

Age and Hemoglobin are Predictors of Febrile Seizure in Children under Five Years

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ABSTRACT

Objectives: In resource-limited settings where the measurement of biomarkers of febrile seizures (FS) is difficult to carry out, it is essential to know the feasible factors associated with FS.

Materials & Methods: This hospital-based case-control study was conducted based on 340 medical records of children aged six to 60 months who were hospitalized at the children's ward of two hospitals in Indonesia, from 2019 to 2020. Data were analyzed using Mann-Whitney U, Chi-square tests, and logistic regression.

Results: The case group had a higher proportion of males (59.4%) and a significantly younger median age of 24.3 months than the control group of 30.3 months ($p = 0.003$). Moreover, the case group had a significantly lower median hemoglobin level of 11.2 g/dL than the control group of 11.5 g/dL ($p = 0.022$). Each month, increasing age was associated with a reduced likelihood of exhibiting FS of 0.980-fold with a 95% CI (0.966, 0.995). Moreover, children with anemia were 2.350 times more likely to exhibit FS than those without anemia, 95% CI (1.399, 3.947).

Conclusion: In low-resource settings. Younger age and reduced hemoglobin levels are useful indicators of febrile seizure risk in children under five, highlighting the importance of early detection and parental education.

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Introduction

Febrile seizures (FS) represent the most prevalent seizure disorder among children aged six to sixty months, occurring in the context of a fever exceeding 38 °C (100.4 °F) and are unrelated to central nervous

system (CNS) infection or metabolic disturbance. The peak incidence is in children aged 12-18 months (1). The prevalence of FS in children under five years old varies, ranging from 2.5% in Burkina Faso (2), 2.57% in India (3), 3.5% in Iran (4), 3.5%-12.8% in Turkey

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(5), 3.55%-3.69% in Denmark (6), 4.61% in Nepal (7), 6.92% in Korea (8), 9.0%-11.2% in Japan (9), and 17.8% in Benin (10).

The pathogenesis of FS is not clearly understood. Previous studies have noted that a systemic infection that elicits an inflammatory response increases cytokine levels in the circulation. The cytokines and pathogen-associated molecular patterns might reduce the permeability of the blood-brain barrier and induce changes in the electroencephalography pattern that trigger seizures (11). Moreover, several factors were reported to be associated with FS, including being male, younger age, low birth weight, a family history of FS, respiratory or enteric viral infection, high fever temperature, cigarette smoking exposure during the prenatal term, neonatal discharge of more than 28 days, low serum zinc, and low serum iron (1,12). A meta-analysis of 20 case-control studies totaling 3,856 children confirmed that iron-deficiency anemia, low mean corpuscular volume, low serum iron, and low ferritin significantly elevate FS risk, with pooled odds ratios ranging from 1.24 to 1.59 (13).

When a child suffers from a seizure, their parents can be emotionally frightened. Parents might panic, thinking that their child might suffer from injury, brain damage, other FS, suffocation, epilepsy, or a developmental disorder. They might check their child's condition thoroughly, put an object inside their child's mouth to prevent injury, or give an antipyretic (14).

In settings with limited resources, where assessing cytokines as biomarkers for FS is challenging, identifying practical and accessible risk factors becomes essential for prevention. This study thus aimed to investigate the relationship between FS and factors such as demographics, birth conditions, and complete blood count at the time of seizure in children under five years old in two hospitals in Indonesia.

Materials & Methods

Study design and sample

This hospital-based case-control study was carried out using medical records of children aged six to 60 months who were hospitalized at the children's ward of S.K. Lerik Regional Public Hospital, Kupang City, East Nusa Tenggara Province, Indonesia, and Dr Ramelan Central Naval Hospital, Surabaya, Indonesia, from 2019 to 2020. The sample size was calculated using the following case-control sample size formula (15), resulting in 170 participants' medical records in each case and control group (a total of 340 participants' medical records), with 104 participants' medical records from S.K. Lerik Regional Public Hospital, and 236 participants' medical records from Dr Ramelan Central Naval Hospital.

$$n = \frac{r + 1}{r} \frac{(p^*)(1 - p^*)(Z_\beta + Z_\alpha)^2}{(p_1 - p_2)^2}$$

The calculation was based on the ratio of controls to cases ($r = 1$), and the prevalence of anemia in the case group ($p_1 = 49.2\%$) (16). The prevalence of anemia in the control group ($p_2 = 34.0\%$) (17); $p^* = (p_1 + p_2) / 2$, with $\alpha = 0.05$ and $\beta = 0.20$.

