcomes as direct lesion resection [8,12,31-35]. If all of the various surgical methods have good seizure outcomes, it is reasonable to choose a method that is relatively less invasive and has fewer adverse effects. In that sense, GKS can be one of the attractive options. GKS presents favorable outcomes for seizure control and several studies reported 60% to 68% improvement in seizure control, with no major adverse effects [30,33-35]. In our study, seven patients underwent GKS as the initial surgical treatment. Among them, three patients (patients 2, 4, and 9; 42.9%) had effective seizure control, with Engel class IA, after a single GKS. Another four patients had additional surgical treatments, such as RFA, second GKS, ED, or SRT, because of persistent seizures after the initial GKS [33].

GKS has the lowest incidence of endocrine dysfunction compared to other methods, because it delivers minimal radiation to

**Fig. 3.** Coronal T2-weighted brain magnetic resonance imaging (MRI) of two patients who underwent repeated gamma-knife radiosurgery (GKS). Brain MRI of patient 11 was obtained (A) at the age of 4 months, (B) at the age of 6 years 10 months (15 months after the first GKS), and (C) at the age of 10 years (14 months after the second GKS). Brain MRI of patient 3, who underwent radiofrequency ablation (RFA) between the first and second GKS (a second GKS 2 years after undergoing RFA), was obtained (D) at the age 6 years, (E) at the age of 15 years (8 years after the first GKS), and (F) at the age of 16 years (1 year after RFA and 1 year before the second GKS).
<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Treatment</th>
<th>Follow-up (median)</th>
<th>No. of patients</th>
<th>Age at operation (median)</th>
<th>Size of HH$^{a}$ (median)</th>
<th>Seizure outcomes$^{b}$</th>
<th>Side effects (no. or % of cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curry et al. [8] (Texas)</td>
<td>2011–2018</td>
<td>LiTT (MRI-guided)</td>
<td>12 mo (12)</td>
<td>71</td>
<td>5 mo–20 yr</td>
<td>4–30 mm</td>
<td>Free from GS: 93% Free from seizure and medication: 12%</td>
<td>DI (1) Severe deficit of short-term memory (1-history of right temporal lobectomy) Episodic hyponatremia (3) Disorders of sodium metabolism (6.9%) Memory disturbance (8.6%) Unfavorable catheter positioning (1.7%)</td>
</tr>
<tr>
<td>Gadgil et al. [31] (Texas)</td>
<td>2011–2017</td>
<td>LiTT (MRI-guided)</td>
<td>1.2 yr (0.6–6.3)</td>
<td>58</td>
<td>5.5 yr (0.4–20.9)</td>
<td>0.52 mL (0.06–14.49)</td>
<td>Engel I+II: 81.1% (69.0%+12.1%)</td>
<td>Disorders of sodium metabolism (6.9%) Memory disturbance (8.6%) Unfavorable catheter positioning (1.7%)</td>
</tr>
<tr>
<td>Ferrand et al. [32] (Rothschild)</td>
<td>1998–2017</td>
<td>ED</td>
<td>37 mo (13–77)</td>
<td>112</td>
<td>7.6 yr (48–133 mo)</td>
<td>NA</td>
<td>Engel I+II: 77.6% (57.1 %+20.5 %)</td>
<td>DI: transient (2), permanent (1) CN III palsy: transient (9), permanent (3) Memory deficit (4), motor deficit (5), SUDPE (1), weight gain (6)</td>
</tr>
<tr>
<td>Shirozu et al. [12] (Niigata)</td>
<td>1997–2013</td>
<td>RFA</td>
<td>3 yr (1–17)</td>
<td>100</td>
<td>10.0 yr (1–50)</td>
<td>15 mm (5–80)</td>
<td>Free from GS: 86.0% Free from other types of seizures: 78.9%</td>
<td>Delayed precocious puberty (9.0%) Pituitary dysfunction (2.0%) Weight gain (7.0%)</td>
</tr>
<tr>
<td>Regis et al. [33] (Marseille)</td>
<td>1999–2007</td>
<td>GKS</td>
<td>71 mo (36–153)</td>
<td>48</td>
<td>16.5 yr (3–50)</td>
<td>9 mm (4–30)</td>
<td>Engel I+II: 69% (47.5%+17.5%)</td>
<td>No permanent neurologic side effectsTransient poikilothermia (6.2%)Transient seizure increase (16.6%)</td>
</tr>
<tr>
<td>Abla et al. [34] (Barrow)</td>
<td>2003–2010</td>
<td>GKS</td>
<td>Mean: 43 mo (18–81)</td>
<td>10</td>
<td>Mean: 15.1 yr (5.7–29.3)</td>
<td>Mean: 0.2 mL (0.14–0.28)</td>
<td>Free from seizures: 60% 50%–90% reduction: 10% 50% reduction: 20%</td>
<td>Short-term memory loss (3) Poikilothermia (1) Increased depression (1) Weight gain/increased appetite (2) Anxiety (1)</td>
</tr>
<tr>
<td>Mathieu et al. [35] (Sherbrooke)</td>
<td>Report from 2010</td>
<td>GKS</td>
<td>36 mo (6–56)</td>
<td>9</td>
<td>23 yr (12–57)</td>
<td>0.6 mL (0.3–1.0)</td>
<td>Engel I: 4 (44.4%) Engel II: 1 (11.1%)</td>
<td>No side effects after the procedure</td>
</tr>
</tbody>
</table>

HH, hypothalamic hamartoma; LiTT, laser interstitial thermal therapy; MRI, magnetic resonance imaging; GS, gelastic seizure; DI, diabetes insipidus; ED, endoscopic disconnection; NA, not available; CN, cranial nerve; SUDPE, sudden unexpected death in epilepsy; RFA, radiofrequency ablation; GKS, gamma-knife radiosurgery.

$^{a}$The size of the HH was measured using the long axis (mm) or volume (mL) on brain MRI; $^{b}$Seizure outcome at the last follow-up.
the targeted tissue [3]. In our study, there were no reported endocrine adverse effects directly associated with GKS. Another advantage of GKS is that its safety is high even when performed multiple times. Second GKS was performed in 58.3% of patients in a previous prospective study [33]. Two of our patients (patients 3 and 11) underwent a second GKS, and there were no remarkable adverse effects. In a previous study consisting of 137 patients (median age: 27.1 years; range: 0.6 to 84.8) with craniopharyngioma, tumor control was better in 25 patients who underwent repeated GKS. Only 10.9% of all patients showed GKS-related complications, including hypopituitarism (8.0%), adverse radiation effects (1.5%), visual deterioration (1.5%), and newly developed cranial nerve palsy (0.7%) [36]. Considering the low adverse effect profile, repeating GKS would be considered for patients who show a partial response to the first GKS to improve the treatment outcome.

GKS has some limitations. It is not recommended for large HH due to biophysical deterioration of the high radiation dose to surrounding tissues in such cases, and other surgical treatments need to be considered for giant HH (>30 mm) [12]. Additionally, the delayed therapeutic effect supports the application of other treatment choices, given that treatment timing might prevent other clinical manifestations of HH. Age can be another obstacle for applying GKS because the skull in younger patients is too brittle for the frame to be applied. More precise stereotactic techniques and suitable frames could help to overcome the limitations of GKS treatment in HH patients.

