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ABSTRACT

Background and Objectives. The most common neurological emergency is status epilepticus (SE). The morbidity and mortality of SE are still high. Immune system activation and inflammation play a role in neuronal death in humans with SE. Interleukin 1β (IL-1β) and interleukin 6 (IL-6) are important pro-inflammatory cytokines in seizure susceptibility and brain cell death. This study aims to compare serum IL-1β and IL-6 levels of between children with SE and non-SE seizures.

Material and Methods. This study employed an analytic observational method at Dr. Soetomo General Academic Hospital Surabaya. The subjects were divided seizures into SE and non-SE seizures. The patients underwent a blood test after the initial assessment in the form of anamnesis, physical examination. Serum IL-1β and IL-6 levels were measured by using enzyme-linked immunosorbent assay (ELISA). Differences between the SE and non-SE groups were determined by using Fisher's exact test or the Mann–Whitney test.

Results. Forty patients participated in the study. In the SE seizure group, the median was 16 months, and the male gender was more prevalent (75%). The clinical manifestations that appeared more frequently in the SE group were fever and decreased consciousness. Most patients were alive at the end of the study: four patients in the SE group died. There

was no significant difference in serum IL-1β between the groups (P=0.372). However, serum IL-6 serum levels were significantly higher in the SE group compared with the non-SE group (P=0.001).

Conclusions Serum IL-1β levels did not differ between the SE and non-SE groups, but serum IL-6 levels were higher in the SE group.

Keywords: IL-1β, IL-6; Morbidity, Mortality, Status Epilepticus

Abbreviations:

SE : Status Epilepticus	MDD : Minimum Detectable Dose
IL-1β : Interleukin 1β	LIF : Leukaemia Inhibitory Factor
IL-6 : Interleukin 6	mRNA : Messenger RNA
TNFα : Tumour Necrosis Factor α	

INTRODUCTION

The most common neurological emergency is status epilepticus (SE). This condition is characterized by epileptic seizures that are sufficiently prolonged or recurrent at short enough intervals to produce an unvarying and long-lasting epileptic condition. The morbidity and mortality of SE are still high despite the implementation of seizure termination measures [1]. Short-duration seizures can cause neurotoxicity, and prolonged seizures cause neuronal damage [2]. Immune system activation and inflammation play a role in neuronal death in humans with SE and in animal models of this condition [3]. Interleukin 1β (IL-1β) and interleukin 6 (IL-6) are important pro-inflammatory cytokines in seizure susceptibility and brain cell death [4]. Cytokines can be used as biomarkers to detect the occurrence of neuroinflammation, which will cause brain cell damage [4].

The incidence of SE in children varies from 10 to 73 per 100,000 per year and is highest in children under 2 years of age, at 135–156 per 100,000 [5]. At Dr Soetomo Hospital Indonesia, there were 24 cases of SE in children in 2016 [6]. SE in rats and humans is followed by an increase in several pro-inflammatory cytokines such as IL-1β and IL-6, tumor necrosis factor-α (TNFα) in the nervous system. Increased inflammatory cytokines in glia lead to neuronal damage due to seizures [7], epileptogenesis [8], and neuronal network excitability [9]. The hypoxia-ischemia process occurring in SE seizures initiates apoptosis, excitotoxicity, and inflammation, leading to cell death [10]. This study aims to compare serum IL-1β and IL-6 levels of between children with SE and non-SE seizures.

MATERIALS AND METHODS

2.1 Design

This study employed an analytic observational method to measure serum IL-1β and IL-6 levels in subjects treated at Dr. Soetomo General Academic Hospital Surabaya from January 2017 to January 2018. The subjects were only observed without giving treatment. Data were collected prospectively on children with SE and non-SE seizures from admission to hospital discharge to assess the clinical outcome.

2.2 Subjects

The sample in this study comprises children aged 1 month to 12 years with SE and non-SE seizures admitted to the Emergency Department at Dr. Soetomo Surabaya and who met the criteria when the study was conducted. The patients were included if they were aged 1 month to 12 years, had SE or non-SE seizures, and were admitted to Dr. Soetomo General Academic Hospital Surabaya, and if their parents or guardians provided written informed consent for them to participate. Patients were excluded if they had a congenital brain disorder; post intracranial surgery; there were brain abnormalities with space descent processes such as intracranial tumors, cerebral hemorrhage, or hydrocephalus; and patients who received steroid therapy before blood sampling.

Seizures are typical clinical signs that are intermittent and paroxysmal; they can be focal followed by generalized. This study divided seizures into SE and non-SE seizures. SE seizures are sustained seizures lasting more than 30 minutes or two or more sequential seizures without recovery of consciousness between seizure episodes [11]. Non-SE seizures last no more than 5 minutes, or patients recover consciousness between two or more seizure episodes. The patients underwent a blood test after the initial assessment in the form of anamnesis, physical examination, and emergency treatment. The clinical outcome after discharge from hospital was alive or dead.

