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Original Article

Soluble transferrin receptor as an indicator of iron deficiency and febrile seizures

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Abstract

Background Iron deficiency (ID) has a high incidence in Indonesia, and is a risk factor for febrile seizures. The most suitable assay to detect iron deficiency in the presence of inflammation has not yet been defined. An indicator of ID unaffected by inflammation is needed, soluble transferrin receptor (sTfR) may be such an indicator.

Objective To evaluate ID as a risk factor for febrile seizures in children with inflammation by sTfR measurements.

Method We conducted an age-matched, case-control study, focused on children experiencing on acute illnesses at the time. Subjects were 80 children matched by age (40 in the case group with febrile seizures, and 40 in the control group who were febrile without seizures) aged 3 months to 5 years in Mohammad Hoesin Hospital, Palembang from February to August 2013. Subjects' clinical data and sTfR levels were recorded. Risk factors were analyzed with odd ratios and 95% confident intervals. The sTfR level cut-off point as a predictor of febrile seizures was also defined. Other risk factors were analyzed with multivariate logistic regression test.

Results Mean sTfR levels were 41.36 (SD 2.04) nmol/L in the case group and 33.09 (SD 1.02) nmol/L in the control group. Multivariate analysis revealed ID and iron deficient anemia (IDA), as measured by sTfR levels, to be risk factors for febrile seizures (adjusted OR=3.9; 95%CI 1.41 to 10.8; P=0.007 and OR 3.27; 95%CI 1.21 to 8.84; P=0.017, respectively). The sTfR level cut-off point that could be used as a predictor of febrile seizures was 37nmol/L.

Conclusion Iron deficiency as measured by increased sTfR is a risk factor for febrile seizures in children. **[Paediatr Indones. 2015;55:95-100]**.

Keywords: *febrile seizures, iron deficiency, sTfR*

Febrile seizures are convulsions associated with a rise in body temperature due to extracranial processes. It is the most common childhood seizure disorder, affecting 2-5% of children under 5 years of age.[1] According to the *Consensus Statement On Febrile Seizure*, it most often occurs between the ages of 3 months to 6 years. Febrile seizures have a good prognosis, but some cases end with neurological deficits, including epilepsy.[2] Iron deficiency (ID) plays role in metabolism of neurotransmitters (GABA/inhibitor and glutamic acid/exitator).[3] Iron deficiency (ID) is the most common nutritional problem worldwide. About 30% of people have anemia, and half of those have hypochromic microcytic anemia.[4] In Indonesia, especially in South Sumatra, the incidence of anemia is 16.5%, and 70% of these have hypochromic microcytic anemia.[5] As such, a large number of Indonesian children are at risk of febrile seizures.

Previous studies reported that iron deficiency to be a risk factor for febrile seizures.[6,7] The gold standard for diagnosing iron deficiency is *Prussian Blue*

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staining of bone marrow iron, but this test is invasive and impractical to perform. Common parameters of iron deficiency used are serum iron, total iron binding capacity (TIBC) and ferritin. However, these three measurements reflect iron stores in plasma, not in bone marrow, have diurnal variations and are directly affected by inflammation. These factors make interpretation difficult in iron-deficient patients who have accompanying inflammation, such as is the case with febrile seizures.[8] On the other hand, a study with animal models of latent iron deficiency showed marked reductions in brain GABA and L-glutamic acid levels.[9] As such, it is important to detect ID early, to prevent anemia. In contrast, soluble transferrin receptor (sTfR) has been shown to be an indicator of iron deficiency and is unaffected by inflammation, with sensitivity 70.8% and specificity 90.6%.[10] The aim of this study was to assess the relationship between sTfR levels as an indicator of iron deficiency and febrile seizures.

