RESEARCH ARTICLE

THE ROLE OF TNF-α AND IL-6 CYTOKINE IN CHILDREN WITH STATUS EPILEPTICUS

Agung Prasetyo Wibowo, Hidayat Sujuti**, Masruroh Rahayu***, Masdar Muid****, Siti Lintang Kawuryan*****

*Magister Program in Biomedical Sciences Faculty of Medicine Brawijaya University, Malang, Indonesia
**Department of Biochemistry Faculty of Medicine Brawijaya University, Malang, Indonesia
***Department of Neurology Faculty of Medicine Brawijaya University, Malang, Indonesia
****Department of Pediatric Faculty of Medicine Brawijaya University, Malang, Indonesia

pISSN : 2407-6724 ● eISSN : 2442-5001 ● http://dx.doi.org/10.21776/ub.mnj.2018.004.02.2 ● MNJ.2018;4(2):53-58
● Received 22 April 2017 ● Reviewed 19 February 2018 ● Accepted 22 February 2018

ABSTRACT

Background. One of pediatric emergencies that has high mortality is status epilepticus (SE). Correlation between tumor necrosis factor (TNF)-α, interleukin (IL)-6, and SE had been reported but human study of it is limited.
Objective. To compare TNF-α and IL-6 level in children with SE to those of children without SE and to find correlation between both cytokines.
Methods. Cross-sectional study was conducted in dr.Saiful Anwar Hospital Malang with 48 children were enrolled in this study. All subjects were divided into three groups, including children who had SE; children who had seizure but not SE; and children who have no seizure. The levels of TNF-α and IL-6 serum were measured by ELISA.
Results. TNF-α and IL-6 serum level were not significantly different between groups (p=0.920, p=0.829). We found interesting fact that the level of IL-6 in children with SE who have no disability was significantly higher than that of children who died or had disability (p=0.015). There was strong correlation between TNF-α and IL-6 in SE group (R^2 = 0.841 and p = 0.0001).
Conclusion. IL-6 serum level was higher in SE children who have no disability and correlate with TNF-α serum level.

Keywords: TNF-α, IL-6, status epilepticus, children

Correspondence: dokterwee@gmail.com

INTRODUCTION

Status epilepticus (SE) is one of the most common pediatric emergencies that contribute high morbidity and mortality in children.[1] Incident of SE is about 10-17/100,000 to 23-58/100,000 children a year.[2] Incident of fatal SE is about 3-15%.[3] Prolonged seizure is difficult to treat with pharmacological intervention because brain damage can already happen in prolonged seizure.[4] Because of this reason, some researchers tried to explore pathophysiological process of prolonged seizure in order to find alternative therapy of it.

Tumor necrosis factor (TNF)-α is one of cytokines that had been studied in pediatric seizure recently, especially in SE.[4,5] This cytokine acts as pro-inflammatory agent or anti-inflammatory one that depends on which receptor that is influenced.[6] Many animal studies showed that TNF-α expression was increased in animal brain during SE.[6,7,8] It has association with seizure through activation of p55 receptor[5], increasing of AMPA, one of glutamate receptors[9], activation of endotelin-1 and p65 NF-kB that cause brain edema.[10,11] However, the other animal studies showed that TNF-α can protect animal from seizure through p75 activation and rearrangement of AMPA structure.[12,13] Unfortunately, Choi *et al.* research, the only one human study, showed that serum TNF-α in children with afebrile SE was slightly higher than this of control group.[14] Interestingly, serum TNF-α is also increased in febrile convulsion children and epilepsy ones, and can act as endogenous pyrogen.[5,15,16,17,18]