Data collection

The case group consisted of children with fever (temperature of more than 38.4 °C) associated with the first episode of simple FS: A generalized seizure event of less than 15 minutes with rapid recovery within one hour; without recurrence in the first 24 hours, evidence of CNS infection, metabolic disorders, or any acute neurological insult (18). The control group consisted of children with fever of unknown etiology without seizures. Children with CNS infections, intracranial abnormalities, a history of FS, or findings of neurologic abnormalities, and those who did not undergo a routine blood test after FS, were excluded. All information, including age, sex, history of asphyxia at birth, diagnosis, and laboratory results, was obtained from the medical records. The complete blood count tests were taken on the first visit to the emergency room at both hospitals.

Complete blood counts were measured from peripheral venous blood samples collected in ethylenediaminetetraacetic acid (EDTA) tubes (19) during admission to the emergency room of both hospitals. The blood samples were taken and analyzed by Abbott CELL-DYN Hematology Analyzers after FS (20). The neutrophil-to-lymphocyte ratio (NLR) was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count, and the mean platelet volume-to-platelet count ratio (MPR) was calculated by dividing the MPV by the platelet count (21).

Ethics considerations

This study adhered to the Declaration of Helsinki and its amendments. Confidentiality was maintained throughout the study. The research protocol received approval from the Research Ethics Committee of Dr Ramelan Central Naval Hospital No. 12/EC/KERS/2020 and the Nusa Cendana University Health Research Ethics Committee No. 84/UN15.16/KEPK/2019.

Statistical analyses

The Kolmogorov-Smirnov test was applied to all continuous scale data to evaluate their normal distribution, resulting in all continuous variables not being normally distributed. For descriptive analysis, continuous variables were presented as median, 25th and 75th percentiles, while categorical variables were

presented as frequencies and percentages. Data were then analyzed using Mann-Whitney U, Chi-square tests, and logistic regression (22). All analyses were performed using IBM® SPSS® Statistics version 26.0 for Windows with an alpha of 0.05

Results

Table 1 compares the case (n = 170) and control (n = 170) groups based on demographic and birth condition characteristics. The case group had a significantly younger median age (24.3 months) than the control group (30.3 months). The case group also

had a higher proportion of children aged less than 24 months (45.9%) than the control group (34.7%).

In addition, of the study participants from the hospital in Surabaya, 16 children (13.6%) in the case group had a first-degree relative with FS, and 13 (11.0%) children in the case group had a history of birth asphyxia. A subset analysis of participants from the hospital in Surabaya (n = 236) indicated a significant correlation between family history of FS and birth asphyxia.

Table 1. Characteristics of study participants [median (25th, 75th percentile), n (%)]

Characteristics	Case (n = 170)	Control (n = 170)	p-value
Age (months) a	24.3 (17.2, 36.3)	30.3 (19.1, 46.5)	0.003
Body temperature (°C) a	38.8 (38.3, 39.3)	38.8 (38.4, 39.3)	0.474
Age category b			0.025
< 24 months	78 (45.9)	59 (34.7)	
24 – 59 months	92 (54.1)	111 (65.3)	
Sex b			0.064
Male	101 (59.4)	84 (49.4)	
Female	69 (40.6)	86 (50.6)	
Family history of FS			< 0.001
No	102 (86.4)	118 (100.0)	
Yes	16 (13.6)	0 (0.0)	
Birth asphyxia b			< 0.001
No	105 (89.0)	118 (100.0)	
Yes	13 (11.0)	0 (0.0)	

Table 2. Complete blood count [median (25th, 75th percentile), n (%)]