2.3 Measurement of serum IL-1β and IL-6 levels

Serum IL-1β and IL-6 levels were measured by using enzyme-linked immunosorbent assay (ELISA), specifically commercially available Quantikine® ELISA kits (R&D System, USA). The minimum detectable dose (MDD) of human IL-1β is < 1 pg/ml and the MDD of human IL-6 is < 0.7 pg/ml. Blood was collected immediately after the initial assessment in the emergency room.

2.4 Statistical analysis

Data were processed with SPSS Statistics Version 23 (IBM Corp, USA). Descriptive characteristics are presented the SE and non-SE groups. Differences between the SE

and non-SE groups were determined by using Fisher's exact test or the Mann–Whitney test, as appropriate. The statistical tests used are indicated in the table notes.

3.5 Ethics

The parents or guardians of all patients in this study provided written informed consent. The Health Research Ethics Committee of Dr. Soetomo General Academic Hospital Surabaya issued the ethical eligibility of the study with no 614/PANKE.KKE/XI/2016.

RESULTS

Forty patients participated in the study. Eight of them were excluded because their parents or guardians did not provide consent for participation, resulting in a total of 32 patients (Figure 1). In the SE seizure group, the median was 16 months, and the male gender was more prevalent (75%). Both groups had a good nutritional status and generalized seizures were more common (Table 1). The clinical manifestations that appeared more frequently in the SE group were fever and decreased consciousness. Most patients were alive at the end of the study: four patients in the SE group died.

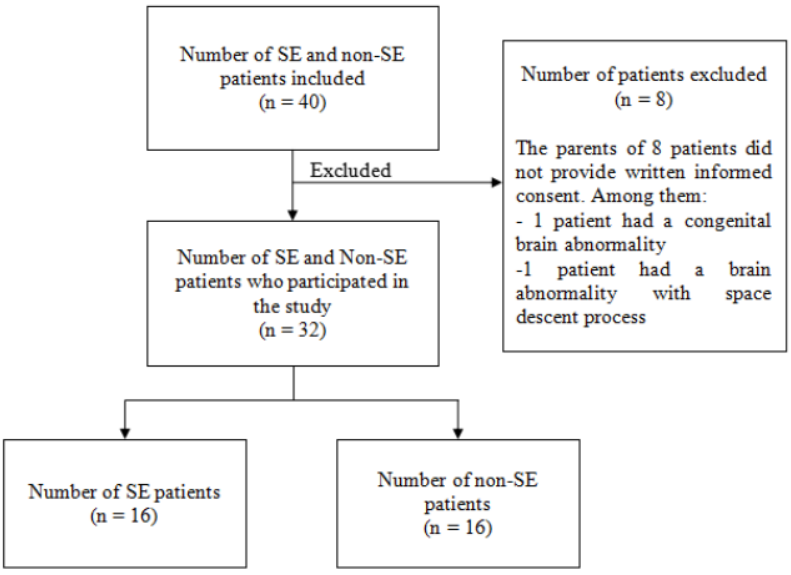

Fig. 1. Subject selection based on the inclusion and exclusion criteria in the study.
SE, Status Epilepticus

Table 1. Baseline characteristics of the subjects.

Characteristic	Status epilepticus [n=16]	Non-status epilepticus [n=16]	*P*
Age, median (range), month	16 (12–84)	8 (3–28)	0.058[2]

Sex, n (%)			
• Male	12 (75.0)	10 (62.5)	1.000[1]
• Female	4 (25.0)	6 (37.5)	
Anthropometry, median (range)			
• Weight (kg)	8.35 (7.5–20)	7.65 (5.2–10)	0.103[2]
• Height (cm)	77.5 (71–123)	69.5 (58–82)	0.074[2]
Nutritional status n (%)			
• Good	14 (87.5)	12 (75.0)	1.000[1]
• Malnutrition (severe and moderate malnutrition)	2 (12.5)	4 (25.0)	
Seizure type, n (%)			
• Generalized	13 (81.3)	12 (75.0)	0.467[1]
• Focal	3 (18.7)	4 (25.0)	
Recurrent seizures, n (%)	12 (75.0)	10 (62.5)	0.200[1]
Previous history of seizure, n (%)	2 (12.5)	3 (37.5)	0.569[1]
Fever	10 (62.5)	8 (50.0)	0.077[1]
Decreased level of consciousness	16 (100)	2 (12.5)	0.001[1]
Lecocytosis	12 (75.0)	10 (62.5)	0.200[1]
Time of blood sampling (hours), median (range)	4.5 (1–18)	4.5 (3–18)	0.672[2]
Outcome			
• Alive	12 (75.0)	16 (100.0)	0.007[1]
• Dead	4 (25.0)	0 (0.0)	

[1]Fisher's exact test

[2]Mann-Whitney U test

There was no significant difference in serum IL-1β between the groups (Table 2). However, serum IL-6 serum levels were significantly higher in the SE group compared with the non-SE group (Table 3).

