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The Correlation between IL-1β and IL-10 Levels in Estimating the Risk of Febrile Seizures

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ABSTRACT

Febrile seizures are the most-common form of seizures in children with fever and evidence from clinical and experimental studies suggests the potential role of immune generated products in their genesis. The balance between pro-inflammatory (IL-1β) and anti-inflammatory (IL-10) cytokines influences the regulation of infections and plays a role in the pathogenesis of febrile seizures. In existing literature, there is no research into the correlation between IL-1β and IL-10 levels as well as ratio of IL-1β to IL-10 in estimating the risk of febrile seizures in seizure-prone children aged 3 months to 5 years. In this study, IL-1β, IL-10 levels and ratio of IL-1β to IL-10 in the risk of febrile seizures were investigated, and respondents were divided into 3 groups, febrile seizures, febrile without seizures and healthy children without histories of febrile seizure. A cross-sectional design was used and each group had 17 co-respondents. Levels of IL-1β and IL-10 were measured using ELISA. Data were analyzed by SPSS 15. Findings showed there were significant differences between IL-1β and IL-10 as well in the ratio of IL-1β to IL-10 between febrile seizures patients and healthy children. It was concluded that there were significant correlations between IL-1β and IL-10 levels as well as IL-1β to IL-10 ratios in estimating the risk of febrile seizures.

Keywords: Febrile seizures, IL-1β, IL-10, IL-1β/IL-10

INTRODUCTION

Febrile seizures (FSs) represent the most common form of childhood seizures and usually occur between 3 months and 5 years of age and are associated with fever but that do not show evidence of intracranial infection or other defined causes. Febrile seizures are classified as either simple or complex [1, 2]. Risk factors such as age, duration of seizures, temperature at the time of seizures and family history can predict the occurrence of future neurological problems [3, 4].

Fever itself is a risk factor in febrile seizures as rising body temperatures provoke seizure threshold and neural excitability as it effects on ion channels, cellular metabolism and ATP production [3, 5, 6].

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Interleukin-1ß (IL-1ß) is a potent pro-inflammatory cytokine that is crucial for host-defense responses to infection and injury and IL-1β may contribute to the hyperexcitability and seizures generated by fever and hyperthermia [5, 7]. Dube *et al* (2000) [8] showed high doses of IL-1β are sufficient to generate seizures even without increased brain temperature [8, 9]. There is an evidence of serial treatment of cells with LPS and ATP provides a very powerful stimulus to induce rapid and efficient release of IL-1β from monocytes, macro-phages and dendritic cells [10, 11]. Research carried out by [5] found elevated levels of IL-1β in patients with febrile seizures when compared to normal ones, febrile patients without seizures and status epilepticus [5].

IL-10 influences three important functions of monocytes/macrophages: the release of immune mediators, the antigen presentation and the phagocytosis. Simply said, it suppresses all functions of monocytes/ macrophages that are responsible for a positive role of these cells in both innate

and specific immunity [12]. At the same time, it enhances the inhibitory, tolerance inducing, and 'scavenger' functions of these cells. In fact, IL-10 inhibits the release of pro-inflammatory mediators from monocytes/ macrophages, and therefore inhibits the LPS- and IFN-γ-induced secretion of TNF-α, IL-1β, IL-6, IL-8, G-CSF, and GM-CSF [6]. IL-10 inhibits the synthesis of IL-12, thereby; it hampering the development of Th1 immunity [13, 14]. Serum IL-10 and sTNFR1 levels may be related to the severity of the neuro-logical symptoms after the initial prolonged feb-rile seizure [7]. The balance between proinflammatory and anti-inflammatory cytokines influences the regulation of infections and could, therefore, play a role in the pathogenesis of FS [15].

In existing literature, there is no research into the correlation between ratios IL-1β to IL-10 and the risk of febrile seizures in seizure-prone children aged 3 months and 5 years.

MATERIALS AND METHODS

Patient information

This study used a cross-sectional study design and compared the level of IL-1β and IL-10 in three groups of 17 children. They were: Group I simple febrile seizures, Group II febrile without seizures, and Group III healthy children without histories of febrile seizures. This study was conducted from 1st June 2013 to 30th November 2013 at the Department of Child Health, Faculty of Medicine, Brawijaya University, Saiful Anwar General Hospital, Malang, Indonesia and Faculty of Medicine, Biomedical Laboratory, Brawijaya University. This study was approved by the Ethics Committee of dr. Saiful Anwar General Hospital, Malang, Indonesia.

The peripheral blood samples were collected from outpatients at the emergency ward or from those admitted to the Department of Child Health Saiful Anwar Hospital. Peripheral blood samples from healthy children without histories of febrile seizures were obtained from patients in the outpatient's clinic who had a history of dengue or dengue hemorrhagic fever during the previous week. Inclusion criteria for Group I included patients diagnosed with simple febrile seizures according to the diagnostic criteria of the National Institutes of Health Consensus Conference; ranging age from 3 months and 5 years; had rectal temperatures of > 38°C and had blood samples taken before 12 hours from the initial seizure, were willing to participate in the study with the informed consent of their parents and had no other causes of seizures other than fever. Inclusion criteria for Group II included patients who had fevers regardless of their cause, and had no history of seizures, ranging in age from 3 months to 5 years, had rectal temperatures of >38°C and who were willing to participate in the study with the informed consent of their parents. Inclusion criteria for Group III included patients who had no fever, had no seizures and no history of seizures and ages ranging from 3 months to 5 years, had rectal temperatures of 36,5 – 37,5°C and who were willing to participate in the study with the informed consent of their parents.