Complete blood count	Case (n = 170)	Control (n = 170)	p-value
Haemoglobin (g/dL) a	11.2 (10.7, 12.1)	11.5 (11.2, 11.9)	0.022
Haemoglobin			< 0.001
Anaemia (< 11 g/dL)	60 (35.3)	29 (17.1)	
Normal (≥ 11 g/dL)	110 (64.7)	141 (82.9)	
Lymphocyte a	2.9 (1.9, 4.3)	2.9 (1.8, 4.3)	0.871
Lymphocyte percent a	27.1 (15.5, 38.6)	29.0 (16.9, 39.9)	0.370
Neutrophil a	6.9 (4.4, 11.1)	6.9 (3.7, 9.8)	0.246
Neutrophil per cent a	61.3 (51.6, 75.5)	59.6 (47.8, 72.9)	0.321
NLR a	2.2 (1.4, 4.9)	2.1 (1.2, 4.1)	0.313
Platelet a	290.5 (214.8, 361.0)	280.5 (217.5, 369.8)	0.705
WBC a	11.6 (8.3, 16.1)	11.1 (7.4, 14.9)	0.200
Monocyte a	0.9 (0.7, 1.5)	1.0 (0.7, 1.4)	0.923
Monocyte percent a	8.7 (6.9, 11.2)	9.2 (7.2, 11.4)	0.345
RBC a	4.7 (4.4, 5.0)	4.7 (4.3, 5.0)	0.856
Haematocrit a	34.5 (32.6, 37.1)	34.9 (32.4, 37.5)	0.380
MCH a	24.3 (2.3, 26.8)	24.5 (2.5, 26.7)	0.808
MCHC a	32.6 (0.3, 33.4)	32.8 (0.3, 33.5)	0.611
MCV a	75.3 (67.6, 80.4)	76.2 (70.3, 80.0)	0.398
MPV a	8.2 (7.1, 9.0)	8.1 (6.7, 8.8)	0.262
MPR	0.03 (0.02, 0.04)	0.03 (0.02, 0.04)	0.943

a Mann-Whitney U test, b Chi-square test

a Mann-Whitney U test, b Chi-square test. Family history of febrile seizure and history of birth asphyxia are only available from the medical records of the hospital in Surabaya.

Table 2 compares the complete blood count analyses. Of all the complete blood count components, the case group had a significantly lower median hemoglobin level of 11.2 g/dL compared to the control group (11.5 g/dL). The case group also had a higher

proportion of anemia (35.3%) than the control group (17.1%).

Table 3 presents a logistic regression with FS as the dependent variable (yes/no), while age in months and hemoglobin category were treated as independent variables. Family history of FS and history of birth asphyxia are not included in the model, since they are only available from the medical records of the hospital

in Surabaya. The reference for the hemoglobin category was the normal range (≥ 11 g/dL). The Hosmer and Lemeshow goodness-of-fit test showed $X^2(8) = 15.060$, $p = 0.058$, meaning that the model estimated fit the data at an acceptable level. The logistic regression model explained a modest proportion of the variance in FS, 8.1% (Nagelkerke R^2), and correctly classified 57.1% of cases.

Table 3. Logistic regression

	B	S.E.	Wald	df	Sig.	Exp(B)	95% CI for Exp (B)	
							Lower	Upper
Age (mos)	-.020	.008	6.638	1	.010	.980	.966	.995
Hb (1)	.854	.265	10.422	1	.001	2.350	1.399	3.947
Constant	.395	.274	2.078	1	.149	1.485		

Discussion

In resource-limited areas, identifying predictors of FS that are feasible and accessible is crucial because of the absence of sophisticated diagnostic equipment. Previous studies found that either the CBC-related parameters (23), the osmolality of blood serum (24), or electrolytes and trace elements testing (25) provide a feasible and effective strategy for assessing the likelihood of FS in children. These can guide early interventions and parental education, without the need for costly or unavailable tests.

