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The role of inflammatory markers and calculated osmotic pressure in the classification of febrile seizures

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Abstract. – OBJECTIVE: Systemic inflammatory response may contribute to the onset of febrile seizures (FSs). The neutrophil-to-lymphocyte ratio (NLR) has been reported to be useful for differentiating simple and complex FSs in children with a first FS. This study aimed to determine whether easily measurable inflammatory markers were useful for distinguishing between the types of FSs in children with FSs not limited to the first FS.

PATIENTS AND METHODS: We conducted a retrospective study of children aged 6-60 months who were presented to the Atsugi City Hospital in Japan for the treatment of FSs between December 2018 and February 2020. A complex FS was defined as a seizure with multiple seizures during the same febrile illness, prolonged seizures and/or focal seizures. A simple FS was defined as a seizure without the characteristics of complex FS. We assessed complete blood count, C-reactive protein, and calculated osmotic pressure.

RESULTS: A total of 205 children with FSs (simple, 139; complex, 66) fulfilled the inclusion criteria. None of the inflammatory markers, including NLR, could predict the FS type. The median osmotic pressure was 279.0, 278.8, 283.3, and 278.3 mOsm/kg H_2O for children with simple, multiple, prolonged, and focal seizures, respectively. Children with prolonged seizures had a significantly higher calculated osmotic pressure than those with simple FSs ($p<0.001$) and multiple seizures during the same febrile illness ($p=0.004$).

CONCLUSIONS: Easily measurable inflammatory markers, including NLR, were not useful for distinguishing between types of FSs in children. Large multicenter studies are needed to evaluate the association between osmotic pressure and FS.

Key Words:
Febrile seizure, Inflammatory markers, Neutrophil-to-lymphocyte ratio, Osmotic pressure.

Introduction

Febrile seizures (FSs) are a common type of childhood seizures globally, affecting 2-11% of children[1,2]. There are two types of febrile seizures: simple and complex. A complex seizure is defined as a seizure with multiple seizure events during the same febrile illness, prolonged seizure lasting ≥15 minutes, and/or focal seizure[1,3,4]. A simple FS is defined as a seizure without the characteristics of a complex FS[1,3,4]. Complex FS represents 20-30% of all FSs[1,3] and has a higher risk of progressing to epilepsy[5].

The mechanisms of FSs are unknown. One hypothesis is that increasing inflammatory cytokines during fever, especially interleukin-1 beta, may contribute to the onset of FSs[6-9]. However, inflammatory cytokines are not routinely measured clinically. Therefore, in patients with simple and complex FSs, alternative inflammatory markers such as C-reactive protein and complete blood count (CBC), including neutrophil-to-lymphocyte ratio (NLR), red blood cell distribution width (RDW), mean platelet volume (MPV), and mean platelet volume/platelet count ratio (MPR). NLR, RDW, and MPV have been found to be significantly higher in children with complex FSs than in those with simple FSs[10-12]. However, previous studies only included children experiencing a first FS. In addition, Liu et al[12] excluded premature infants (age at birth <37 weeks and age under one year). Therefore, the aim of this study was to evaluate the utility of easily measurable inflammatory markers to distinguish between various types of FSs in children, not limited to the first FS. We compared children with simple and complex (overall) FSs. We also compared simple FSs with each type of complex FSs because of the possible differences

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in the pathophysiological mechanisms of the different types of complex FSs.

Patients and Methods

Patients and Study Design

We conducted a retrospective study at Atsugi City Hospital in Kanagawa, Japan. We included patients who visited the emergency department (ED) of our hospital for treatment of FSs between December 1, 2018, and February 29, 2020. FS was defined according to the criteria of the Japanese Society of Child Neurology as "a seizure accompanied by fever (body temperature ≥38.0°C), without central nervous system infection, that occurs in infants and children 6 through 60 months of age"[4]. A complex FS was defined as a seizure with multiple seizures during the same febrile illness, prolonged seizures (seizure lasting ≥15 minutes) and/or focal seizures[4,5]. A simple FS was defined as a seizure without the characteristics of complex FSs[4]. Children with epilepsy, gastroenteritis, chromosomal abnormalities, inborn errors of metabolism, perinatal abnormalities, delayed psychomotor development, hydrocephalus, brain tumor, intracranial hemorrhage, or history of intracranial surgery were excluded. In addition, children fulfilling any of the following criteria were excluded: diazepam administration before visiting the ED or on discharge; treatment of seizures with anticonvulsant drugs before getting blood samples for laboratory tests; and those without laboratory test results available.