In conclusion, seizures in HH are mostly intractable and constitute a major motivation for surgical treatment. There are many surgical options for HH, for each of which several factors should be considered, including the degree of invasiveness, associated adverse effects, and availability. This study demonstrated a favorable overall outcome of GKS without associated adverse effects in controlling HH-related seizures. Considering its safety and comparable efficacy, GKS can be considered as the preferential surgical treatment option for HH patients with intractable epilepsy. In addition, regarding its delayed effect, GKS can be chosen in cases with a poor response after other surgical treatments. Moreover, repeated GKS could be considered if the first GKS shows a partial response. For optimal guidance for surgery in patients with HH, further research with more cases from multiple centers and systematic evaluations is needed.

Supplementary materials

Supplementary materials related to this article can be found online at https://doi.org/10.26815/acn.2021.00360.

Conflicts of interest

Jeehun Lee is an editorial board member of the journal, but he was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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Author contribution

Conceptualization: JL and JL. Data curation: JYS and JL. Formal analysis: JYS. Methodology: JIL, HJS, JL, and JL. Project administration: JL and JL. Visualization: JYS. Writing-original draft: JYS. Writing-review & editing: JL and JL.

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Clinical and Genetic Characteristics of Young Children with Fragile X Syndrome

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Purpose: This study analyzed the clinical and genetic characteristics of young children with fragile X syndrome (FXS) and evaluated the significance of FXS genetic (FX) testing for children with global developmental delay (GDD).

Methods: FX testing was performed in 324 children aged <5 years and their family members between 2007 and 2020. Fourteen children (10 boys, four girls) with abnormal results were finally included in this report. We retrospectively reviewed their medical records and categorized them based on genetic test results. The results of an analysis of the expanded cytosine-guanine-guanine trinucleotide (CGG) alleles of fragile X mental retardation 1 (FMR1) were divided into four groups: normal, intermediate (IM), premutation (PM), and full mutation (FM).

Results: Twelve of the 14 children presented with FM (nine boys, three girls), and one each with PM and IM, respectively. Five of the children with FM and the one with PM belonged to two families. At the initial visit, the mean age of the nine boys with FM was 24.8±9.7 months. They presented with significant GDD and markedly delayed language development. Most of them had subtle physical features. Two girls with FM presented with less severe developmental delay than the boys with FM, and they were identified via sibling studies. However, one girl presented with FM resulting from maternal uniparental disomy and severe developmental delay.

Conclusion: Even in the absence of a family history, physicians should consider FX testing for children with unexplained GDD. If there is a family history of FXS, FX screening tests should be performed for all family members.

Keywords: Fragile X syndrome; Genetic testing; Intellectual disability

Introduction

Global developmental delay (GDD) is defined as a significant developmental delay in two or more of the following: gross or fine motor movement, speech/language, cognition, social/personal skills, and activities of daily living. The term GDD is reserved for children under the age of 5, and the term intellectual disability (ID) is applied to older children who can be evaluated using a relatively reliable intelligence test. Thus, GDD is considered a predictor of the future diagnosis of ID [1].

Fragile X syndrome (FXS, OMIM*300624) is considered the most common inherited cause of ID, and is the most common monogenic cause of autism [2]. It is associated with attention deficit, hyperactivity, social deficit, autistic-like behavior, and psychiatric problems. Physical features include a long narrow face, prominent forehead, high arched palate, large ears, macrocephaly, promi-
nent jaw, flexible fingers, flat feet, and pubertal macroorchidism [3]. It can be difficult to detect, especially in young children, because the physical features of the disease are subtle and vague [2]. FXS results from the loss of function of the fragile X mental retardation 1 (FMR1) gene (OMIM*300624), caused by an expansion of cytosine-guanine-guanine trinucleotide (CGG) repeats in this gene. This is associated with hypermethylation of the FMR1 promoter, leading to silencing of the FMR1 gene and the consequent loss of its product, the fragile X mental retardation protein (FMRP). FMRP is a major regulator of the translation of many mRNAs involved in synaptic plasticity [3]. According to guidelines from the American College of Medical Genetics and Genomics, expanded CGG alleles can be divided into four groups: normal (6 to 44 CGG repeats), intermediate or gray zone (IM or GZ; 45 to 54 repeats), premutation (PM; 55 to 200 repeats), and full mutation (FM; > 200 repeats) [2]. Those with the PM or IM allele usually lack the typical clinical features of FXS. PM is meiotically unstable and therefore prone to expansion to FM when transmitted from the mother to the next generation. The risk of FM expansion thus increases with increasing numbers of CGG repeats. The presence of AGG interruptions in the CGG sequence influences FMR1 stability, and the loss of these AGG interruptions appears to increase the instability of CGG repeats [4-6]. FXS tends to be diagnosed later because of the subtle clinical findings in young children with FXS. A late diagnosis can result in a lack of early intervention for rehabilitation and genetic counseling of family members [7].

In all children with unexplained GDD/ID, chromosomal microarray (CMA) and FXS genetic (FX) testing are recommended as first-tier diagnostic tests [1]. CMA provides a high diagnostic yield (15% to 20%) when used in the genetic testing of patients with unexplained GDD/ID or autistic spectrum disorder (ASD). In contrast, the FX test provides a low diagnostic yield (1% to 2%). Therefore, some reports have suggested that it may not be necessary to include FX testing in first-line diagnostic evaluations [8].

We retrospectively analyzed the medical records of children diagnosed with FXS between 2007 and 2020. This study aimed to analyze the clinical and genetic characteristics of young children with FXS, and to evaluate the significance of FX testing for children with unexplained GDD.

Materials and Methods

1. Subjects
FX testing was performed in 323 children (246 males and 77 females) with GDD under the age of 5 and their family members between 2007 and 2020. Of these 323, only 13 children (10 boys and three girls) had abnormal results. One girl without GDD was tested because her sister was diagnosed with FXS. Thus, 14 children were included in the study.

2. Methods
We retrospectively reviewed the medical records of the 14 children with abnormal FMR1 CGG test results. Medical and physical examinations were performed, and the language and cognitive abilities of the children were examined. The Bayley Scales of Infant Development (BSID-II) were used to assess cognitive function, which provided the mental development index (MDI) and the psychomotor development index (PDI). A significant delay was defined as a development index (DI) < 70, and a mild delay was defined as 70 ≤ DI < 85. A DI ≥ 85 was considered normal [9]. The Korean Social Maturity Scale, a measure of personal and social skills needed in everyday life, was used to obtain the social maturity quotient (SQ), upon which basis social maturity was classified as normal (SQ ≥ 85), mild deficit (70 ≤ SQ < 85), or significant deficit (SQ < 70) [10]. Language abilities were assessed using the Sequenced Language Scale for Infants. A significant speech development delay was defined as a developmental score two standard deviations (SDs) away from the mean score, and a mild speech developmental delay was defined as a developmental score between 1 SD and 2 SDs from the mean [11]. The Korean version of the Childhood Autism Rating Scale (CARS) was used to assess ASD. A score of 30 or above on CARS was considered clinically significant [12]. The (CGG)n repeats of the FMR1 gene were analyzed by polymerase chain reaction (PCR) using Amplicon PCR CE/FMR1 (Asuragen, Austin, TX, USA) and the products were separated by capillary electrophoresis with ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The results were analyzed using the GeneMapper version 4.0 software (Applied Biosystems).

