

## Differences in the Levels of Interleukin-1 $\beta$ (IL-1 $\beta$ ) and Interleukin-1 Receptor Antagonist (IL-1ra) in Children with Status Epilepticus and Febrile Seizure

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### ABSTRACT

Proinflammatory cytokines are elevated in status epilepticus and febrile seizure and associated with tissue damage. This study aimed to investigate the differences in interleukin 1 beta (IL-1 $\beta$ ) and interleukin 1 receptor agonist (IL-1ra) levels in status epilepticus as compared with febrile seizure and febrile. This cross-sectional study was designed to include 45 subjects divided equally into three groups (status epilepticus, febrile seizure, and febrile). Both IL-1 $\beta$  and IL-1ra were measured by using an ELISA method. Results showed that IL-1 $\beta$  levels were significantly higher in the status epilepticus group as compared with the febrile seizure and febrile groups ( $p < 0.05$ ). IL-1ra levels in the status epilepticus group were significantly lower compared with the febrile seizure group ( $p = 0.04$ ). Consistently, the IL-1 $\beta$ /IL-1ra ratio in the status epilepticus group was substantially higher as compared with the febrile seizure group ( $p = 0.01$ ). We concluded that IL-1 $\beta$  and the IL-1 $\beta$ /IL-1ra ratio were significantly higher in status epilepticus. IL-1ra levels were considerably greater in the febrile seizure group

**Keywords:** Febrile seizure, IL-1 $\beta$ , IL-1ra, status epilepticus

### INTRODUCTION

Status epilepticus is defined as a seizure that lasts for more than 30 minutes. Status epilepticus in children is an emergency condition that can be life-threatening. The incidence rate of status epilepticus is 10 – 58 per 100 000 people per year in the US population. The annual incidence rate of status epilepticus in children is 20 per 100,000 people in developing countries [1, 2].

A seizure is a clinical manifestation of neurologic dysfunction and is usually found in the emergency room. In the pediatric population, seizures are mostly caused by febrile seizure [3]. A febrile seizure is defined as a seizure associated with fever and age without intracranial infection or impairment of the central nervous system [4]. The peak incidence of febrile seizure occurs at 18 months of age (age range 3 months to 5 years) [4, 5]. The incidence of febrile seizure in US and Europe ranges from 2% to 5%, whereas in the Asian population,

it is twice that of the US and Europe (Japan 8.3%–9.9%, India 10.1%, and Guam 14%) [8]. The mortality rate of febrile seizure is 0.64% – 0.75%, and most patients recover without neurologic sequelae [4, 6].

Recently, abnormalities in proinflammatory cytokine expression were found in patients with seizures [7, 8]. An imbalance of pro- and anti-inflammatory cytokines could lead to progressive damage of the brain parenchyma and could be clinically related to complications [9]. Both febrile seizure and status epilepticus are distinguished on the basis of clinical manifestation. However, the prognosis in status epilepticus is worse than that in febrile seizure [10].

Interleukin-1 (IL-1) is a proinflammatory cytokine that has been studied and reflects inflammation of the central nervous system [9, 11]. A previous study showed that increased levels of interleukin 1 beta (IL-1 $\beta$ ) and interleukin-1 receptor antagonist (IL-1ra) were associ-

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ated with seizure and febrile conditions [9, 11]. Another research demonstrated that IL-1 $\beta$  and IL-1ra levels were elevated in patients with febrile seizures compared with controls [7]. This study aimed to investigate the differences in IL-1 $\beta$  and IL-1ra levels in status epilepticus as compared with febrile seizure.

## MATERIALS AND METHODS

This was a cross-sectional study designed to compare IL-1 $\beta$  and IL-1ra levels in patients with status epilepticus and febrile seizure. This study was conducted in the emergency ward of the Pediatric Department, Dr. Saiful Anwar Hospital Malang, and the Biochemistry Laboratory, Faculty of Medicine, Brawijaya University Malang. This study was approved by the Ethical Committee of the Faculty of Medicine, Brawijaya University (400/86/K.3/302/2015).