As per the protocol of the Japanese Society of Child Neurology, routine electroencephalogram and cerebrospinal fluid analyses were not routinely performed[4]. We, therefore, used clinical symptoms and signs to exclude patients with central nervous system infections, such as meningitis and encephalitis. Additionally, patients were followed up using an outpatient service or telephone calls to ensure the well-being of the patients and that no symptoms suggestive of central nervous system infection were present. Otherwise, patients who did not visit or call the ED after FS onset were considered not to have central nervous system infections.

Variables and Data Collection

Children were categorized into simple and complex FSs groups. We compared the laboratory test results of the two groups. In addition, we compared the results of children with simple FSs and each complex FS type.

The time from the onset of fever to arrival at the ED, and the total number of past FSs were determined by interviewing the parents/caregivers.

Peripheral venous blood samples were collected in EDTA tubes during the ED visit and sent to the laboratory for testing. Results of the following laboratory tests were included in the analysis: CBC, including white blood cell count, hemoglobin (Hb), platelet count (PLT), neutrophils, lymphocytes, and monocytes counts and percentage, MPV, and RDW. The NLR was calculated by dividing the absolute neutrophil count by absolute lymphocyte count. Then, MPR was calculated by dividing the MPV by the PLT. We also assessed osmotic pressure because FSs have been shown to correlate with hyponatremia[13-17] and osmotic pressure[18]. Osmotic pressure was calculated using the formula: osmotic pressure (mOsm/kg H_2O) = sodium (mEq/L) × 2 + glucose (mg/dL)/18 + blood urea nitrogen (mg/dL)/2.8.

Statistical Analysis

Statistical analysis was performed using the Stata version 15.1 (StataCorp, College Station, TX, USA) software package. Categorical variables were expressed as frequencies and percentages. Continuous variables were expressed as medians and interquartile ranges (IQR). Comparisons between groups were made using the chi-square or Fisher's exact test for categorical variables and Mann-Whitney *U*-test for continuous variables. Statistical significance was set at $p < 0.05$.

For comparison of the simple and complex seizure groups, a two-step analysis was adopted. First, we compared simple and complex (overall) FSs. Second, we compared children with simple FSs to those with the different types of complex FSs (the different types of seizures were stratified into multiple seizures during the same febrile illness, prolonged seizures, and focal seizures) using Kruskal-Wallis tests with Bonferroni post hoc tests. $p<0.0083$ was considered statistically significant.

Ethical Approval and Informed Consent

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and with the ethical guidelines for epidemiological studies issued by the Ministry of Health, Labor and Welfare, Japan. This study

was approved by the Atsugi City Hospital Institutional Review Board (R2-04). Informed consent was not deemed necessary because the data were obtained retrospectively from the patient charts.

Results

Patients Characteristics

A total of 292 children with FSs were treated at the ED during the study period. Of these children, 205 children fulfilled the inclusion criteria. The remaining 87 patients were excluded (39 due to missing laboratory test results, 13 for diazepam administration before visiting the ED, 15 for diazepam administration on discharge, 14 for gastroenteritis, three for perinatal abnormality, one for clinically mild encephalitis/encephalopathy with a reversible splenial lesion, one for trisomy 21 with epilepsy, and one for hydrocephalus). None of the patients who fulfilled the inclusion criteria had acute symptomatic diseases that cause childhood seizures, such as bacterial meningitis or acute encephalitis/encephalopathy.

Of the 205 participants, 139 (67.8%) had simple FSs, and 66 had complex FSs, including 42 with multiple seizures during the same febrile illness, 18 with prolonged seizures, and six with focal seizures. The patient characteristics and the laboratory test results stratified by type of FSs (simple vs. complex) are shown in Table I. The characteristics of the different types of complex seizures are summarized in Table II.

Comparison of Laboratory Test Results of Children with Simple Versus Complex Febrile Seizures

There were no statistically significant differences in the laboratory test results between children with simple and complex FSs (Table I).

Comparison of Laboratory Test Results of Children with Different Seizure Types

The osmotic pressure differed significantly according to the seizure type (p <0.01) (Table II). Patients with prolonged FSs had a significantly higher osmotic pressure than those with simple FSs (p <0.001) and multiple seizures during the same febrile illness (p =0.004). However, none of the other laboratory variables differed significantly according to the seizure type.