3. Ethical statement
The study was approved by the Institutional Review Board of Daegu Catholic University Medical Center, Daegu, Korea (IRB No. CR-20-076). The requirement for informed consent was waived because of the retrospective nature of the study.

Results

1. Study subjects
In total, nine boys and three girls tested positive for the FM allele. Two of the three girls (patients 10 and 12) had an FM allele and a normal allele, but one girl (patient 11) presented with only FM alleles, resulting from maternal uniparental disomy (UPD) of the

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tire X chromosome. The mother of the girl with only FM alleles had one normal allele (30 CGG repeats) and one PM allele (65 CGG repeats). Her father had a normal 32-CGG-repeats allele. CMA analysis and short tandem repeat analysis confirmed the maternal inheritance of the duplicated X chromosome, consistent with maternal UPD. In addition, one girl (patient 13) presented with the PM allele, and one boy (patient 14) with the IM allele. FX testing was also performed on six mothers of the children with the FM allele. All of them had a PM allele.

This study focused on 12 children with FM (Table 1). They were full-term with a mean birth weight of 3.6 ± 0.44 kg. There were no specific perinatal problems in any of the children. The mean age at the first visit of the nine boys was 24.8 ± 9.7 months (range, 11 to 45), and the mean age of the three girls was 34.3 ± 8.3 months (range, 25 to 41). The initial presentations included gross motor developmental delay in two children and language developmental delay in the other 10 children. The nine boys with FM began walking independently after 12 months (mean age, 16.9 ± 3.0; range, 15 to 24). A girl with FM (patient 10) presented with delayed language development and none of the typical clinical features of FXS. She was evaluated using the FX test because her younger brother (patient 6) had been diagnosed with FXS at our clinic; she was found to be heterozygous for FM at the FMR1 locus. Later, her male cousin (patient 7) visited our clinic for delayed development and was also diagnosed with FXS. The remaining three girls (patients 11, 12, and 13) belonged to one family. The first sister (patient 11) had only FM alleles (288 and 288 repeats), the second (patient 12) was heterozygous for the FM allele (30 and 282 repeats), and the third (patient 13) presented with one PM and one normal allele (31 and 99 repeats) (Table 2).

2. Analysis of clinical and genetic characteristics of 14 children

Medical records revealed that all nine boys presented with both large ears and a prominent forehead, consistent with the physical features of children with FXS. However, we could not detect these

Table 1. Demographic data of the 12 study participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>9/3</td>
</tr>
<tr>
<td>Age at first visit (mo)</td>
<td>27.1 ± 9.9 (11–45)</td>
</tr>
<tr>
<td>Male</td>
<td>24.8 ± 9.7 (11–45)</td>
</tr>
<tr>
<td>Female</td>
<td>34.3 ± 8.3 (25–41)</td>
</tr>
<tr>
<td>Initial presentation</td>
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<tr>
<td>Delayed motor development (n)</td>
<td>2</td>
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<tr>
<td>Delayed language development (n)</td>
<td>10</td>
</tr>
<tr>
<td>Birth history</td>
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<tr>
<td>Gestational age (wk)</td>
<td>39.5 ± 1.2</td>
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<tr>
<td>Birth weight (kg)</td>
<td>3.6 ± 0.44</td>
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<tr>
<td>Developmental history</td>
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<tr>
<td>Age of independent walking (mo)</td>
<td>Male 16.9 ± 3.0 (15–24)</td>
</tr>
<tr>
<td></td>
<td>Female 14.0 ± 3.6 (11–18)</td>
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<tr>
<td>Maternal FX test</td>
<td>6</td>
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<tr>
<td>Family history of FXS</td>
<td>6 (2 family)</td>
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</table>

Values are presented as mean ± standard deviation (range).

FX, fragile X; FXS, fragile X syndrome.

Table 2. Molecular diagnostic findings and results of psychological testing for all 14 patients evaluated in this study

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (yr)</th>
<th>Sex (CGG)</th>
<th>Maternal (CGG)</th>
<th>Walking alone</th>
<th>Age (yr)</th>
<th>MDI</th>
<th>PDI</th>
<th>SO</th>
<th>L-R</th>
<th>L-E</th>
<th>CARS</th>
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<tr>
<td>1</td>
<td>11</td>
<td>M</td>
<td>342</td>
<td>24/98</td>
<td>24</td>
<td>22</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>49.5</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>M</td>
<td>&gt;450</td>
<td>NA</td>
<td>18</td>
<td>40</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>55</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>M</td>
<td>209</td>
<td>30/84</td>
<td>15</td>
<td>20</td>
<td>50</td>
<td>60</td>
<td>65</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>M</td>
<td>353</td>
<td>31/86</td>
<td>15</td>
<td>25</td>
<td>&lt;50</td>
<td>65</td>
<td>64</td>
<td>9</td>
<td>8</td>
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<td>5</td>
<td>25</td>
<td>M</td>
<td>274</td>
<td>NA</td>
<td>15</td>
<td>27</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>65.1</td>
<td>14</td>
<td>10</td>
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<tr>
<td>6</td>
<td>25</td>
<td>M</td>
<td>275</td>
<td>30/88</td>
<td>15</td>
<td>27</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>54.1</td>
<td>13</td>
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<td>7</td>
<td>25</td>
<td>M</td>
<td>278</td>
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<td>17</td>
<td>26</td>
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<td>&lt;50</td>
<td>61.6</td>
<td>10</td>
<td>9</td>
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<td>8</td>
<td>32</td>
<td>M</td>
<td>294</td>
<td>29/92</td>
<td>18</td>
<td>33</td>
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<td>&lt;50</td>
<td>65.7</td>
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<td>M</td>
<td>284</td>
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<td>48</td>
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<td>&lt;50</td>
<td>69.4</td>
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<td>25</td>
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<tr>
<td>10</td>
<td>41</td>
<td>F</td>
<td>26/271</td>
<td>30/88</td>
<td>13</td>
<td>43</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>67.94</td>
<td>15</td>
<td>16</td>
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<td>11</td>
<td>37</td>
<td>F</td>
<td>288/288</td>
<td>30/65</td>
<td>18</td>
<td>38</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>68.2</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>12</td>
<td>25</td>
<td>F</td>
<td>30/282</td>
<td>30/65</td>
<td>11</td>
<td>27</td>
<td>62</td>
<td>65</td>
<td>88</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>13</td>
<td>7</td>
<td>F</td>
<td>31/89</td>
<td>30/65</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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</tr>
<tr>
<td>14</td>
<td>46</td>
<td>M</td>
<td>52</td>
<td>NA</td>
<td>16</td>
<td>47</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>66.2</td>
<td>24</td>
<td>24</td>
</tr>
</tbody>
</table>

CGG, cytosine-guanine-guanine trinucleotide; MDI, mental development index; PDI, psychomotor development index; SO, social maturity quotient; L-R, language-receptive; L-E, language-expressive; CARS, Childhood Autism Rating Scale; NA, not available.

| 'Age (months) at initial visit,' | 'Age (months) of walking alone,' | 'Age (months) at psychological testing,' | 'L-R, L-E as developmental age (months).' |

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findings in some children at their initial visit. The girl with only FM alleles (288 and 288 repeats) (patient 11) presented with FXS features including a prominent forehead, large ears, and significant GDD with inattentive, hyperactive behavior. She began to walk independently at approximately 18 months of age. The other two girls who presented with one FM and one normal allele (patients 10 and 12) did not show any physical features of FXS. These girls reached gross motor developmental milestones on time. They began to walk independently at approximately 12 months. However, their language development was delayed, but the degree of the delay in language development was less severe than that of the boys with FM.