### Population and subjects

The research subjects included in this study were divided into 15 patients per group. Inclusion criteria were as follows: having status epilepticus, febrile seizure, or febrile and permission from the subject's parents to join this study (informed consent). Criteria for inclusion in the status epilepticus group were seizures that lasted for more than 30 minutes or 2 minutes or more seizures with decreased consciousness between seizures, and age > 1 month or < 18 years. Criteria for inclusion in the febrile seizure group were simple or complex febrile seizures. The criterion for inclusion in the febrile patient groups was body temperature  $\geq 38.5^{\circ}\text{C}$ . Criteria for exclusion from the febrile seizure group were severe clinical conditions and previous chronic disease.

### Blood sampling

Blood sampling was performed in the emergency room and pediatric ward of dr. Saiful Anwar Public Hospital Malang. Blood sampling was performed by a well-trained nurse. A 2-mL venous blood sample was mixed with EDTA as an anticoagulant, stored in a cooler box, and maintained at  $4^{\circ}\text{C}$ . The mixture of venous blood and EDTA was immediately transported to the Biomedical Laboratory, Medical Faculty of Brawijaya University. In the laboratory, specimens were centrifuged at 1000 rpm for 30 minutes. Plasma (supernatant) was separated from blood cells (pellet) and stored for a later experiment.

### Measurement of IL-1 $\beta$ and IL-1ra

Both IL-1 $\beta$  and IL-1ra levels were measured by using

an ELISA (enzyme-linked immunoassay) method. After all serum samples from 24 patients had been collected, measurement of IL-1 $\beta$  and IL-1ra was performed. The extracellular antigen of IL-1 $\beta$  and IL-1ra were dissolved in coating buffer and 50  $\mu\text{L}$  was dispensed into an ELISA microplate (1 : 50) and incubated at  $4^{\circ}\text{C}$  overnight. The antigen suspension was washed twice with buffer solution (PBS-T) for 5 min each and incubated with 1% PBS-T for 45 minutes. After this process, the antigen was washed twice for 5 minutes each. The next step was incubation with anti-IL-1 $\beta$  and anti-IL-1ra primary antibody for 60 minutes. The suspension was removed, and the plate was washed twice with buffer solution for 5 minutes each. The next step was incubation with secondary antibody (anti-mouse) for 60 minutes. The suspension was removed, and the plate was washed twice with phosphate buffer solution for 5 minutes each, followed by addition of streptavidin-horseradish peroxidase (SA-HRP) and then incubated for 60 minutes. The reaction was washed twice with buffer solution for 5 min each. Tetramethylbenzidine (TMB) substrate was added to the microplate and then incubated for 30 minutes. The reaction was stopped by addition of 1 N HCl for 15 minutes. The absorbance was read at a wavelength of 492 nm in an ELISA microplate reader [16].

### Statistical analysis

Levels of IL-1 $\beta$  and IL-1ra were analyzed for distribution and homogeneity. Differences of IL-1 $\beta$  and IL-1ra levels in the status epilepticus group, febrile seizure group, and the febrile group was analyzed by using ANOVA (confidence interval 95%). Data were analyzed by using software SPSS (statistical product and service solution) for Windows version 16.0.

## RESULTS AND DISCUSSION

The normal mature erythrocyte of *E. cyanophlyctis* is an oblong-oval shape with a centric nucleus (Figure 1a). In the present study, we observed five majors nuclear Baseline characteristics

In this study, we included 45 subjects divided equally into three groups (status epilepticus, febrile seizure, and febrile groups). As described in Table 1, 17 subjects were male, and the rest were female. The average age of subjects was 29.4 months (2 years, 6 months) in all groups. Good nutritional status was present in 24 of 45 subjects. Average seizure duration in the status epilepticus group (55 minutes) was longer than in the febrile seizure group (4.3 minutes). Eleven