The present study found that the case group had a significantly younger median age (24.3 months) compared to the control group (30.3 months). Each month, increasing age was associated with a reduced likelihood of exhibiting FS of 0.980-fold, with a 95% CI (0.966, 0.995). Notably, the odds of FS were 2.35 times higher in children with anemia than in their non-anemic counterparts (95% CI: 1.399–3.947). Unlike earlier investigations (26,27), no significant association was found between sex and FS risk. The findings of the present study were in line with previous studies, indicating that younger children (less than 24 months) had a higher risk of developing their first FS (26,28). Further, a younger age at the first FS of less than 18 months was one of several risk factors for the recurrence of FS. The first FS is more prevalent in early childhood, associated with the stage of brain development, a lower seizure threshold, a higher occurrence of infections, and a stronger fever response in the body (29,30).

In this study, no significant differences were observed in various leukocyte parameters, including NLR and MPR, commonly used indicators of inflammation, generally serving as baseline reference markers in individuals with FS (26). NLR, in particular, reflects the cellular immune system activation and tends to rise during the hyperdynamic phase of early inflammation. Neutrophils play a key role in initiating

inflammatory responses through mediators like TNF- α and IL-1 β , associated with FS. Additionally, some neutrophils are capable of expressing voltage-gated sodium channel 1.3 (NaV1.3); when activated, NaV1.3 can generate sustained high-frequency sodium currents (31).

In contrast, of all complete blood count parameters evaluated in this study, only hemoglobin levels exhibited a correlation with FS. A higher proportion of children with FS (35.3%) had lower hemoglobin levels. Although this difference reached statistical significance, the absolute difference between groups was relatively small. Therefore, the clinical significance of this finding should be interpreted cautiously, as the modest reduction in hemoglobin may not necessarily translate into a meaningful effect on seizure susceptibility at the individual patient level. Hemoglobin is the chief oxygen transporter within the body, and lack of sufficient amounts could interfere with oxygen supply. If there was decreased oxygen availability in febrile conditions, there might be interference in neuron function due to reduced adenosine triphosphate generation, which is necessary for maintenance of ion gradient across the cell membrane (32,33). Consequently, lower hemoglobin levels may contribute to a reduced seizure threshold in susceptible children. However, this mechanism was not directly evaluated in the present study. Previous studies have reported an association between iron deficiency anemia and FS. Nevertheless, iron status was not assessed in this study using indicators such as serum transferrin, soluble transferrin receptor, or transferrin saturation. Therefore, it was not possible to determine whether iron deficiency contributed to the observed association between hemoglobin levels and FS (13). Any mechanistic interpretation involving iron deficiency should thus be made with caution. Moreover, dehydration, commonly accompanying febrile illnesses in children, may contribute to cellular

dehydration and electrolyte disturbances, which could further lower the seizure threshold (34).

A prior case-control study conducted in 75 cases and 75 controls of children aged 6 months to 6 years found a significantly higher proportion of iron deficiency anemia in 48.0% of cases and 22.0% of controls. Moreover, the mean serum ferritin was significantly lower in cases (39.54 ± 24.55) than in controls (51.29 ± 20.26), $p < 0.01$ (35). A systematic review and meta-analysis of 17 studies, including 2,416 children with FS and 2,387 controls highlighted a significant association between iron deficiency and FS in young children. Despite similar ages in the case and control groups, this study found that iron deficiency anemia was present in 35.2% of cases and 29.6% of controls, indicating a significant association with FS. The combined OR was 1.98 (95% CI: 1.26, 3.13, p -value = 0.003) (36). Another systematic review and meta-analysis employing 30 case-control studies with a total of 4,158 children reported significantly lower ferritin and serum iron in the cases than in controls, with the standardized mean difference, $SMD = -0.79$ (95% CI: -1.15, -0.430, $p < 0.0001$) and $SMD = -0.60$ (95% CI: -1.04, -0.16, $p = 0.007$), respectively. In addition, this study indicated that the zinc level was lower in cases than in controls (37).