Table 2. Serum interleukin 1β (IL-1β) levels

	n	Median (range)	Mean (SD)	*P*
Status epilepticus	16	7.85 (3.2–81.1)	17.68 (26.04)	0.372*
Non-status epilepticus	16	14.6 (5.5–24.2)	14.36 (7.42)	

*Mann–Whitney U test

Table 3. Serum interleukin 6 (IL-6) levels

	n	Median (range)	Mean (SD)	*P*
Status epilepticus	16	170.3 (23.3–442.4)	220.6 (188.8)	0.001*
Non-status epilepticus	16	10.4 (1.6–24.5)	10.7 (6.8)	

*Mann–Whitney U test

DISCUSSION

There was no difference in serum IL-1β levels between the SE and non-SE group. There is some evidence of increased expression of pro-inflammatory cytokines in the brain after seizures in experimental studies in experimental animals. IL-1β, TNFα, IL-6, and leukemia inhibitory factor (LIF) messenger RNA (mRNA) is expressed in the hippocampus, cerebral cortex, hypothalamus, and striatum of experimental animals 1–3 hours after experiencing SE seizures due to kainic acid induction [12]. Peak IL-1β mRNA expression is reached at 12 hours and peak IL-1ra expression is reached at 12–14 hours after SE seizures induced by kainic acid [13]. On the other hand, peak IL-6 mRNA expression occurs after 12 hours in the SE model with pilocarpine induction [14]. IL-1β mRNA expression begins within 1 hour after hypoxia, and peak levels are reached in 4–12 hours; then, within 24 hours, IL-1β returns to normal levels [15].

Research on peripheral cytokine levels after seizures in humans is currently limited to febrile seizures and epilepsy. A meta-analysis on febrile seizures analyzed four research articles including 114 patients with febrile seizures and 199 patients with fever without seizures (controls). The authors found no significant difference in serum IL-1β levels between the groups (standardized mean difference [SMD] 0.624, 95% confidence interval [CI] -0.561 to 1.809, P = 0.302) [16].

The results of the present study are also similar to several previous studies that found no difference in IL-1β levels after seizures [17–20]. Peltola et al. [19] compared plasma cytokine levels of patients 72 hours after primary generalized seizures or secondary tonic-clonic generalized seizures with those patients who had experienced seizures more than 2 weeks earlier. There was no difference in IL-1β levels due to the short half-life of the cytokine.

In the present study, the mean serum IL-6 levels were significantly higher in the SE group compared with the non-SE group. In their meta-analysis on epilepsy, Yu et al. [21] examined plasma IL-6 levels from eight research articles including 261 subjects with epileptic seizures and 564 control subjects. There was a significant difference between the groups (SMD 1.27 pg/ml, 95% CI 0.72–1.82, P<0.0001). Another meta-analysis evaluated 10 research articles with 386 patients with epilepsy and 463 control subjects. Plasma IL-6 levels in the epileptic seizure group were significantly higher than in non-epileptics. The SMD was 1.084 (95% CI 0.646–1.523, P = 0.000) [16].

Increased plasma and cerebrospinal fluid IL-6 levels have been found in patients with epilepsy after a seizure compared with healthy controls [17,18,22]. Lehtimaki et al. [18] proved that elevated plasma IL-6 levels are directly related to seizures. An investigation on febrile seizures and acute encephalopathy in children showed that cytokine levels change dynamically during the early stages, within 72 hours of onset. Most cytokines increase immediately after onset, and some cytokines (IL-1β, IL-4, IL-6, IL-8, and IL-17), including pro-inflammatory cytokines, peak within 12 hours after onset and decrease to approximately control levels within 48 hours after onset [23]. Fadl et al. [24] reported a significant difference in IL-6 and IL-1β between febrile status seizures and control group. Genetic risk factors for febrile seizures in children may be related to promoter variation in the IL1B gene rather than the IL6 or HMGB1 genes.

This research has several limitations that may be considered when conducting similar future research. First, the time of blood collection in this study was not the same, and there was no periodic check of serum IL-1β and IL-6 levels. Blood was collected simultaneously with examinations for other diagnostic processes when the subject was in the emergency care facility. Second, there are numerous confounders that are difficult to control, such as the variety of underlying diseases that cause acute symptomatic SE seizures, the presence of other diseases as comorbid factors, and several complications.

CONCLUSION

Serum IL-1β levels did not differ between the SE and non-SE groups, but serum IL-6 levels were higher in the SE group. Additional research is recommended to evaluate the role of IL-1β and IL-6 in the prognosis of patients with SE.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR'S CONTRIBUTIONS

Conceptualization, P.I.G., R.N., S.M.S; methodology, P.I.G., R.N.; validation, P.I.G; formal analysis, P.I.G., R.N., S.M.S; investigation, R.N., S.M.S.; data curation, P.I.G.; writing—original draft preparation, P.I.G., R.N., S.M.S; writing—review and editing, P.I.G., R.N., S.M.S; visualization, P.I.G., R.N; supervision, P.I.G; project administration, P.I.G., R.N., S.M.S. All authors have read and agreed to the published version of the manuscript.

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