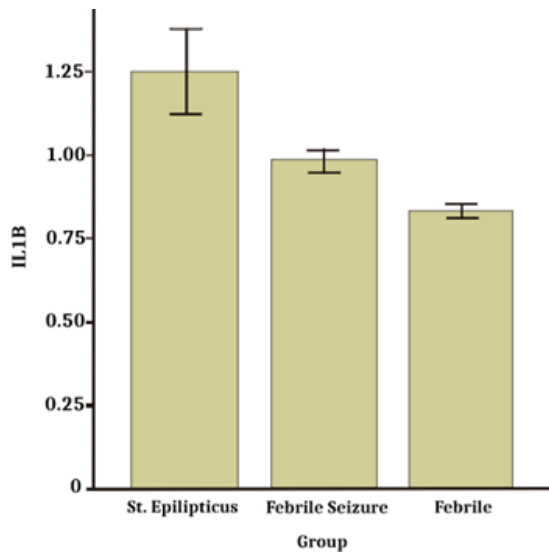
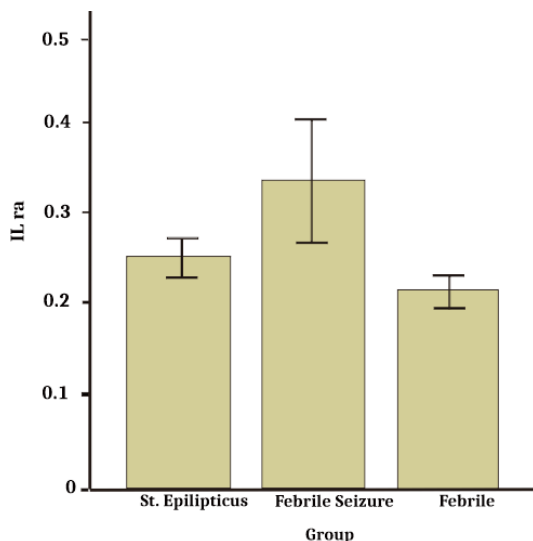
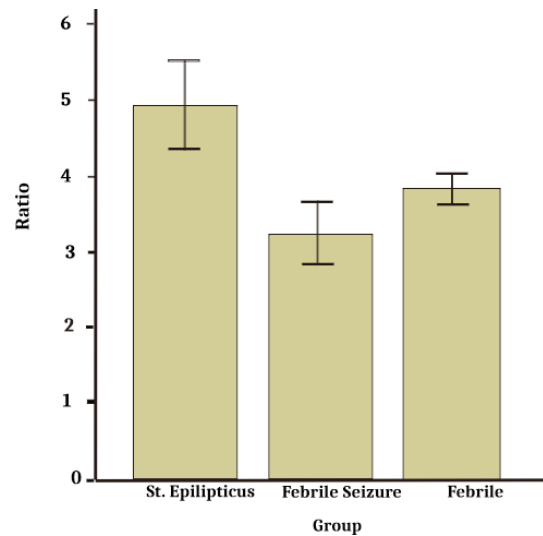
Figure 1. The level of IL-1 $\beta$  (pg/mL) in each group

Figure 2. The level of IL-1ra (ng/mL) in each group

of 15 subjects in the status epilepticus group died, but no one died in the febrile seizure group.

This was a cross-sectional study design including 45 subjects divided equally into three groups: status epilepticus, febrile seizure, and febrile groups. In the seizure group, 12 of 30 subjects were male, whereas, in the febrile seizure group, 8 of 15 subjects were female. A previous study showed similar characteristics [7]. The average age in this study was 29.4 months. In the febrile seizure group, the average age of subjects was 24.2 months. These data were in accordance with a previous study [7]. Febrile seizures often occur in children aged 17 – 23 months. In early life, neuronal cells in the brain tissue are in a developmental window; therefore, the febrile condition as a traumatic insult could provoke a seizure [7, 10].

Figure 3. The level of IL-1 $\beta$ /IL-1ra (ng/mL) in each group

### Levels of IL-1 $\beta$ and IL-1ra

Results showed that IL-1 $\beta$  levels were significantly higher in the status epilepticus group than in the febrile seizure and febrile groups (one-way ANOVA,  $p < 0.05$ ) (Figure 1). IL-1ra levels in the status epilepticus group were significantly lower than in the febrile seizure group (one-way ANOVA,  $p < 0.05$ ) (Figure 2). Consistently, the IL-1 $\beta$ /IL-1ra ratio in the status epilepticus group was significantly higher than in the febrile seizure group (one-way ANOVA,  $p < 0.05$ ) (Figure 3).