Interleukin (IL)-6 is the other cytokine that had been studied in pediatric seizure not only in SE, but also in non SE seizure, such as febrile convulsion and epilepsy.[4,5] It also can act as endogenous pyrogen.[18] Like TNF-α, IL-6 has dual role: as pro-inflammatory agent or anti-inflammatory one.[19] Moreover, IL-6 acts as neuroprotectan in the brain by protecting brain tissue from excitotoxicity and oxidative stress, inhibiting TNF-α and diapedesis of netrofil, and promoting adaptive immune system and brain healing.[20] However, the role of IL-6 in SE is in debate. Previous animal studies showed that IL-6 expression in brain was increased in animal SE[7] and seizure was prolonged and became severe after intranasal IL-6 administered.[5] The other studies showed that there was no difference between plasma IL-6 in rat SE group and rat control group[21]; and glutamate sensitivity was increased in IL-6 knock out mice.[20] Sinha *et al.* showed that there was correlation between serum IL-6 and SE in adult[17], but Choi *et al.* showed that this correlation was absent in children.[14] Most studies in children with febrile convulsion or epilepsy, showed that serum IL-6 in these children was higher than this of control.[14,17,20,23,24] It seems that IL-6 has more role in seizure non SE than SE itself. Human study that correlates IL-6 with SE is limited too.

The aims of this study were to compare TNF-α and IL-6 level in children with SE to those of children without SE, and to find correlation between these cytokines in children with SE Our hypothesis, these cytokine had higher level in children who has SE, and correlated each other in children with SE.

METHODS

Research design. This study was cross-sectionally design to compare level of TNF-α and IL-6 in three groups: first group who had SE and second group who had seizure but not SE, and third who had no seizure. The study was conducted since July 2015 until January 2016 in pediatric ward Saiful Anwar General Hospital Malang and Physiologic Department, Faculty of Medicine, Brawijaya University Malang. This study had been approved by Ethical Committee of Saiful Anwar General Hospital Malang.

Population and Subject. Sixteen subjects were included in each group of this study. All groups had age between 1 to 14 year old, and had been allowed by his/her parents to joint this study. All children in the first group met SE criteria according to International League Against Epilepsy 1981: a single seizure or recurrent seizures lasting for more than 30 minutes during which consciousness is not regained.[1] All children in the second group had seizure but did not meet SE criteria, such as febrile convulsion and epilepsy. We called this second group as control 1. The third group had no seizure at all, and called as control 2. Exclusion criterias of all groups were autoimmune disease such as SLE, nephrotic syndrome, AIHA; malignancy; severe malnourishment and immunocompromized conditions such as HIV infection. Age, gender, presence of fever (body temperature more than 38°C), and the etiology of seizure were recorded. We also recorded the outcome of children in SE group after one month therapy, such as mortality and disability.

Blood Sampling and Cytokine Measurement. Peripheral blood samples were collected from pediatric ward or emergency ward, 12-24 hours after the last seizure. The blood samples were placed into EDTA vacutainer, and centrifuged to get supernatan part or serum. The serum was placed then into eppendorf tube and stored at -20°C refrigerator. After all sample had been collected, cytokine measurement was performed. Both TNF-α and IL-6 were measured by ELISA method based on biotin double antibody sandwich technology. We used Human TNF-α ELISA kit and Human IL-6 ELISA kit that produced by Bioassay Technology Laboratory.

Statistical Analysis. We used Chi square, Fisher exact test, or ANOVA to compare clinical characteristic between all group. ANOVA test was used to compare level of TNF-α and IL-6 serum between groups. For these 3 variables, the data was express as means. Linear regression analysis was used to find correlation between TNF-α and IL-6 in SE group.

We used SPSS 16 program to analyze the data. Statistical difference was set at $p<0.05$ for all the tests.

RESULTS

Characteristics of Subjects. The study involved 48 subjects, 16 subjects in each group. The characteristics of the subjects are described in Table 1.

Correlation between TNF-α and IL-6 in SE group. The correlation between level of TNF-α and IL-6 in SE group was shown in Figure 3. There was positive correlation between TNF-α and IL-6 in the SE group (R^2 = 0,841 and p = 0.0001). It means that the higher TNF-α.