Cytokine measurement

Levels of cytokines including IL-1β and IL-10 were measured using Human IL-1β and IL-10 Quantikine ELISA kits according to the R&D Systems (Minneapolis, MN, USA) manufacturer's instructions. Samples were analyzed in duplicate and compared with control samples. The detection limits were 0.27pg/mL for IL-1β and 0.22pg/mL for IL-10.

Statistical Analysis

The Kruskal-Wallis and Mann-Whitney test were used to compare the clinical characteristics between febrile seizure patients, febrile without seizure patients and the controls. The Mann-Whitney test was also used to compare plasma cytokine levels, and laboratory findings between a) control and febrile seizure patients; b) febrile without seizures and febrile seizure patients and c) febrile without seizures and controls. The Spearman correlation test was employed to calculate any significant correlations between cytokine levels. Values were expressed as means, and statistical significance of differences was set at $p<0.05$ for all tests.

RESULTS AND DISCUSSION

Patient characteristics

Table 1 summaries the patient's clinical data. The mean age of febrile seizure patients was 26.8±22.61 months. Boys were more prevalent than girls. This finding is consistent with previous study [1]. There was no significant different

in ages or gender between the 3 groups (Table 3 and 5). Lewis (2002) [16] with 41 children in each group, had no significant age difference between febrile seizure and controls [5]. In Fuadi *et al* (2010) [17] research, age was correlated between febrile seizure patients and controls (CI 1,39-8.30, p<0.01) [17].

There were significant differences in temperature (Table 2) between the 3 group (p<0.05). The mean temperature in febrile seizure group was 39,55 ± 0,49°C. From Table 5, there was significant correlation between high febrile temperatures and seizures (r=0.940; p<0.05). In Fuadi *et al* (2010) [17] found significant temperature difference (p<0.05) and temperature correlations between febrile seizure and healthy children (CI 2.33-10.83; p<0.01) [17].

Table 1. Clinical characteristics of febrile seizures, febrile without seizures and healthy children groups

	Clinical characteristics	Mean ± Standard Deviation	Sample
Temperature (°C)	Febrile seizures	39,55 ± 0,49	17
	Febrile without seizure	38,47 ± 0,16	17
	Healthy children	36,19 ± 0,16	17
Age (months)	Febrile seizures	26,82 ± 22,61	17
	Febrile without seizure	22,18 ± 19,48	17
	Healthy children	35,29 ± 19,28	17
IL-1β (pg/ml)	Febrile seizures	11,24 ± 13,07	17
	Febrile without seizure	7,54 ± 4,70	17
	Healthy children	4,72 ± 2,50	17
IL-10 (pg/ml)	Febrile seizures	258,61 ± 527,08	17
	Febrile without seizure	67,27 ± 51,95	17
	Healthy children	15,12 ± 6,02	17
IL-1β / IL-10	Febrile seizures	0,21 ± 0,38	17
	Febrile without seizure	0,15 ± 0,09	17
	Healthy children	0,35 ± 0,20	17

Table 2. Gender characteristics of febrile seizures, febrile without seizures and healthy children groups.

			Controls	
		Febrile seizures (n = 17)	Febrile without seizure (n = 17)	Healthy children (n = 17)
Gender	Male	12 / 17	14 / 17	13 / 17
	Female	5 / 17	3 / 17	4 / 17

p values were calculated by Mann-Whitney test

The IL-1β in febrile seizures (11.24±13.07 pg/ml) was higher than that in febrile without seizure (7.54±4.70 pg/ml) and healthy children groups (4.72±2.50 pg/ml) (Table 1). Comparisons of IL-1β levels between febrile seizure to healthy children and febrile without seizure to healthy children groups showed significantly higher than febrile seizure to febrile without seizure group (Table 4, p<0.05). IL-1β plasma levels were significantly correlated to febrile seizures (Table 5; r=0.335; p<0.05). These findings are consistent with the study which found that IL-1β

febrile seizure plasma patient levels were 12.0 ±5.3 pg/mL, significantly higher ($p<0.05$) than these for febrile without seizure group. Lewis *et al* (2002) [16] also found a significant positive correlation between IL-1β and febrile seizures [5].