Many factors can alter the seizure threshold, one of which is iron deficiency. Iron is a vital nutrient necessary for proper growth and development in children, especially around 4-12 months. Iron is essential for neurodevelopment and cytochrome c oxidase (CytOx), which has a heme pocket acting as a binding site for copper and iron. Mild or moderate iron deficiency can cause low CytOx, altering the metabolism of neurons and leading to poor myelination. Several neurotransmitters in the brain will be unstable, and the cell membrane will release more dopamine, leading to seizures (38). An iron deficiency can impair the functioning of multiple organs, resulting in anemia, developmental delays, behavior issues, cognitive impairment, disrupted temperature regulation, reduced physical capacity, and weakened immune function (39). Moreover, iron deficiency can negatively affect brain development by disrupting neuronal growth in the hippocampus, impairing energy metabolism, delaying myelination, and reducing the responsiveness of auditory and visual systems, also altering neurotransmitter systems, including dopamine, GABA, norepinephrine, and glutamate. As a treatable condition, iron deficiency is considered a potential contributing factor to FS in children (40).

A subset analysis of this study indicated that a family history of FS and birth asphyxia was correlated with the occurrence of FS, consistent with

other studies (41). Previous studies have found genetic variability in familial FS (FEB1-FEB11) and identified genetic polymorphisms in cytokine-encoding genes linked to FS (42). Seizures are abnormal and uncontrolled neural activity resulting in heightened metabolic demands, prompting neuronal glycolysis and increased energy depletion. Birth asphyxia is considered a perinatal brain injury, leading to the undermining of foundational structures and function of the brain, making it more vulnerable to future brain injuries. In brains already weakened by birth asphyxia, the negative effects of FS can be significantly intensified, indicating a greater risk of worsening neurological damage in these susceptible individuals. Possibly, neonatal seizures could further worsen neuronal injury in a brain that is already energy-depleted and damaged from initial perinatal asphyxia (43). Therefore, health education on FS should be delivered to parents and caregivers.

The current study had strengths, including addressing a critical health issue in resource-limited settings and focusing on easily measurable and clinically relevant factors, such as age and hemoglobin levels. It also used a hospital-based case-control design with a relatively large sample size ($n = 340$), as well as the application of non-parametric tests and logistic regression for handling data that do not follow a normal distribution. However, this study had some limitations that need to be acknowledged. This research was limited to two hospitals in Indonesia, which may affect the generalizability of the findings to other populations or regions. The current study relied on medical records. Another limitation is that family history of FS and history of birth asphyxia were not incorporated into the regression model due to their limited availability, as they were recorded only in the medical records of the hospital in Surabaya. The present study only evaluated the complete blood count and did not include a blood smear examination to characterize the type of anemia. Additionally, iron status was not assessed, making it impossible to differentiate iron deficiency anemia from other types of anemia. Consequently, the potential role of iron deficiency in the observed association between hemoglobin levels and FS could not be evaluated directly. Moreover, the modest effect size observed for hemoglobin differences may limit the immediate clinical applicability of this finding. Besides, this study lacked information regarding medication given to the children before they were taken to the hospital and the history of infections preceding the FS. Further, the wide age span (6-60 months) may mask significant age-specific trends, particularly since FS incidence is highest between 12 and 18 months. Additionally, potential confounding variables, such as nutritional

status and pre-existing infections, may not have been adequately accounted for. To enable a more thorough and scientifically robust evaluation of the risk factors identified in this study, future research could involve a prospective, multicenter study with a larger sample size.

In Conclusion

In settings with limited resources, age and hemoglobin level are practical and accessible predictors of FS in children under five. Younger age and the presence of anemia significantly increase the risk of FS. These findings highlight the importance of early screening and health education for parents and caregivers to support prevention and timely intervention.

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Authors' Contributions

Wienta Diarsvitri, Pramita Anindya Nugraheni, Verna Biutifasari, Angela Puspita and Maria Steffany Lainama contributed to the design, data collection, and manuscript preparation and revision. Wienta Diarsvitri analyzed the data and supervised the project. Vita Safira, Nurlaili Istiqomah and Nauroh Firdaus participated in data collection, interpretation, and manuscript preparation. Ika Apriyanti Arum Putri, Belinda Anasthasya Tansi and Annisa Haslidianingsih assisted with data interpretation, manuscript preparation, and revision. All the authors have read and approved the final submitted manuscript.

Conflict of Interest

None declare

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