DISCUSSION

There were many differences of characteristics of the subjects in this study: age, etiologies of seizure, and presence of fever. This facts might be caused by 'the dominancy' of febrile convulsion as the most common etiology in control 1 (14/16) and epilepsy in SE group (7/16). The peak incidence of febrile convulsion is at 17-23 months old children[24], while epilepsy is older than 4 years old.[2,25] According to Ng *et al.*, about twenty percent of status epilepticus is caused by epilepsy.[25] However the incident of epilepsy in SE group in this study was higher.

The average of age of subjects in SE groups was higher than in the previous study. In Salim *et al*. (2018), the average of age of subjects in SE groups was 23 month.[26] These facts might be also caused by 'the dominancy' of epilepsy as the most common etiology in SE group in our study, so that the average of age became higher.

The level of TNF-α and IL-6 serum in all groups were similar. These facts are resembled to the result in the previous study. In Choi *et al.* (2011) research, the level of TNF-α and IL-6 in afebrile SE children were not different significantly ($p<0.05$) than those of control, eventhough the level of TNF-α in SE group was slightly higher than this of control one.[14] But the facts of our study were different from the facts in almost animal studies.[5,6,7,8] Unfortunately we didn't separate the type of SE of our subjects, it was febrile or afebrile. The level of TNF-α might be higher in febrile SE than in control because infection processes could enhance the expression of TNF-α in the brain.[27]

Our study was the first study that showed correlation between TNF-α and IL-6 serum in children with SE. The previous study said that in inflammation process, TNF-α could enhance the forming of IL-6[28]; while IL-6 could block synthesis and activation of TNF-α.[20,29] We thought that inflammation was happened in the brain while SE happened, eventhough the etiology of SE was not always an infection of central nervous system.

Interesting facts in this study was that the level of IL-6 in children who did not have any disability was significantly higher than children who died or had any disability, in children with SE. These facts resembled to the previous animal studies. This cytokine could protect brain of rat from damage that caused by stroke, by mediating STAT3 dan Mn-SOD pathways.[30] Another study said that IL-6 deficient-mice had more severe brain damage after having kainic acid-inducted seizure.[31] Our study was the first human study that showed this condition.

This study had some limitations. First, we used serum TNF-α and IL-6 for the sample not TNF-α and IL-6 of liquor cerebrospinalis (LCS). The most animal studies that shown correlation between these cytokines and SE showed the cytokines expression in the brain tissue not in plasma so that it is better if we use LCS for this kind of study than using serum. Second, we did not control fever as the confounding factor. These two cytokines are also increased at fever condition because both

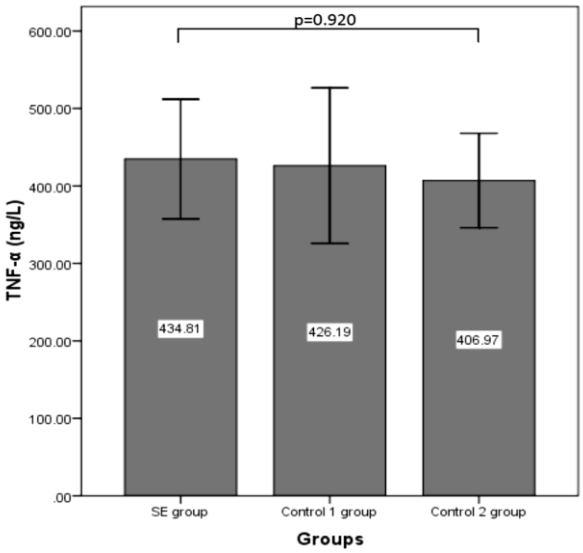
cytokines have role as endogenous pyrogen [18]. We did not control this confounding factor because we thought febrile SE was the most common form in SE. We should find match subject in control group for each subject in SE group to get better results. Logistic regression could be used to control this factor but it may need greater number of samples.