The girl with PM (31 and 99 repeats) (patient 13) did not show any developmental delays. She was tested at 7 months because her two older sisters both presented with an FM allele. The boy with an IM allele (52 repeats) (patient 14) demonstrated a prominent forehead, large ears, and significant GDD. His clinical presentation was indistinguishable from that of the boys with FM. However, this patient was lost to follow-up and therefore we could not evaluate other factors contributing to his development.

The nine boys with FM and the girl with only FM alleles all had significant GDD with an MDI of < 70, PDI of < 70, SQ of < 70, and markedly delayed language development. Among the 12 children, there was one boy with a CARS score > 30. Increased severity of autistic behavior, as measured by CARS, was strongly associated with more severe delays across all domains, particularly in the communication and fine motor domains [13]. Even after 2 years of age, the boys with FM could only say two to three meaningful words at best. They were inattentive and hyperactive, had difficulty remaining still, and were unaware of danger. They were highly dependent on adults and anxious about being separated from their parents. Even after 3 years of age, they communicated using single words or simple phrases, and toilet training had not been completely established. The girls with one FM allele (patients 10 and 12) showed delayed language development, but the degree of the delay was less severe than that of the boys with FM.

Discussion

FXS is found in many populations across the world; however, there is wide variation in its prevalence based on differences in the study subjects and diagnostic methods. This makes global prevalence estimates difficult. The prevalence of FXS is estimated to be ~1/4,000 to 1/7,000 in males, and ~1/6,000 to 1/11,000 in females. In addition, approximately 1/500 to 1/800 males and 1/200 to 1/300 females are PM carriers [14]. Similarly, the exact prevalence of FXS and PM carriers in the Korean population has not been well established. Based on the results of studies on preconception or pregnant women, the prevalence of PM carriers is estimated to be around 1/700 to 1/800 Korean women, which is lower than that of Western populations and higher than that of other Asian populations [4,15,16]. Several studies in Korea have shown that approximately 1% to 3% of all children with GDD/ID or ASD are found to have FXS [17-21]. Hong et al. [17] reported a 1.6% prevalence of FXS in children with pervasive developmental disorders. Han et al. [21] reported a 3% prevalence of FXS in children with GDD/ID. However, there are no studies reporting the prevalence of FXS in children under 5 years of age in Korea.

In this study, there were 14 children (10 boys and four girls) with abnormal FX test results over a 13-year evaluation period. The mean age of the nine boys with FM at the initial visit was 24.8 ± 9.7 months (range, 11 to 45). Only one boy visited our clinic before the age of 12 months. All the boys with FM and the girl with only FM alleles presented with significant GDD involving difficulties with attention and inhibition control. It has been reported that boys with FXS are more inattentive, overactive, and impulsive than boys with other types of ID [22]. In boys with FXS, delayed motor and language development manifests in the first several years of life. A longitudinal study of boys with FXS (range, 24 to 72) reported that their overall development was significantly delayed, and that their rate of development was approximately half that of age-matched peers. Deficits in nonverbal communication and cognition have also been reported [23].

In this study, there were two girls with an FM allele and a normal allele, who did not show any typical features of FXS or delayed gross motor development. However, they could not reach language developmental milestones on time. There is considerable variability in FXS symptoms. Individuals producing higher levels of FMRP are typically less affected. Females with FM have a higher degree of variability in their FMRP production due to compensation by the second X chromosome with a healthy FMR1 gene, individual differences in inactivation of the fragile X chromosome, or the presence of somatic mosaicism. Individuals presenting with mosaicism are likely to produce more FMRP. Both repeat-size mosaicism (e.g., FM/PM, FM/IM) and methylation mosaicism have been described [3]. Therefore, confirmation of methylation status should be complemented with analysis of the repeat number of CGG of the FMR1 gene.

There was an unusual case of a 3-year-old girl with typical FXS features, as a result of homozgyosity for the FM allele of the FMR1 gene (288/288 CGG repeats), resulting from maternal UPD of the entire X chromosome. Her mother, who was found to be a heterozygous carrier of PM (30/65 CGG repeats), had no clinical phenotype. The mother’s PM was transmitted to her offspring with
expansion to FM. UPD is a genetic condition in which two homologous chromosomes or chromosomal regions/segments are inherited from only one parent, and not from both parents [24]. Thus, UPD should be considered a possible cause in female patients with unexplained severe manifestations of GDD/ID.

Those with the PM allele do not have the phenotype of ID, but often have adult-onset phenotypes, including fragile X-associated primary ovarian insufficiency before age 40, as seen in approximately 20% of female carriers. In addition, fragile X-associated tremor/ataxia syndrome is observed in approximately 40% of male and 16% of female cases with the PM allele.

This is related to excessive FMR1 gene activity and the deleterious consequences of elevated production of FMR1 mRNA, leading to anxiety, attention deficit-hyperactivity, and social deficits. Approximately 10% of males with the PM allele meet the diagnostic criteria for ASD, which is usually recognized in childhood [3].

Despite delayed attainment of developmental milestones, the absence of malformations, significant medical problems, or characteristic physical features has traditionally caused FXS to be diagnosed later in childhood. The average age of diagnosis for FXS has been reported to be around 36 months of age for boys and rather later for girls [7]. FXS has a significant impact on individuals and their families; however, the diagnosis is difficult due to its subtle clinical features.

Despite the advent of CMA, next-generation sequencing testing expands the understanding of genetic causes of GDD/ID or ASD, and we therefore suggest that physicians should consider the FX PCR test as a part of first-line testing in children with unexplained GDD/ID or ASD, despite a low diagnostic yield, to prevent underdiagnosis of this disorder, even in the absence of a family history. If there is a family history of FXS, FX testing should be screened in all family members, especially on the maternal side.

This study has several limitations. First, we did not perform FX testing on all the children who presented with GDD. Therefore, our study may have underdiagnosed the number of FXS children as we excluded those without clinical suspicion. Second, our abnormal FX genetic test results included a patient with IM alleles who could not be assessed for other diseases that could cause GDD, due to follow-up loss. Third, this was a retrospective chart review study, increasing the possibility of research bias in these results. Although our study is limited by size and researcher bias, our findings will be useful for the early diagnosis of FXS.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

References


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Author contribution

Conceptualization: JKK. Data curation: SHL and JEJ. Formal analysis: SHL, JEJ, and YYJ. Methodology: YYJ. Writing-original draft: SHL. Writing-review & editing: JKK.