Table I. Baseline characteristics of patients with simple and complex febrile seizures.

	Total (N = 205)	Simple (N = 139)	Complex (N = 66)	*p*-value
Sex, n (%)				0.60
Male	122 (59.5%)	81 (58.3%)	41 (62.1%)	
Female	83 (40.5%)	58 (41.7%)	25 (37.9%)	
Age (mo), median (IQR)	23 (17-36)	23 (17-36)	22 (15-33)	0.36
Number of previous FSs, n (%)				0.56
1	131 (64.2%)	90 (65.2%)	41 (62.1%)	
2	45 (22.1%)	28 (20.3%)	17 (25.8%)	
3	19 (9.3%)	14 (10.1%)	5 (7.6%)	
≥ 4	9 (4.4%)	6 (4.3%)	3 (4.5%)	
Duration from fever to seizure onset (hr), median (IQR)	10.1 (3.4-18.0)	8.9 (3.0-16.7)	12.1 (4.1-22.4)	0.21
White blood cells ($\times 10^3/\mu L$), median (IQR)	10.6 (7.3-13.6)	10.4 (7.3-13.8)	11.0 (7.2-13.6)	0.91
Hemoglobin (g/dL), median (IQR)	11.8 (11.3-12.3)	11.8 (11.3-12.3)	11.9 (11.3-12.3)	0.94
Platelets ($\times 10^4/\mu L$), median (IQR)	27.3 (22.2-32.9)	27.1 (21.9-33.2)	28.1 (23.1-32.9)	0.63
Neutrophils (%), median (IQR)	70.5 (62.1-78.1)	71.3 (63.2-78.4)	69.5 (58.8-78.0)	0.29
Lymphocytes (%), median (IQR)	19.0 (12.0-25.5)	18.5 (12.0-24.8)	19.4 (11.9-27.85)	0.37
Monocytes (%), median (IQR)	9.0 (6.3-11.5)	8.7 (6.0-11.1)	9.4 (7.0-12.1)	0.22
NLR, medial (IQR)	3.71 (2.49-6.33)	3.75 (2.63-6.58)	3.56 (2.11-6.27)	0.35
MPV (fL), median (IQR)	7.2 (6.7-7.7)	7.2 (6.7-7.7)	7.0 (6.7-7.6)	0.67
RDW (%), median (IQR)	13.9 (13.35-14.8)	13.8 (13.4-14.7)	14.1 (13.3-15.3)	0.27
MPR, median (IQR)	0.27 (0.21-0.33)	0.28 (0.21-0.33)	0.26 (0.21-0.31)	0.39
CRP (mg/dL), median (IQR)	0.57 (0.17-1.30)	0.68 (0.17-1.42)	0.37 (0.17-0.79)	0.07
Calculated osmotic pressure (mOsm/kg H_2O), median (IQR)	279.2 (275.4-282.7)	279.0 (275.6-281.8)	280.1 (275.4-283.7)	0.14

CRP, C-reactive protein; ED, emergency department; IQR, interquartile range; MPV, mean platelet volume/platelet count ratio; MPV, mean platelet volume; NLR, neutrophil-to-lymphocyte ratio; RDW, red blood cell distribution width.

Table II. Baseline characteristics of patients with simple and each type of complex febrile seizures.