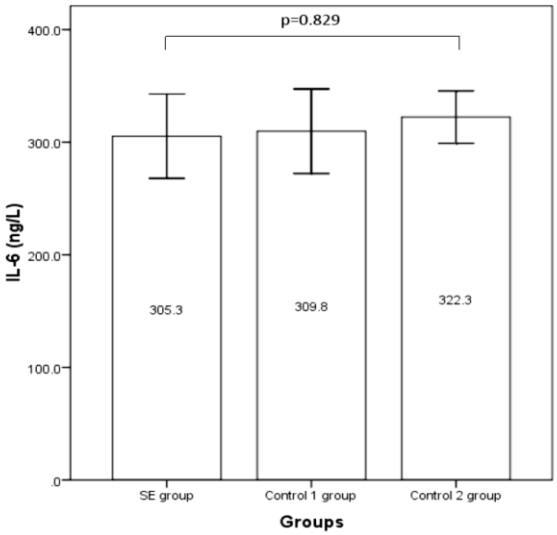
Table 1. Characteristic of the Subjects

Parameters of the Subjects	SE group (n=16)	Control 1 group (n=16)	Control 2 group (n=16)	p-value
Age (month), mean ± SD	62.25±43,20	26.25±18,70	79.5±56,09	0.001***
Gender				0.556*
Male, n	7 (7/16)	10 (10/16)	9 (9/16)	
Female, n	9 (9/16)	6 (6/16)	7 (7/16)	
Seizure etiologies				0.001**
Febrile seizure, n	1 (1/16)	14 (14/16)	0	
CNS infection, n	7 (7/16)	0	0	
Epilepsy, n	7 (7/16)	2 (2/16)	0	
Hydrocephalus, n	1 (1/16)	0	0	
Non CNS infection,n	0	0	13 (13/16)	
Hematological disorder, n	0	0	2 (1/16)	
Heart disorder, n	0	0	1 (1/16)	
Fever, n	9 (9/16)	16 (16/16)	11 (11/16)	0.013**

CNS= Central Nervous System, n= number, x = mean, SD = standard of deviation (*) = analysis with Chi square test, (**) = analysis with Fisher test, (***) = analysis with ANOVA test, significant p-value is if p-value<0.05.

Comparison of TNF-α and IL-6 serum level between groups. The comparison of TNF-α and IL-6 serum level between groups was shown in Figure 1. There was no significant difference of TNF-α, IL-6 serum level between group (p=0.920 and 0.829 respectively).





Comparison of TNF-α and IL-6 serum level between SE outcome subgroup. We divided children in SE group into 2 subgroups based on the outcome: children who died or had disability and children who had no disability at all. The level TNF-α in both subgroup were similar (p=0.051). Interesting facts in this study was that the level of IL-6 in children who did not have any disability was significantly higher than children who died or had any disability (p=0.015) (Figure 2).