Clinical Value of Magnetic Resonance Spectroscopy in the Initial Evaluation of Patients with Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-Like Episodes

Hyunjoo Lee, MD, Je Hee Shin, MD, Ji-Hoon Na, MD, Young-Mock Lee, MD
Department of Pediatrics, Yonsei University College of Medicine, Seoul, Korea

Purpose: Magnetic resonance spectroscopy (MRS) is a diagnostic tool used to detect abnormal accumulation of lactate in the brain parenchyma in various metabolic diseases. This study evaluated the clinical roles of brain MRS in the initial assessment of mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) caused by impairment of the mitochondrial respiratory chain.

Methods: Twenty-five patients with the A3243G mutation among 34 MELAS patients referred to the pediatric neurology clinic of Gangnam Severance Hospital between January 2006 and December 2020 were included. In this retrospective study, demographic, clinical, laboratory (serum lactate and lactate-to-pyruvate ratio), magnetic resonance imaging (MRI), and initial MRS (presence of lactate peak and abnormal N-acetylaspartate [NAA]) data were reviewed.

Results: Brain MRI showed cortical lesions in 24 of 25 genetically confirmed A3243G MELAS patients with neurologic symptoms in this study. On MRS, 18 patients (72%) had increased lactate peaks, depicting anaerobic energy metabolism, and 17 patients (68%) had decreased NAA levels, indicating neuronal integrity. Ten patients underwent MRS in the acute stage (within 2 weeks of symptoms). Unlike patients who underwent MRS more than 2 weeks after symptom onset, a lactate peak on MRS was observed in all patients in the acute stage ($P=0.011$).

Conclusion: Elevated lactate peaks in acute cerebral infarctions are highly suggestive of mitochondrial encephalopathy. MRS alone is insufficient to diagnose MELAS, but it is valuable as a noninvasive supplemental diagnostic tool in combination with genetic testing.

Keywords: Magnetic resonance spectroscopy; Mitochondrial encephalomyopathies; MELAS syndrome; Mitochondrial diseases; Acidosis, lactic

Introduction

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome is a rare neurodegenerative disorder caused by the impairment of the mitochondrial respiratory chain. Approximately 80% of cases have an A to G mutation at nucleotide 3243 (A3243G) in the mitochondrial DNA molecule (MT-TL1), and heteroplasmy causes different mutational burdens within tis-
sues, resulting in diverse clinical phenotypes. Patients usually present with stroke-like episodes (hemianopia, cortical blindness, and dysarthria), seizures, recurrent headaches, or muscle weakness. Elevated lactate levels in blood and cerebrospinal fluid (CSF) and a change in the lactate-to-pyruvate ratio indicate respiratory chain dysfunction [1-3]. Typical magnetic resonance imaging (MRI) findings in an acute stroke-like episode of MELAS include cortical lesions. Magnetic resonance spectroscopy (MRS) is a noninvasive method that reveals metabolic information regarding brain tissue. Increased lactate levels reflect anaerobic glycolysis, and N-acetylaspartate (NAA) is a marker of neuronal integrity that represents neuronal injury associated with infarction. MRS has been used to diagnose stroke-like episodes in MELAS, with elevated lactate peaks and low NAA. Several researchers have previously reported that MRS is a useful tool for monitoring the progress of disease in patients with mitochondrial disorders [4-7]. This study aimed to evaluate the role of MRS in the initial assessment of MELAS.

Materials and Methods

The data of MELAS patients who were admitted to the pediatric neurology department at Gangnam Severance Hospital (Seoul, Korea) between January 2006 and December 2020 were reviewed. In total, 34 patients had findings compatible with the MELAS clinical diagnostic criteria, as enumerated by Bernier et al. [8]. This was a retrospective study of patients with MELAS who met the following inclusion criteria: genetically confirmed diagnosis of a pathogenic variant associated with MELAS (MT-TL1, m.3243A>G) and MRS performed at our center. Mutation carriers without neurological symptoms were excluded.

To identify the A3243G mutation, polymerase chain reaction amplification and restriction fragment length polymorphism analysis of total DNA extracted from leukocytes were performed. Based on this analysis, 25 patients with pathogenic mutations were included among the 34 symptomatic patients in this study. Demographic, clinical, laboratory, and MRI data were reviewed. This study was approved by the Institutional Review Board of the Gangnam Severance Hospital, Yonsei University College of Medicine (3-2017-0168). Informed consent for this retrospective study was waived by the board.

1. Mitochondrial characteristics of MELAS with A3243G

The laboratory tests consisted of blood lactate level (normal < 2 mmol/L) and the lactate-to-pyruvate ratio (normal < 25). Muscle biopsy samples were obtained from some patients and processed through routine morphological and histochemical staining, including periodic acid-Schiff, modified Gomori trichrome, ATPase 9.4, nicotinamide adenine dinucleotide tetrazolium reductase, and succinate dehydrogenase stains. All samples were examined for changes such as pleoconia and megaconia using electron microscopy. We investigated the initial MRS data to diagnose mitochondrial disease. MRS was performed on a GE 750 W 3.0T scanner (GE Medical Systems, Milwaukee, WI, USA) with a 16-channel head-neck combined coil including T2-weighted sequences to obtain lactate at 1.3 ppm and NAA at 2.02 ppm. The voxels were placed in the frontal and occipital gray matter and basal ganglia of the bilateral hemispheres.

The clinical severity of the patients was graded as follows: mild, self-ambulatory, with or without independence for daily activities; moderate, full-time wheelchair-bound, or partially dependent for daily activities, with the ability to engage in brief communication; and severe, bedridden, totally dependent for daily activities, or dead. All analyses were conducted using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Categorical variables were compared using the chi-square test and the Fisher exact test. Differences were considered statistically significant at a P value of < 0.05.

Results

1. Demographics and mitochondrial characteristics of patients with A3243G MELAS

To evaluate the role of MRS in the initial evaluation process for the diagnosis of MELAS, this study adopted a retrospective design involving a total of 25 patients (13 boys/men and 12 girls/women) who presented with neurological symptoms and in whom a genetic analysis identified the pathogenic variant A3243G in MT-TL1 (Table 1). The median age upon first clinical presentation was 13.3 years (range, 0.2 to 45.6), and the median time interval from the first clinical presentation to the diagnosis of MELAS was 4.8 months. The first symptoms of the disease were seizures (36%), visual impairment (28%), and motor weakness (18%). All patients exhibited neurological symptoms, followed by symptoms involving the orbital, endocrine, cardiac, auditory, psychological, myocardial, gastrointestinal, and renal systems.

The median serum lactate level was 4.3 mmol/L (range, 0.9 to 13.5), and 92% of patients had a value of 2 mmol/L or higher. An elevated lactate-to-pyruvate ratio was found in only 52% of the patients (Table 2).

2. MRI findings in patients with A3243G MELAS

MRI studies performed to assess acute neurologic episodes identified cortical lesions in 24 (96%) of the 25 patients. Seventeen patients (68%) showed old infarctions with volume loss and diffuse cerebral atrophy, and 16 patients (64%) demonstrated diffuse cere-
bellar atrophy. Nine patients (36%) had lesions in the basal ganglia, and two patients (8%) had lesions in the thalamus.