This study showed that IL-1 $\beta$  levels in the status epilepticus group (1.25 pg/mL) were significantly higher than in the febrile seizure (0.98 pg/mL) and febrile groups (0.83 pg/mL). However, in both groups, IL-1 $\beta$  levels were elevated above the reference value of the normal level (0.3 pg/mL). These data were similar to a previous study, which demonstrated that IL-1 $\beta$  levels were higher in febrile seizure. Viruses as being increasingly implicated as causative agents of febrile seizures. Neurotropic viruses, such as the herpesviruses and influenza A, are commonly associated with febrile seizures in the United States and Asia. Fever induced by viral infection is regulated by components of the immune response, particularly proinflammatory cytokines. Proinflammatory cytokines are higher in influenza-associated febrile seizures, further suggesting a causative role for cytokines in the pathogenesis of febrile seizures, its make level of IL-1 $\beta$  was higher than others [9], epileptic seizure [8], and status epilepticus [12] conditions. Heida and colleagues showed that IL-1 $\beta$  levels were elevated in peripheral inflammation in both infection and an LPS-induced animal model [12]. In this study, in most cases of status epilepticus, the ethology was meningoencephalitis.

Zhang and colleagues also showed that HFMD cases with clinical meningoencephalitis caused by viral infection (enterovirus and coxsackie virus) showed higher levels of IL-1 $\beta$  than those without meningoencephalitis [13]. A previous study demonstrated that bacterial meningoencephalitis (meningococcus or pneumococcus) caused an elevation of IL-1 $\beta$  [8]. IL-1 $\beta$  might be associated with tissue damage, which is the underlying cause of morbidity and mortality in meningoencephalitis [14].

This study also showed that IL-1ra levels were elevated in all groups (reference value 0.2 ng/mL). However, IL-1ra levels in the febrile seizure group were significantly higher than in the status epilepticus group. Other types of seizure, such as epileptic seizure, have shown an elevation of IL-1ra levels [8]. However, Virta and colleagues showed different results regarding IL-1ra levels. They demonstrated that both IL-1ra levels and the IL-1ra/IL-1 $\beta$  ratio were significantly higher in the febrile seizure group than in the control group [15]. Theoretically, elevated IL-1ra in febrile seizure might be caused by compensatory mechanisms of body systems that act to stop IL-1 $\beta$  action and thus prevent prolonged and recurrent seizure. Scheld and colleagues showed elevated IL-1ra levels in the meningoencephalitis group [14]. Griffiths and colleagues also demonstrated that IL-1ra levels were lower in HFMD with viral encephalitis (enterovirus) as compared with HFMD without encephalitis [11]. Later data indicated that IL-1ra might have the beneficial effect of decreasing the severity of disease associated with overexpression of IL-1 $\beta$  [11]. Consistent with previous results, the IL-1 $\beta$ /IL-1ra ratio was significantly higher in the status epilepticus group than in the febrile seizure group. An elevated IL-1 $\beta$ /IL-1ra ratio was also shown in other types of seizure, such as epileptic seizure [8], febrile seizure [7], and viral meningoencephalitis [11].

The IL-1 $\beta$ /IL-1ra ratio in the status epilepticus group was significantly higher than in the febrile seizure and febrile groups. The lowest IL-1 $\beta$ /IL-1ra ratio was shown in the febrile seizure group. These data were similar to a previous study by Virta, which demonstrated that the IL-1 $\beta$ /IL-1ra ratio was decreased in a febrile seizure [15]. Another also demonstrated that the IL-1 $\beta$ /IL-1ra ratio was decreased in the febrile group compared with the febrile seizure group [7].

## CONCLUSION

We concluded that IL-1 $\beta$  and the IL-1 $\beta$ /IL-1ra ratio were significantly higher in the status epilepticus group compared with the febrile seizure and febrile groups. IL-

1ra levels were substantially greater in the febrile seizure group compared with the status epilepticus group.

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