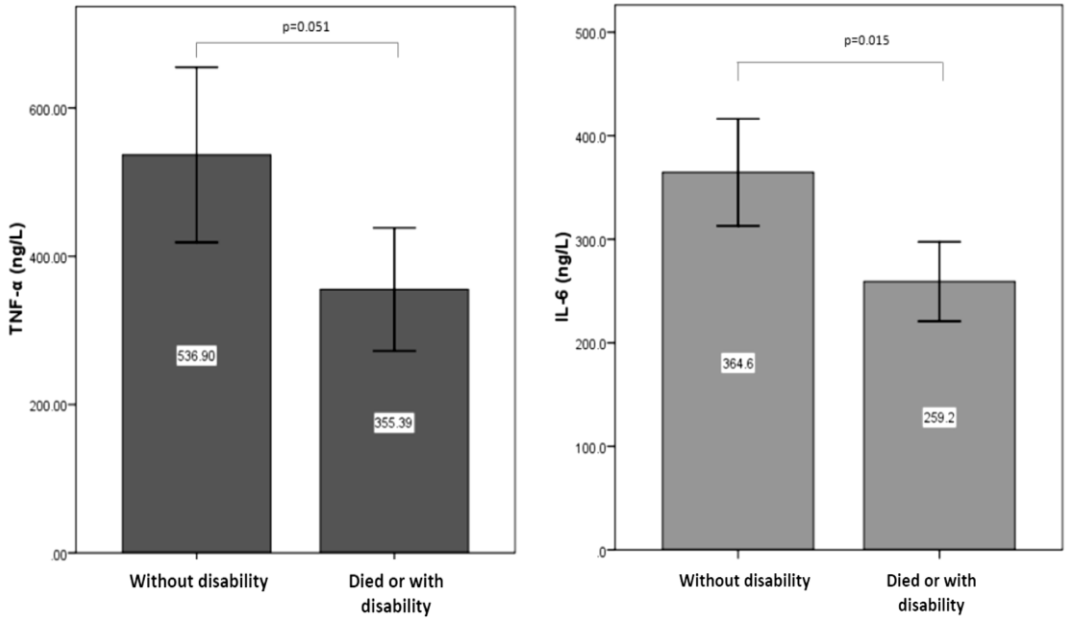


Figure 2. Comparison of TNF-α and IL-6 serum level in chlidren who died or had any disability compared to children without disability in SE group.

CONCLUSION

We conclude that there is no significant difference between TNF-α and IL-6 in children who have SE and who did not have. Those cytokines correlate each other in children with SE and IL-6 may have neuroprotective effect in these children. Further study which has better method is needed to get better result.

REFERENCES

1. Behera MK, Rana KS, Kanitkar M, Adhikari KM. Status Epilepticus in Children. MJAFI 2005; 61: 174-178
2. Mastrangelo M, Celato A. Diagnostic work-up and therapeutic options in management of pediatric status epilepticus. World J Pediatr 2012; 8(2): 109-115
3. Pujar SS, Neville BG, Scott RC, Chin RF. Death within 8 years after childhood convulsive status epilepticus: a population-based study. In: Mastrangelo M, Celato A. Diagnostic work-up and therapeutic options in management of pediatric status epilepticus. World J Pediatr 2012; 8(2): 109-115
4. Miskin C, Hasbani DM. Status epilepticus: Immunologic and inflammatory mechanism of epilepsy. Seminars in Pediatric Neurology 2014: 1-15. DOI:http://dx.doi.org/10.1016/j.spen.2014.09.001
5. Li G, Bauer S, Nowak M, *et.al.* Cytokines and epilepsy. Seizure 2011; 20: 249-256
6. Balosso S, Ravizza T, Aronica E, Vezzani A. The dual role of TNF-α and its receptors in seizures. Experimental Neurology 2013; 247: 267-271
7. Rizzi M, Perego C, Aliprandi M. Glia activation and cytokine increase in rat hippocampus by kainic acid-induced status epilepticus during postnatal development. Neurobiology of disease 2003; 14: 494-503
8. Johnson EA, Kan RK. The acute phase response and soman induced status epilepticus: temporal, regional and cellular changes in rat brain cytokine concentrations. Journal of neuroinflammation 2010; 7(40): 1-9
9. Bernardino L, Xapalli S, Silva AP, *et.al*. Modulator effects of interleukin-1 beta and tumor necrosis faktor alfa on AMPA-induced excitotoxitiy in mouse organotypic hippocampal slice cultures. The Journal of Neuroscience 2005; 25(29): 6734-6744
10. Kim JE, Ryu HJ, Kang TC. Status epileptikus Induces vasogenic edema via tumor necrosis factor-alfa/endotelin-1 mediated two different pathways. Plos One 2013; 8(9): e74458-e74471