3. MRS

Remarkably, the MRS results did not show statistically significant differences between patients in whom MELAS was genetically confirmed and those without genetic confirmation (Table 3). MRS alone was insufficient for identifying patients with MELAS as an initial diagnostic marker.

Increased lactate peaks were observed in 18 of 25 MRS studies (72%), and decreased NAA peaks were observed in 17 (68%) (Table 2). The acute stage was defined as clinical staging within the first 2 weeks of neurologic symptoms [9,10]. MRS studies were performed in the acute stage in 10 of the 25 patients with A3243G after neurologic episodes (seizures in three patients, visual disturbances in three patients, confusion in two patients, motor weakness in one patient, and headache in one patient). Fifteen of the 25 initial MRS studies were performed to screen patients with mitochondrial disease who did not show acute neurologic deterioration. Elevated lactate peaks were found in all 10 genetically con-

### Table 1. Clinical characteristics of MELAS (A3243G) patients (n=25)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male:female)</td>
<td>0.55</td>
</tr>
<tr>
<td>Age at first clinical presentations (yr)</td>
<td>14.2 ± 10.9</td>
</tr>
<tr>
<td>Age at diagnosis of MELAS (yr)</td>
<td>16.7 ± 10.6</td>
</tr>
<tr>
<td>Time interval from first clinical presentation to the diagnosis of MELAS (yr)</td>
<td>0.9 ± 1.3</td>
</tr>
<tr>
<td>Familial history of mitochondrial disease</td>
<td>4 (16)</td>
</tr>
<tr>
<td>Presentation symptoms at disease onset</td>
<td></td>
</tr>
<tr>
<td>Seizure</td>
<td>9 (36)</td>
</tr>
<tr>
<td>Visual disturbance</td>
<td>7 (28)</td>
</tr>
<tr>
<td>Motor weakness</td>
<td>4 (16)</td>
</tr>
<tr>
<td>Headache</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Loss of consciousness</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Delayed development</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Organ involvement</td>
<td></td>
</tr>
<tr>
<td>Central nervous system</td>
<td>25 (100)</td>
</tr>
<tr>
<td>Endocrinologic</td>
<td>16 (64)</td>
</tr>
<tr>
<td>Hearing</td>
<td>15 (60)</td>
</tr>
<tr>
<td>Myopathy</td>
<td>14 (56)</td>
</tr>
<tr>
<td>Eye</td>
<td>13 (52)</td>
</tr>
<tr>
<td>Cardiologic</td>
<td>13 (52)</td>
</tr>
<tr>
<td>Psychological</td>
<td>11 (44)</td>
</tr>
<tr>
<td>Gastrointestinal system</td>
<td>9 (36)</td>
</tr>
<tr>
<td>Renal system</td>
<td>8 (32)</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation or number (%). MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes.

### Table 2. Mitochondrial characteristics of MELAS (A3243G) patients (n=25)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum lactate, initial (mmol/L)</td>
<td>4.3 (0.9–13.5)</td>
</tr>
<tr>
<td>Lactic acidosis (&gt; 2 mmol/L)</td>
<td>23 (92)</td>
</tr>
<tr>
<td>Serum lactate-to-pyruvate ratio, initial</td>
<td>27.5 (11.3–52.8)</td>
</tr>
<tr>
<td>Increased (&gt; 25)</td>
<td>13 (52)</td>
</tr>
<tr>
<td>Magnetic resonance imaging</td>
<td></td>
</tr>
<tr>
<td>Infarction</td>
<td>24 (96)</td>
</tr>
<tr>
<td>Cortex signal abnormality</td>
<td>24 (96)</td>
</tr>
<tr>
<td>Diffuse cerebral atrophy</td>
<td>17 (68)</td>
</tr>
<tr>
<td>Cerebellar atrophy</td>
<td>16 (64)</td>
</tr>
<tr>
<td>Basal ganglia signal abnormality</td>
<td>9 (36)</td>
</tr>
<tr>
<td>Thalamus signal abnormality</td>
<td>2 (8)</td>
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<td>White matter signal abnormality</td>
<td>19 (76)</td>
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<tr>
<td>Magnetic resonance spectroscopy</td>
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<td>Increased lactate peak</td>
<td>18 (72)</td>
</tr>
<tr>
<td>Decreased NAA</td>
<td>17 (68)</td>
</tr>
<tr>
<td>Muscle biopsy obtained (n = 7)</td>
<td>7 (28)</td>
</tr>
<tr>
<td>Light microscopic changes (+)</td>
<td>3 (43)</td>
</tr>
<tr>
<td>Electron microscopic changes (+)</td>
<td>2 (29)</td>
</tr>
<tr>
<td>Clinical severity</td>
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<tr>
<td>Mild</td>
<td>15 (60)</td>
</tr>
<tr>
<td>Moderate</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Severe</td>
<td>4 (16)</td>
</tr>
<tr>
<td>Death</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Delayed development or mental retardation</td>
<td>17 (85)</td>
</tr>
<tr>
<td>Regression/deterioration</td>
<td>13 (52)</td>
</tr>
<tr>
<td>Oxygen dependency</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Enteral tube feeding</td>
<td>4 (16)</td>
</tr>
</tbody>
</table>

Values are presented as median (interquartile range) or number (%). MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; NAA, N-acetylaspartate.

### Table 3. Magnetic resonance spectroscopy findings in MELAS patients

<table>
<thead>
<tr>
<th>A3243G mutation (+)</th>
<th>A3243G mutation (-)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased lactate peak (+)</td>
<td>18/25</td>
<td>4/9</td>
</tr>
<tr>
<td>Decreased NAA peak (+)</td>
<td>17/25</td>
<td>5/9</td>
</tr>
</tbody>
</table>

MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; NAA, N-acetylaspartate.
Increased lactate peaks shown on magnetic resonance spectroscopy in mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS; A3243G) patients (n=25).

Discussion

In this study, we evaluated the role of MRS in the initial diagnosis of MELAS. Most initial MELAS evaluations begin with a series of biochemical studies of blood, urine, and CSF. Elevated lactate levels have long been associated with mitochondrial diseases. False elevations of plasma lactate are common because of improper specimen collection and handling. An elevated lactate-to-pyruvate ratio provides a more useful tool than serum lactate values for indicating respiratory chain dysfunction. CSF lactate levels are not influenced by the collection technique and are elevated in mitochondrial disorder patients with predominant brain manifestations.

Suomalainen et al. [11] reviewed the sensitivity (52%) and specificity (92%) of lactate measurements in patients with genetically confirmed mitochondrial disease. Blood lactate levels can be normal in MELAS patients; however, even in the presence of normal serum lactate, the CSF lactate level may be a more reliable diagnostic marker for MELAS.

Tsujikawa et al. [12] reported that the concentration of CSF lactate was closely correlated with that of brain tissue lactate obtained via MRS. Elevations of brain lactate levels are generally indicative of increased anaerobic glycolytic rates. NAA reduction reflects neuronal injury and impaired mitochondrial dysfunction after recurrent stroke-like episodes.