	Simple (N = 139)	Complex (n = 66)			p-value
		Multiple seizures (N = 42)	Prolonged seizures (N = 18)	Focal seizure (N = 6)	
Sex, n (%)					0.76
Male	81 (58.3%)	25 (59.5%)	11 (61.1%)	5 (83.3%)	
Female	58 (41.7%)	17 (40.5%)	7 (38.9%)	1 (16.7%)	
Age (mo), median (IQR)	23 (17-36)	23 (15-33)	21 (17-42)	20.5 (12-28)	0.52
Number of previous FS, n (%)					0.69
1	90 (65.2%)	25 (59.5%)	12 (66.7%)	4 (66.7%)	
2	28 (20.3%)	12 (28.6%)	4 (22.2%)	1 (16.7%)	
≥ 3	20 (14.4%)	5 (11.9%)	2 (11.1%)	1 (16.7%)	
Time from onset of fever to arrival at the ED (hr), median (IQR)	8.9 (3.0-16.7)	14.3 (4.1-26.4)	5.3 (2.1-12.8)	13.0 (11.7-24.6)	0.14
White blood cells ($\times 10^3/\mu L$), median (IQR)	10.4 (7.3-13.8)	11.4 (7.1-13.6)	11.2 (8.3-13.7)	7.7 (6.4-9.7)	0.33
Hemoglobin (g/dL), median (IQR)	11.8 (11.3-12.3)	11.8 (11.2-12.3)	12.0 (11.4-12.2)	11.8 (10.4-12.9)	0.85
Platelets ($\times 10^4/\mu L$), median (IQR)	27.1 (21.9-33.2)	27.5 (23.1-31.6)	29.0 (22.5-33.5)	26.9 (23.7-31.0)	0.85
Neutrophils (%), median (IQR)	71.3 (63.2-78.4)	70.8 (62.3-78.0)	67.4 (49.6-78.8)	65.4 (57.0-74.1)	0.38
Lymphocytes (%), median (IQR)	18.5 (12.0-24.8)	19.0 (10.8-25.3)	21.7 (14.0-34.2)	23.9 (12.7-27.4)	0.30
Monocytes (%), median (IQR)	8.7 (6.0-11.1)	9.5 (7.2-11.7)	8.3 (6.5-12.8)	11.4 (9.6-12.4)	0.43
NLR, medial (IQR)	3.75 (2.63-6.58)	3.69 (2.43-7.00)	3.14 (1.48-5.71)	2.81 (2.08-5.83)	0.31
MPV (fL), median (IQR)	7.2 (6.7-7.7)	7.0 (6.7-7.5)	7.2 (6.9-7.6)	7.3 (6.9-7.9)	0.81
RDW (%), median (IQR)	13.8 (13.4-14.7)	14.1 (13.5-15.6)	14.1 (13.2-14.5)	14.8 (13.6-16.1)	0.27
MPR, median (IQR)	0.28 (0.21-0.33)	0.27 (0.21-0.31)	0.25 (0.20-0.31)	0.25 (0.25-0.30)	0.79
CRP (mg/dL), median (IQR)	0.68 (0.17-1.42)	0.49 (0.20-1.24)	0.37 (0.13-0.60)	0.19 (0.09-0.72)	0.12
Calculated osmotic pressure ($mOsm/kg\ H_2O$), median (IQR)	279.0* (275.6-281.8)	278.8† (275.0-283.0)	283.6*† (280.3-285.4)	278.3 (274.-280.3)	0.005

*p <0.001, †p = 0.004. CRP, C-reactive protein; ED, emergency department; IQR, interquartile range; MPV, mean platelet volume/platelet count ratio; MPV, mean platelet volume; NLR, neutrophil-to-lymphocyte ratio; RDW, red blood cell distribution width.

Discussion

This study revealed that children with prolonged seizures had significantly higher osmotic pressure than those with simple FSs and multiple seizures during the same febrile illness. In contrast, none of the laboratory test results, including NLR, RDW, and MPV, differed significantly between children with simple and complex FSs.

The reasons why our findings differ from those of previous studies may be because of differences in the inclusion criteria and sample size. This current study was more representative of real-world clinical environments than previous studies because children with a history of FSs, preterm infants, and infants aged less than one year were included. The sample size of this report (139 with simple FSs, 66 with complex FSs) was larger than that of two previous studies[10,11], but less than that of a study by Liu et al[12] (167 with simple FS, 82 with complex FS). In addition, the results of this research were more precise than those of previous studies because the interval from fever onset to seizure onset was recorded in this investigation.

To our knowledge, this is the first study to compare inflammatory markers, which are readily available in the clinical environment, in children with simple FSs and the various types of complex FSs. These results suggest that inflammatory markers may not predict the FS type. Notably, children with prolonged seizures had significantly higher osmotic pressure than those with simple FSs and other types of complex FSs, although the median osmotic pressure for all seizure types was within the normal range. We did not measure osmotic pressure directly. However, this is unlikely to have introduced bias because there were no factors (such as mannitol treatment and ethanol) which might have caused the calculated osmotic pressure to differ from the actual osmotic pressure. Andrew[18] found that lower osmotic pressure contributed to the onset of seizures. The results of this study suggest that

prolonged seizures might be triggered by other mechanisms. Therefore, we suggest reviewing the classification of FSs to differentiate between prolonged seizures and other FSs.