11. Kim JE, Ryu HJ, Choi SY, Kang TC. Tumor necrosis factor alfa mediated threonine 435 phosphorylation of p65 nuclear factor-kB subunit in endothelial cells induces vasogenic edema and neutrophil infiltration in the rat piriform cortex following status epilepticus. Journal of Inflammation 2012; 9: 1-13
12. Balosso S, Ravizza T, Perego C, *et.al*. Tumor necrosis factor alpha inhibits seizures in mice via p75 receptors. In: Li G, Bauer S, Nowak M, *et.al.* Cytokines and epilepsy. Seizure 2011; 20: 249-256
13. Stellwagen D, Beattie EC, Seo JY, Malenka RC. Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-alpha. In: Li G, Bauer S, Nowak M, *et.al.* Cytokines and epilepsy. Seizure 2011; 20: 249-256
14. Choi J, Min HJ, Shin JS. Increased levels of HMGB1 and pro-inflammatory cytokines in children with febrile seizures. Journal of Neuroinflammation 2011; 8(135): 1-9
15. Haberlandt. Proinflammatory cytokines in children with febrile seizures. Neuropediatrics 2008; 39: V11
16. Nurindah D, Muid M, Retoprawiro S. Hubungan antara Kadar *Tumor Necrosis Factor Alpha* (TNF-α) Plasma dengan Kejang Demam Sederhana pada Anak. Jurnal Kedokteran Brawijaya 2014; 28: 115-119
17. Sinha S, Patil SA, Jayalekshmy V, Satishchandra P. Do cytokines have any role in epilepsy? Epilepsy Research 2008; 82: 171-176
18. Abbas AK, Lichtman AH. Cellular and Molecular Immunology, Fifth edition. Elsevier 2007:303-306
19. Scheller J, Chalaris A, Arass DS, John SR. Review: The pro and anti-inflammatory properties of the cytokine interleukin-6. Biochimica et Biophysica Acta 2011: 878-888
20. Erta M, Quintana A, Hidalgo J. Interleukin-6, a Major Cytokine in the Central Nervous System. Int J Biol Sci 2012; 8(9): 1254-1266
21. Holtman L, van Vliet EA, Aronica E. Blood plasma inflammation markers during epileptogenesis in post-status epilepticus rat model for temporal lobe epilepsy. Epilepsia 2013; 54: 589-595
22. Ichiyama T, Suenaga N, Kajimoto M, *et al*. Serum and CSF levels of cytokines in acute encephalopathy following prolonged febrile seizures. Brain & Development 2008; 30: 47-52
23. Uludag IF, Bilgin S, Zorlu Y, Tuna G, Kirkali G. Interleukin-6, interleukin-1 receptor antagonist levels in epileptic seizures. Seizure 2013; 22: 457-461
24. Al-Ajlouni SF, Kodah IH. Febrile convulsions in children. Neuroscience 2000; 5: 151-155
25. Ng YT, Maganti R. Status epilepticus in childhood. J Paediatr Child Health 2013; 49(6): 432-437
26. Salim, I., Muid, M., & Sujuti, H. Levels' Influence of IFN-γ and IL-10 in Children with Epilepticus Status. Malang Neurology Journal, 4(1), (2017). 19-24. doi:http://dx.doi.org/10.21776/ub.mnj.2018.004.01.4
27. Santoso, G., Sujuti, H., & Hidayati, D. The Effect of Infection of of Mycobacterium Tuberculosis Strain H37RV towards the Expression of TNF- α in the Brain. Malang Neurology Journal, 3(1), (2017). 12-16. doi:http://dx.doi.org/10.21776/ub.mnj.2017.003.01.3
28. Tanabe K, Nishiwaki RM, Yamaguchi S, Iida H, Dohi S, Kozawa O. Mechanism of tumor necrosis factor-α-induced interleukin-6 synthesis in glioma cells. Journal of Neuroinflammation 2010; 7: 1-8
29. Starkie R, Ostrowski SR, Jauffred S, Febraio M, Pedersen BK. Exercise and IL-6 infusion inhibit endotoxin-induced TNF-α production in humans. The FASEB Journal 2003: 1-10
30. Jung JE, Kim GS, Chan PH. Neuroprotection by IL-6 Is Mediated by STAT3 and Antioxidative Signaling in Ischemic Stroke. Stroke 2011; 42: 3574-3579
31. Penkowa M, Molinero A, Carrasco J, and Hidalgo J. Interleukin-6 deficiency reduces the brain inflammatory response and increases oxidative stress and neurodegeneration after kainic acid-induced seizures. Neuroscience 2001: 805-818