Previous studies have reported that brain metabolites could be evaluated using MRS to determine disease progression and perform treatment monitoring in patients with mitochondrial disorders [5,13]. Heteroplasmy results in a variety of clinical phenotypes, from asymptomatic carriers to fully symptomatic patients, as well as different mutational burdens between and within tissues. Weidusch et al. [7] recently reported that metabolic abnormalities detected by MRS in asymptomatic A3243G carriers have potential clinical value as biomarkers of disease progression and therapeutic response. A recent study comparing the efficacy of MRS after acute neurologic symptoms in patients with acute ischemic stroke (AIS) and patients with MELAS found a lactic acid peak in only 69.2% of AIS patients, whereas a lactic acid peak was found in all patients with MELAS [14,15]. Therefore, MRS findings can reflect a lack of energy production due to impaired oxidative phosphorylation caused by nonspecific ischemia and hypoxia in the acute phase, and the relatively high sensitivity of MRS makes it a useful tool for the diagnosis of MELAS patients.

This study aimed to evaluate the clinical value of non-invasive MRS to detect brain metabolites in the initial diagnosis of genetically confirmed A3243G MELAS patients. Among 34 MELAS cases, no significant difference was found for increased lactate (P > 0.05) or decreased NAA peaks (P > 0.05) in MRS between the groups with and without genetic confirmation. Twenty-five of the 34 patients were diagnosed with genetically confirmed MELAS with the A3243G pathogenic mutation. Only 18 (72%) of those 25 patients had a lactate peak on initial MRS.

The detection of a lactate peak on MRS is dependent on the timing or severity of the disease. In the 25 genetically confirmed MELAS patients, the timing of the initial MRS test was divided into groups according to whether MRS was performed in the acute stage, despite the limited number of samples, and lactic acid peaks were found in all MRS data performed within 2 weeks after the first neurologic symptoms. In this study, the neurological symptoms of MELAS most commonly presented as stroke-like episodes, and elevated lactate peaks in MRS were identified in all MELAS patients in the acute stage with or without serum lactic acidosis. Elevated lactate peaks in initial MRS were not identified in patients with resolving first stroke-like symptoms, who showed symptoms at least 28 days before MRS. However, elevated lactate peaks on MRS and severe diffuse cerebral atrophy on MRI were shown in eight of 15 patients who underwent MRS more than 2 weeks after initial onset, and those patients had at least two recurrent stroke-like episodes despite the current absence of acute symptoms. Decreased NAA peaks were found in 17 of the 25 MELAS patients. The decreasing trend in NAA concentration reflects brain damage due to recurrent stroke-like attacks during prolonged periods with or without acute symptoms.

In conclusion, although initial MRS alone is not enough to definitively diagnose MELAS, it is worthwhile as a non-invasive complementary diagnostic tool along with genetic testing, as well as for monitoring the disease progress of MELAS.

Conflicts of interest

Young-Mock Lee is an editorial board member of the journal, but he was not involved in the peer reviewer selection, evaluation, or
decision process of this article. No other potential conflicts of interest relevant to this article were reported.

**Author contribution**

Conceptualization: HL and YML. Data curation: HL and JHS. Formal analysis: HL and JHN. Methodology: YML. Project administration: JHS and YML. Visualization: HL and JHN. Writing-original draft: HL. Writing-review & editing: HL and YML.

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**References**


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Jacobsen syndrome is a rare genetic disorder caused by partial deletions in the long arm of chromosome 11. The deletion size ranges from 7 to 20 Mb, with a breakpoint at 11q23.3, in 70% to 80% of cases. Affected individuals can express a wide variety of phenotypes, including delayed physical growth and psychomotor development, dysmorphic features, congenital heart malformations, and thrombocytopenia [1,2]. Few reports have described white matter abnormalities in Jacobsen syndrome. Herein, we describe a case of a boy diagnosed with Jacobsen syndrome with white matter abnormalities on brain magnetic resonance imaging (MRI). This study was reviewed and approved by the Institutional Review Board of the Gachon University Gil Hospital (GFIRB 2020-458). Due to its retrospective nature, the study was exempt from requiring informed consent from the participants.

A 6-day-old neonate was brought to our institution because of jaundice. He was born at 37 weeks of gestation without perinatal asphyxia. His birth weight was 2,980 g (22nd percentile), height was 46 cm (2nd percentile), and head circumference was 33 cm (13th percentile). Physical examination revealed a cephalohematoma. Laboratory tests showed hyperbilirubinemia, hypothyroidism, neutropenia, and thrombocytopenia. The total bilirubin level was 25.5 mg/dL. The white blood cell count was 2,070/μL, hemoglobin was 10.7 g/dL, and platelet count was 9,000/μL. During 1 month of hospitalization, thrombocytopenia persisted despite treatment with intravenous immunoglobulin, steroids, and transfusion. After discharge, the patient’s blood cell counts were regularly monitored through follow-up. A chromosomal study was not performed at the time.

He visited our neurology clinic because of developmental delay at 2 years of age. As he grew up, his height remained below the 3rd percentile. At 2 years of age, his height was 81 cm (2nd percentile), weight was 11 kg (19th percentile), and head circumference was 47 cm (18th percentile). He had a dysmorphic face, including a high prominent forehead, down-slanting palpebral fissures, short nose, flat nasal bridge, and thin upper lip. He could speak only the word “mama” unclearly. He started walking at 19 months of age. The Bayley scale was used to assess his degree of development at 25 months of age, and showed that his cognition and motor skills were at the 1st percentile, whereas language was below the 0.1 percentile.

An assessment of language development via the Sequenced Language Scale for Infants, conducted at 25 months of age, showed that the patient’s language development was equivalent to that of a 6-month-old child.

Brain MRI performed at 24 months of age showed focal and patchy T2 high-signal-intensity lesions in the bilateral frontal and parietal sub-
Cortical white matter (Fig. 1). Electroencephalography revealed no abnormalities. Echocardiography revealed a small atrial septal defect (secundum type). Abdominal ultrasonography showed no visceral malformations including the liver, kidney, and intestines. Ophthalmologic evaluations and hearing tests were normal.

Chromosomal microarray analysis confirmed a 12.6-Mb deletion in 11q24.1q25, which resulted in the diagnosis of Jacobsen syndrome. Therefore, we could conclude that Jacobsen syndrome was the reason for the patient’s developmental delay, intellectual disability, and physical growth retardation due to the deletions in chromosome 11.

Currently, at the age of 4 years and 10 months, his height is 102 cm (6th percentile), body weight is 19.4 kg (60th percentile), and head circumference is 50.1 cm (25th percentile). He can speak about five words, including “mama” and “papa.” According to the most recent laboratory tests, his white blood cell count was 4,500/μL, hemoglobin was 11.9 g/dL, and platelet count was 119,000/μL.

Jacobsen syndrome is caused by partial deletion of genes on the long arm of chromosome 11 [1]. Its prevalence has been estimated at 1/100,000 births, with a female-to-male ratio of 2:1 [3]. Common clinical features of Jacobsen syndrome include physical growth retardation, psychomotor retardation, facial dysmorphism, and thrombocytopenia or pancytopenia [3]. Some patients have visceral malformations involving the heart, kidney, gastrointestinal tract, genitalia, or central nervous system. Ocular, hearing, immunological, and hormonal problems may also occur [1,2]. During the neonatal period, most patients are hospitalized for prolonged periods due to feeding difficulties, cardiac problems, or bleeding diathesis [3]. Our patient had a prolonged hospitalization due to thrombocytopenia. He had growth retardation, psychomotor retardation, facial dysmorphism, thrombocytopenia, neutropenia, and white matter changes on brain MRI. The characteristic clinical aspects and chromosomal analysis led to the diagnosis.