Methods

This case control study included subjects from the Pediatric Emergency, Neuropediatric, and Infection Wards at Dr. Mohammad Hoesin Hospital, Palembang, from February to September 2013. The subjects were children aged 3 months to 5 years with acute fever for less than 7 days. Subjects were consecutively allocated to the case group if their body temperature was 37.5°C or higher and had seizures, or to the control group if their body temperature was 37.5°C or higher without seizures. The ratio of subjects in the case and control groups was one-to-one and subjects were matched for age. Other inclusion criteria were no history of epilepsy, no anti-convulsive medication used, not malnourished, not having chemotherapy or repeated transfusions, nor any chronic diseases. Direct explanations were given to parents and they provided written informed consent prior to subject enrollment. The study protocol was approved by the Ethics Committee of Sriwijaya University. Data was collected by questionnaire, physical examination and laboratory findings. Gender, body temperature, family history of febrile seizures, intrauterine and labor history, and cause of fever, were also recorded as confounding factors. Subjects were classified as ID if their sTfR levels were ≥ 29.5 nmol/L and hemoglobin levels > 11 g/dL. They were classified as iron deficiency anemia (IDA) if their sTfR levels were > 29.5 nmol/L and hemoglobin levels was ≤ 11 g/dL.

Data are presented as proportion, percentage, mean (standard deviation) and median (range). Differences between groups were analyzed with Chi-square and Fischer's exact tests for categorical variables, and unpaired T-test and Mann-Whitney U-test for continuous variables. The association between sTfR levels and febrile seizures was expressed with odds ratio. A P value < 0.05 was considered to be statistically significant. Confounding factors were controlled by multivariate linear regression resulting in adjusted ORs. Statistical analysis was performed by using *SPSS 15* software package.

Results

Ninety-five patients met the inclusion criteria, but 15 were excluded due to electrolyte imbalance (2 patients) and intracranial infection (13 patients). The total number of subjects was 80, with 40 subjects in each group (**Figure 1**). The baseline characteristics of subjects are shown in **Table 1.**

Table 2 shows that mean hemoglobin, serum iron (SI), TIBC, transferrin saturation (trans SAT), and ferritin levels were lower in the case group than in the control group, but were not statistically significant different. The only significant difference was that the mean sTfR level was higher in the case group than in the control group [41.36 (SD 2.04) nmol/L vs. 33.09 (SD 1.02) nmol/L; respectively, (P = 0.007)].

Bivariate analysis revealed that increased sTfR level was a risk factor for febrile seizures (OR 2.5; 95%CI 1.02 to 6.19; P=0.04), when combined with anemia, the risk increased (OR 3.3; 95%CI 1.28 to 8.85; P= 0.012) (**Table 3**).

To assess the validity of sTfR measurement as a predictor of febrile seizures, we performed a receiver operator curve (ROC) analysis (**Figures 2** and **3**). We found that the area under curve was 78.9% and the cut-off point was 37 nmol/L, with sensitivity of 77.0% and specificity of 72.6%.

Based on our cut-off point, we found that subjects with sTfR level above 37 nmol/L were 5.6 times at higher risk of having febrile seizures (OR