IL-1β in febrile seizures (11.24±13.07 pg/ml) was higher than that in febrile without seizure (7.54±4.70 pg/ml) and healthy child groups (4.72±2.50 pg/ml) (Table 5). Comparisons of IL-1β levels between febrile seizure and healthy child groups showed significantly higher levels in febrile seizures (Table 6, $p<0.05$), but not between febrile seizure and febrile without seizure groups (Table 6, $p>0.05$). IL-1β plasma levels were significantly correlated to febrile seizures (Table 7; $r=0.77$; $p<0.01$). These findings are consistent with Lewis *et al* (2002) study which confirms that IL-1β febrile seizure plasma patient levels are 12.0±5.3 pg/mL, significantly higher ($p<0.05$) than these for febrile without seizure group and also find a significant positive correlation bet-ween IL-1β and febrile seizures [16].

Table 1. Comparisons of age, temperature, IL-1β, IL-10 and IL-1β/IL-10 between febrile seizure, febrile without seizures and healthy children groups.

			Controls		*p*
		Febrile seizures (n = 17)	Febrile without seizure (n = 17)	Healthy children (n = 17)	
Gender	Male	12 / 17	14 / 17	13 / 17	0,726
	Female	5 / 17	3 / 17	4 / 17	
Age (months)		26,8 ± 22,61	22,2 ± 19,48	35,3 ± 19,28	0,103
Temperature (°C)		39,55 ± 0,49	38,72 ± 0,40	36,19 ± 0,16	0,001
IL-1β (pg/ml)		11,24±13,07	7,54±4,70	4,72±2,50	0,015
IL-10 (pg/ml)		258,61±527,08	67,27±51,95	15,12±6,02	0,001
IL-1β / IL-10		0,21±0,38	0,15±0,09	0,35±0,20	0,001

p values were calculated using Kruskal-Wallis test

Table 2. Comparison of IL-1β, IL-10 and ratio IL-1β / IL-10 between febrile seizures to febrile without seizures, febrile seizures to healthy children and febrile without seizures to healthy children

	Febrile seizures	Febrile without seizure	*p*	Febrile seizures	Healthy children	*p*	Febrile without seizure	Healthy children	*p*
IL-1β	11,24±13	7,54±4,7	0,92	11,24±13,1	4,72±2,5	0,03	7,54±4,7	4,72±2,5	0,01
IL-10	258,61±527	67,27±52	0,18	258,61±527	15,12±6	0,00	67,27±52	15,12±6	0,01
IL-1β / IL-10	0,21±0,4	0,15±0,1	0,09	0,21±0,4	0,35±0,2	0,00	0,15±0,1	0,35±0,2	0,01

p value was calculated using Mann-Whitney test

The mean IL-10 level for febrile seizures was 258.61±527 pg/mL, that in febrile without seizures was 67.27±52 pg/mL, and that in healthy children was 15.12±6 pg/mL (Table 1). Comparisons of IL-10 levels between febrile seizure to healthy children and febrile without seizure to healthy children groups showed significantly lower than febrile seizure to febrile without seizure group (Table 4, $p<0.05$). IL-10 plasma levels were significantly correlated to febrile

seizures (Table 5; r=0.731; p<0.05). Straussberg *et al* (20012) also came to the conclusion, that IL-10 febrile seizure levels were significantly higher than those for control, but used short stature patients with-out fever, anemia, constipation and growth failure as controls [18]. Our finding agrees with [18] explanation that IL-10 levels increase few hours after febrile seizure because of IL-10 activity inhibits the production of IL-1β. This explanation could answer why there were significantly higher levels of IL-1β and IL-10 in the febrile seizure than healthy children groups [18].

Table 3. The correlations between the various ages, genders, temperatures, IL-1β, IL-10 and IL-1β/IL-10

	r	p
Ages	-0,209	0,142
Genders	0,057	0,693
Temperatures	0,940	0,001
IL-1β	0,335	0,016
IL-10	0,731	0,001
IL-1β / IL-10	0,573	0,001

Another study found the same increase in IL-1β and IL-10 and concluded that IL-1β and IL-10 may act as "enhancers" and "attenuators" of febrile seizure susceptibility. On the one hand, IL-1β may promote the fever response during acute infections, resulting in activation of temperature elevation and subsequent increased susceptibility to febrile seizure. On the other hand, IL-10 may suppress the fever response, resulting in inhibition of temperature elevation and subsequent decreased febrile seizure susceptibility. However, considering the results of animal experiments under high-temperature, it is possible that IL-1β and IL-10 may directly induce and reduce neuronal hyperexcitability, respectively, leading to decreased and increased seizure thresholds [19].

In the present study, IL-1β/IL-10 plasma levels were significantly correlated to febrile seizures (Table 5; r=0.573; p<0.05) These inflammation cytokine (IL-1β) and anti-inflammation cytokine (IL-10) ratio correlation was inconsistent with [16] findings, but [11] study used IL-1β as an inflammation cytokine and IL-1RA as an anti-inflammation cytokine [16].

CONCLUSION

In conclusion, there were significant differences and correlations between IL-1β and IL-10 as well as IL-1β/IL-10 in estimating the risk of febrile seizures. The methodological limitation was small sample size, as the numbers of febrile seizure patients and control in our study were only 17 for each group, further studies with larger groups are needed to confirm our findings. The IL-10 levels were higher in febrile seizures than in healthy children groups possibly because blood samples were taken late.

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