Approximately 65% of patients with Jacobsen syndrome have a structural abnormality of the brain such as ventriculomegaly, cerebral atrophy, agenesis of the corpus callosum, or pachygyria [3]. Cerebral white matter abnormalities in Jacobsen syndrome have also been reported, but detailed data regarding the pathophysiology, radiological characteristics, and clinical course are lacking.

White matter abnormalities in Jacobsen syndrome are thought to be associated with the deletion of HEPACAM located on 11q24.2, which causes white matter water retention and intra-mylelinic edema [4]. HEPACAM is the causative gene in megalencephalic leukoencephalopathy with subcortical cysts (MLC) type 2A and 2B. MLC is characterized by chronic white matter edema, macrocephaly, and subcortical cysts [5]. Although Jacobsen syndrome does not present other features of MLC except white matter edema, HEPACAM haploinsufficiency in Jacobsen syndrome might have a potential role in the pathogenesis of white matter changes. Fujino et al. [5] documented serial white matter changes from the neonatal period to young childhood. Brain MRI taken before the age of 2 years shows more extensive or diffuse white matter changes. In contrast, after 2 years of age, white matter changes are mostly mild or partial [5]. White matter abnormalities and progression of myelination improve over time.

In conclusion, it should be noted that when clinical features suggest the presence of Jacobsen syndrome, the patient may also have nonspecific white matter abnormalities on brain MRI. Awareness of the presentation of Jacobsen syndrome on brain MRI is important to prevent unnecessary evaluation.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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Conceptualization: HJK. Data curation: SA, ISJ, DWS, KJA, KIL, and HJK. Formal analysis: SA and HJK. Methodology: SA, ISJ, DWS, KJA, KIL, and HJK. Project administration: HJK. Visualization: SA and HJK. Writing-original draft: SA. Writing-review & editing: HJK.
References


Instructions to authors

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Observations pertaining to the results of research and other related materials should be interpreted for your readers. Emphasize new and important observations; do not merely repeat the contents of the results. Explain the meaning of the observed opinion along with its limits, and within the limits of the research results connect the conclusion to the purpose of the research. In a concluding paragraph, summarize the result and its meaning.

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Examples of reference styles

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The relationship between initial body mass index and body
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agonist therapy in idiopathic true precocious puberty girls. J
2. Wheless JW, Treiman DM. The role of the newer antiepileptic
drugs in the treatment of generalized convulsive status
epilepticus. Epilepsia 2008;49 Suppl 9:74-8
Psychopharmacol 1988;8(4 Suppl):31S-37S.
4. Nikitovic M, Wodchis WP, Krahn MD, Cadarette SM. Direct
health-care costs attributed to hip fractures among seniors: a

2) Book
- Book
5. Volpe JJ. Neurology of the newborn. 2nd ed. Philadelphia, PA:
- Book chapter
6. Pan ES, Cole FS, Weintrub PS. Viral infections of the fetus
  and newborn. In: Taeusch HW, Ballard RA, Gleason CA,
editors. Avery’s diseases of the newborn. 8th ed. Philadelphia:
- Abstract book or conference proceedings
  community response. Proceedings of the First AMA National
  Conference on Child Abuse and Neglect; 1984 Mar 30-31;
- Thesis
8. Youssef NM. School adjustment of children with congenital
  heart disease (dissertation). Pittsburgh, PA: Univ. of
  Pittsburgh; 1988.

3) Website
  Third Korea National Health and Nutrition Examination
  Survey (KNHANES III) [Internet]. Seoul: Ministry for

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1) Each table should be inserted on a separate page, with the table
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   The first character should be capitalized.
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   in figures.
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6) Unnecessary longitudinal lines should not be drawn.
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트리렙탈은 NICE GUIDELINE에서 성인 및 소아부분 발작 환자에서 일차 단독요법 또는 보조요법으로 권고되고 있습니다.1

1) 자살충동과 자살행동 : 항전간제를 복용한 환자에서 자살충동 또는 자살행동을 보이는 위험성이 증가되므로 항전간제를 치료받은 환자는 자살충동 또는 자살행동, 우울증의 발현 또는 악화 및 기분과 행동의 비정상적 변화에 대하여 모니터링 되어야 한다. 항전간제를 처방받는 간질과 다른 많은 질병은 그 자체가 이환 및 사망, 치료기간 동안의 자살충동과 자살행동의 위험성 증가와 관련된다. 따라서, 처방자는 항전간제 처방시 환자의 치료기간 동안 자살충동 또는 자살행동과 치료될 질병간의 연관성 유무 및 이 약의 유효성을 함께 고려한다.

2) 저나트륨혈증 (hyponatremia) : 이 약의 치료 중 혈청 나트륨치의 감소가 나타날 수 있으므로 치료 전과 치료 개시 후 정기적으로 혈청 나트륨치를 측정해야 한다. 특히 이미 많은 수분 섭취가 필요한 신장애를 갖고 있거나, 혈청 나트륨치가 낮은 환자, 혈청 나트륨을 감소시킬 수 있는 약물(예: 이뇨제, 데스모프레신)을 투여하거나 NSAIDs(예: 인도메타신)로 치료받는 환자, 혹은 저나트륨 혈증의 증상(예: 구역, 권태, 두통, 졸음증, 혼란, 둔감 또는 발작 빈도 혹은 정도의 증가)을 보이는 환자에게는 특별한 주의가 요구된다. 3) 신부전, 간부전 또는 심부전 환자나 고령자에게는 이상반응의 위험성이 더 높아지므로 특별한 주의가 요구된다. 4) 카르바마제핀에 대해 알레르기가 있는 환자에게 사용할 경우 이러한 환자 중 약 25~30%에서 교차 알레르기 반응이 보고되었으므로 주의가 요망된다. 이는 두 약물의 구조적 유사성 때문인 것으로 추정된다. 5) 중대한 피부반응: 피부점막안증후군(스티븐스-존슨 증후군) 및 독성표피괴사용해(리엘증후군)를 포함하는 중대한 피부반응이 소아와 성인에서 이 약의 사용과 관련하여 보고되었다. 보고된 증례의 발현시간(중앙값)은 19일이었다. 이러한 중대한 피부반응은 생명을 위협할 수 있으며, 몇 명 환자는 치명적인 결과로 입원이 필요했던 보고가 매우 드물게 있었다. 이 약을 재투여하였을 때 중증의 피부반응의 재발 또한 보고되었다. 6) 모든 항전간제와 마찬가지로 이 약은 발작횟수의 증가 가능성을 최소화하기 위해 점차적으로 중단되어야 한다. 7) 모든 환자나 개발단계의 중증 및 중등도의 증상의 발생률이 증가할 수 있으므로 주의가 요구된다. 8) 고령자, 특히 신장 기능 장애가 있는 고령의 환자: 처방하시기 전, 상세 제품정보를 참조하시기 바랍니다.
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