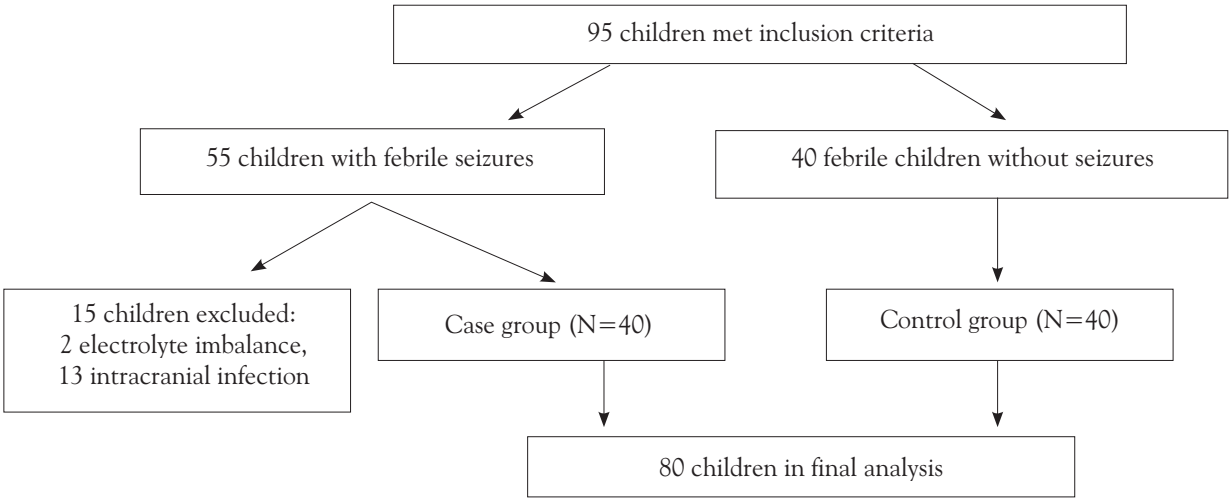

Figure 1. Subjects recruitment scheme

Table 1. Baseline characteristics of subjects

Characteristics	Case group (n= 40)	Control group (n= 40)
Gender, n		
Male	14	31
Female	26	19
Median age (range), months	20.58 (3-60)	21.68 (3-60)

5.571; 95%CI 2,119 to 14,647; P=0.000).

To control for confounding factors, we performed a multivariate logistic regression analysis. **Table 4** shows the risk factors: family history, health state, intrauterine history, and sTfR itself. The following formula: $P=\frac{1}{1+e^{-y}}$ and $y = a + b_{x1} + b_{x2} + b_{x3} + etc$,

Table 2. Iron deficiency parameters

Variables	Group						P value
	Case			Control			
	Mean	SD	95% CI	Mean	SD	95% CI	
Hb, g/dL	10.2	1.8	9.7 to 10.8	10.8	1.9	10.2 to 11.4	0.20
SI, µg/dL	59.70	4.96	43.80 to 75.59	63.45	5.91	44.53 to 82.36	0.85
TIBC, µg/dL	271	7.41	247 to 295	296	4.45	153 to 438	0.73
TransSAT, %	26	0.29	17 to 36	31	0.36	20 to 43	0.35
Ferritin, µg/dL	364	67	147 to 580	801	145	361 to 1266	0.09
sTfR, nmol/L	41.36	2.04	34.8 to 47.9	33.09	1.02	14.4 to 36.4	0.007

SI=serum iron, TIBC=total iron binding capacity, transSAT=transferrin saturation, sTfR= soluble transferrin receptor

Table 3. Relationship between the ID or IDA and febrile seizures

Risk factors	Group				OR (95%CI)	P value
	Case		Control			
	n	%	n	%		
ID					2.5 (1.02 to 6.19)	0.04
Positive	26	65	17	42.5		
Negative	14	35	23	57.5		
IDA					3.3 (1.28 to 8.85)	0.012
Positive	21	52.5	10	25		
Negative	19	47.5	30	75		

ID=iron deficiency; IDA=iron deficiency anemia

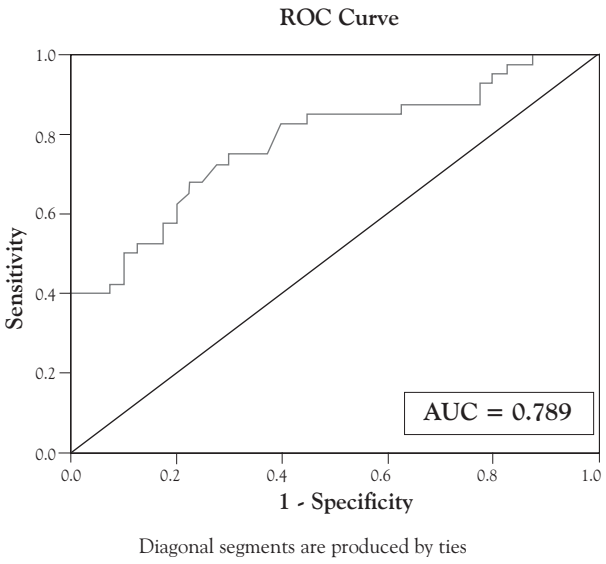

Figure 2. Receiver operator curve (ROC) of sTfR level

revealed that the probability of a child with an sTfR level above 29.5 nmol/L, without any other factors, for having febrile seizures was 97%. If that sTfR level was accompanied by all other risk factors, the probability of having febrile seizures increased to 99.9%.