This paper had two main limitations. First, the sample size, especially of children with focal seizures, was small. Second, it was conducted in a single center. It would be useful to confirm the association between osmotic pressure and FSs in a large multicenter study.

Conclusions

To sum up, easily measurable inflammatory markers, including NLR, were unable to differentiate between the various types of FSs in children. Children with prolonged FSs had significantly higher osmotic pressure than children with other FS types. This point may contribute to understanding the pathogenic mechanism of FSs. Large multicenter researches are needed to evaluate the association between osmotic pressure and FSs to gain insight into the pathogenic mechanisms.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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References

1) Patel N, Ram D, Swiderska N, Mewasingh LD, Newton RW, Offringa M. Febrile seizures. BMJ 2015; 351: h4240.
2) Kubota J, Higurashi N, Hirano D, Isono H, Numata H, Suzuki T, Kakegawa D, Ito A, Yoshihashi M, Ito T, Hamano SI. Predictors of recurrent febrile seizures during the same febrile illness in children with febrile seizures. J Neurol Sci 2020; 411: 116682.
3) Berg AT, Shinnar S. Unprovoked seizures in children with febrile seizures: short-term outcome. Neurology 1996; 47: 562-568.
4) Natsume J, Hamano SI, Iyoda K, Kanemura H, Kubota M, Mimaki M, Niijima S, Tanabe T, Yoshinaga H, Kojimahara N, Komaki H, Sugai K, Fukuda T, Maegaki Y, Sugie H. New guidelines for management of febrile seizures in Japan. Brain Dev 2017; 39: 2-9.
5) Nelson KB, Ellenberg JH. Predictors of epilepsy in children who have experienced febrile seizures. N Engl J Med 1976; 295: 1029-1033.
6) Dube C, Vezzani A, Behrens M, Bartfai T, Baram TZ. Interleukin-1beta contributes to the generation of experimental febrile seizures. Ann Neurol 2005; 57: 152-155.
7) Dube CM, Brewster AL, Baram TZ. Febrile seizures: mechanisms and relationship to epilepsy. Brain Dev 2009; 31: 366-371.
8) Deng H, Zheng W, Song Z. The genetics and molecular biology of fever-associated seizures or epilepsy. Expert Rev Mol Med 2018; 20: e3.
9) Zhu G, Okada M, Yoshida S, Mori F, Ueno S, Wakabayashi K, Kaneko S. Effects of interleukin-1beta on hippocampal glutamate and GABA releases associated with Ca2+-induced Ca2+ releasing systems. Epilepsy Res 2006; 71: 107-116.
10) Goksugur SB, Kabakus N, Bekdas M, Demircioglu F. Neutrophil-to-lymphocyte ratio and red blood cell distribution width is a practical predictor for differentiation of febrile seizure types. Eur Rev Med Pharmacol Sci 2014; 18: 3380-3385.
11) Yigit Y, Yilmaz S, Akdogan A, Halhalli HC, Ozbek AE, Gencer EG. The role of neutrophil-lymphocyte ratio and red blood cell distribution width in the classification of febrile seizures. Eur Rev Med Pharmacol Sci 2017; 21: 554-559.
12) Liu Z, Li X, Zhang M, Huang X, Bai J, Pan Z, Lin X, Yu D, Zeng H, Wan R, Ye X. The role of mean platelet volume/platelet count ratio and neutrophil to lymphocyte ratio on the risk of febrile seizure. Sci Rep 2018; 8: 15123.
13) Maksikharin A, Prommalikit O. Serum sodium levels do not predict recurrence of febrile seizures within 24 hours. Paediatr Int Child Health 2015; 35: 44-46.
14) Sharawat IK, Singh J, Dawman L, Singh A. Evaluation of risk factors associated with first episode febrile seizure. J Clin Diagn Res 2016; 10: SC10-13.
15) Hugen CA, Oudesluys-Murphy AM, Hop WC. Serum sodium levels and probability of recurrent febrile convulsions. Eur J Pediatr 1995; 154: 403-405.
16) Kiviranta T, Airaksinen EM. Low sodium levels in serum are associated with subsequent febrile seizures. Acta Paediatr 1995; 84: 1372-1374.
17) Millichap JG, Millichap JJ. Role of viral infections in the etiology of febrile seizures. Pediatr Neurol 2006; 35: 165-172.
18) Andrew RD. Seizure and acute osmotic change: clinical and neurophysiological aspects. J Neurol Sci 1991; 101: 7-18.