As an additional result of this study, we found that if ID and IDA were assessed by SI, TIBC and ferritin levels, the prevalence of ID and IDA were lower than if assessed by sTfR level, and there was no significant correlation between ID/IDA and febrile seizure (**Table 5**). Based on SI and TIBC, subjects were classifed as ID if transferrin saturation (percentage of SI/TIBC) was <20% and Hb level was >11 g/dL; and subjects were classified as IDA if transferrin saturation was <10% and Hb level ≤ 11 g/dL. Based on ferritin level, subjects were classified as ID if ferritin level was <12 mg/dL and Hb level > 11g/dL, and IDA if ferritin was < 12mg/dL and Hb level ≤ 11 g/dL. By the standard parameter prevalence if iron deficiency and iron deficiency anemia is lower.

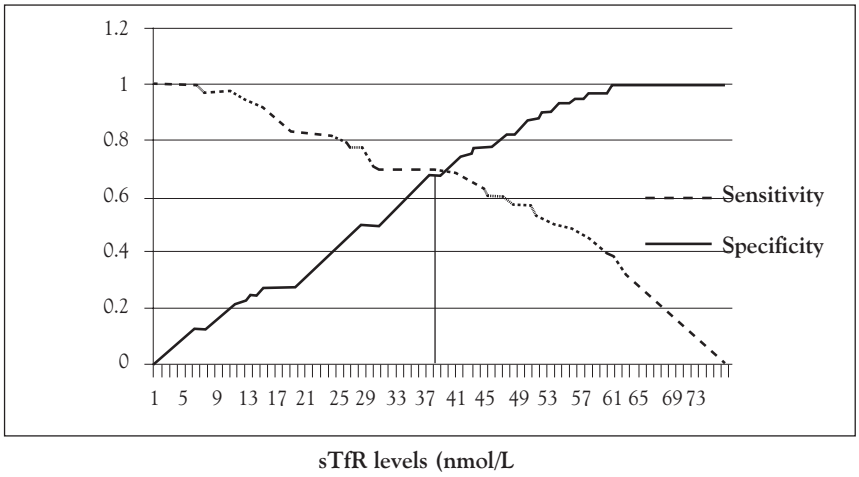

Figure 3. Cut-off point of sTfR level to predict febrile seizures

Table 4. Multivariate logistic regression analysis of confounding factors (N=80)

Risk factors	Adjusted OR	95% CI		P value
		Lower	Upper	
Health status	3.42	0.80	14.68	0.09
Family history	16.39	1.64	16.60	0.01
sTfR level	3.9	1.43	10.79	0.00
Intrauterine history	2.53	0.89	7.09	0.07
Constant	-0.35			

Table 5. Comparison of odds ratios of several parameters of iron deficiency

Variables	Group				P value	OR (95% CI)
	Case	%	Control	%		
sTfR level						
Iron deficiency					0.04*	2.55
Positive	26	65	17	42.5		(1.02 to 6.19)
Negative	14	35	23	57.5		
Iron deficiency anemia					0.012	3.3
Positive	21	52.5	10	25		(1.28 to 8.85)
Negative	19	47.5	30	75		
Transferrin saturation level						
Iron deficiency					1.00	1.00
Positive	18	37.5	18	37.5		(0.41 to 2.41)
Negative	22	62.5	22	62.5		
Iron deficiency anemia					0.23	2.03
Positive	9	22.5	5	12.5		(0.61 to 6.71)
Negative	31	77.5	35	87.5		
Ferritin level						
Iron deficiency					1.00	1.00
Positive	3	7.5	3	7.5		(0.18-5.28)
Negative	37	92.5	37	92.5		
Iron deficiency anemia					0.55	0.48
Positive	1	2.5	2	5		(0.04 to 5.59)
Negative	39	97.5	28	95		

*T-test, Mann Whiney

Discussion

Using sTfR levels as an indicator of ID and IDA, we found ID and IDA to be risk factors for febrile seizures. The mean sTfR level of the case group was significantly higher than that of the control group. Subjects with ID, as diagnosed by sTfR level ≥29.5 nmol/L, had a 3.9 times higher risk of febrile seizure than those without ID. In addition, subjects with IDA, as diagnosed by sTfR level < 29.5 nmol/L, had a 3.3 times higher risk of febrile seizure than those with IDA (OR 3.3; 95% CI 1.28 to 8.85; P= 0.012). Similarly, a previous study found a highly significant association between iron deficiency and simple febrile seizures in both univariate and multivariate analyses. Their crude odds ratio was 5.34 (95%CI 3.27 to 8.73; P<0.001) and the adjusted odds ratio from their logistic regression analysis was 4.5 (95% CI 2.69 to 7.53; P<0.001).[11] Ramadanti (2007) also found the similar results.[7]

Some earlier studies showed that in iron deficiency anemia and latent iron deficiency, both excitatory and inhibitory neurotransmitters are affected. Mittal and Agarwal found significantly decreased GABA and increased glutamic acid in an animal study with iron deficient rats. The changes induced in the fetal brain were irreversible on rehabilitation.[15] The role of intraneuronal iron in metabolism is varied and involves the following: incorporation of iron into enzymes of oxidation-reduction or electron transport; synthesis and packaging of neurotransmitters; as well as uptake and degradation of the neurotransmitters into other iron-containing proteins that may directly or indirectly alter brain function through peroxide reduction, amino acid metabolism and fat desaturation, thus altering membrane functioning.[12]

Because standard tests of iron status are affected by inflammation, hindering clinical interpretation, we used soluble transferrin receptor (sTfR) as the parameter of iron deficiency, as it is unaffected by inflammation. In the early stages of iron deficiency, sTfR level increases progressively.[10] We found significantly higher mean sTfR level in the case group than in the control group. Another study also reported similar results, as his univariate, bivariate and multivariate analyses showed that iron deficiency as defined by sTfR measurements was significant as a risk factor for febrile seizures (OR=25.1; 95%CI 5.1 to 122.6; P<0.001).[13]

The transferrin receptor is a transmembrane cellular protein primarily expressed in cells that require iron. The soluble form is elevated in serum and plasma in

cases of iron deficiency, even in stage 2. The circulating sTfR concentration is proportional to cellular expression of the membrane-associated TfR.[10] Ahluwalia[8] proposed that use of sTfR and the sTfR index improves detection of IDA, particularly in situations where routine markers provide inferior results. Findings demonstrated a significant advantage in the simultaneous determination of ferritin, sTfR, and sTfR index. Obtaining a ferritin level alone may delay diagnosis of combined IDA and anemia of chronic disease (ACD).[8] In addition, we found that other parameters such as SI, TIBC and ferritin failed to reliably indicate iron deficiency in our study population. Further study with a larger sample is needed to confirm this finding.

To assess the validity of sTfR level as a predictor of febrile seizures, we used a ROC analysis, with the following results: area under curve was 78.9%, and the cut-off point was sTfR of 37 nmol/L with sensitivity 77% and specificity 72.55%. Khanis found an sTfR cut-off point of 2.55 μg/mL as an indicator of febrile seizures.[13]

In conclusion, increased sTfR level as an indicator of iron deficiency is a significant risk factor for febrile seizures. Follow-up studies with a prospective design and larger sample size are required.

Conflict of interest

None declared

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