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INTERLEUKIN-1β, INTERLEUKIN-6, AND ANTAGONIST INTERLEUKIN-1RECEPTOR AS MEMORY IMPAIRMENT RISK FACTOR IN COMPLEX PARTIAL EPILEPSY

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ABSTRACT

Memory impairment is one of the most common adverse following epilepsy, particularly complex partial epilepsy. Cytokines physiologically play an important role in memory impairment by preventing long term potentiation process in hypocampus. Several literatures have mentioned that IL-1β, IL-6 and antagonist receptor IL-1Ra are crucial cytokines in complex partial epilepsy. This study aims to find out whether high level of IL-1β and IL-6 as well as low level of IL-1Ra might be risk factors of memory impairment in complex partial epilepsy patient.

This was a case control study, enrolling 30 complex partial epilepsy patients with memory impairment as case group and 30 complex partial epilepsy patients without memory impairment as control group.

In this study, it was obtained that the mean of IL-1β level in case group was significantly higher compared to the control (2.74 ± 4.36 vs. 0.42 ± 0.18 pg/ml, p = 0.007). The mean of IL-6 in case group was significantly higher compare to control (5.89 ± 6.32 vs. 2.34 ± 1.80 pg/ml, p = 0.006). The mean of IL-1Ra level of the case group was not significantly higher compared to the control (519.81 ± 262.64 vs. 413.28 ± 106.85, p = 0.767). By applying bivariate analysis, McNemar's test, we observed that IL-1β with cut off point 0.63 pg/ml and OR = 70 is a risk factor of memory impairment in complex partial epilepsy indicated by p = 0.001. Similar result was also gained for IL-6 with cut off point 2.87 pg/ml and OR = 4.57 as a risk factor of memory impairment in complex partial epilepsy indicated by p = 0.007. Meanwhile, IL- 1Ra with cut off point 471 pg/ml and OR = 0.727 was not as a risk factor of memory impairment in complex partial epilepsy indicated by p = 0.573.

It can be concluded that the high level of IL-1B and IL-6 were the risk factors of memory impairment in complex partial epilepsy patients. High level 1L-1B patient was 70 times higher risk of becoming memory impaired. High IL-6 patients will have the risk nearly 5 times higher. The low level of IL-1Ra does not as a risk factor in epilepsy patients for having the following memory impairment.

Keyword: Memory, impairment, complex partial epilepsy, IL-1β, IL-6, IL-1Ra.

INTRODUCTION

Epilepsy is a condition of unprovoked recurrent epileptic seizures at least 24 hours apart. Epileptic seizure manifests abnormal electrical discharge brain dysfunction. Prevalence of epilepsy based on epidemiology study was in the range of 0.5% - 2%. Assuming that Indonesian population is 250.000.000 hence the number of people with epilepsy will reach 1.250.000 – 5.000.000.[1]

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Epilepsy causes brain dysfuction which in turn emerge some following neurological ailments such as mental disorders and social problems. Individuals with epilepsy will deal with work place, litigation, education, career and marriage issues as following social problems of their illness.

The effect on intellectual development of epilepsy in majority would be living with cognitive function and behaviour problems. Furthermore, epilepsy patients will live with social stigma attached to being abnormally low intelligence. A study of 30 generalized and 14 partial epilepsy patients which are on antiepilepsy drugs (AED) had been conducted by Sidiarto, Neurology Departement of FK UI/RS CM. This

study concluded that 75% of epilepsy patients have retrieval memory impairment and AEDs do not interfere with memory impairment. The National Child Development Study, United Kingdom in 1985 mentioned that impaired memory as one of cognitive function has decreased intellectual ability in 30 patients with epilepsy and cognitive function began to gain more concern lately in 20 century. The advance in neuroscience particularly in molecular biology has drawn great attention of researchers to investigate on epilepsy which in turn disclosed the role of neurotransmitter in epilepsy.

Glutamate has been known as excitatory neurotransmitter in pathophysiology of epilepsy, influx of Ca ion will occur in the excited post synaptic neuron, result in depolarisation. If this processes overexcite the neuron, the seizure occurs. Beside Glutamate the other neurotransmitter is Gamma Amino Butiric Acid (GABA). GABA acts as an inhibitory neurotransmitter through Ion Cl^- channel induces by hyperpolarisation of the post synaptic membrane to complete inhibition process.[2,3]

Despite its role in the pathophysiology of epilepsy, neurotransmitter has significant role in the memory and learning processes. Based on the model researches, memory process (to recall) is formed by the synapse activities through repetitive stimulation (exprience-dependent synaptic trnsmission). These result in phosphorilation of protein membrane and followed by Long Term Potentiation (LTP) in Hypocampus.[2,4]

Individuals with epilepsy release an excessive amount of glutamate by which create neurotoxicity and damage to the neurons. The injured neurons release proinflammatory cytokine particularly IL-1, IL-6 and TNF-α. IL-1ß subset of IL -1 is a proconvulsive cytokine and it interfere with LTP by disrupting protein kinase activity and calcium-calmodulin dependent protein kinase II (Ca MK II). Disrupting this process will prevent the synapses potentiation and LTP process in creating memory. At first these affect the immidiate and short term memory and later eventually affect longterm memory.[5,6] In normal condition IL-1 and IL-1Ra have an activity as an inhibitory and equally maintained. Decrease of IL-1Ra will be followed by decrease of inhibitory activity.[4,7,8]

During seizure glutamate is released excessively, since glutamate induce neurotoxicity, this will lead to neuronal and glial (astrocyte) injury followed by proinflammatroy cytokine release such as large amount of IL-1ß and IL-6. This leads to increase of antagonist receptor IL-1Ra expression. In certain condition when increase of IL-1ß and IL-6 did not followed by increase of antagonist receptor of IL-1Ra, the LTP proscess in hypocampus will be disrupted as well as the formation of memory. Hence seizure cascade result in memory impairment. In certain condition if the increase of IL-1ß and IL-6 is not equall with increase of antagonist receptor of IL-1Ra, It will result in memory impairment. This study aims to find out whether increase of IL-1ß, IL-6 and decrease of IL-1Ra will impair the memory of complex partial epilepsy individual.

PATIENTS AND METHOD

The study design is match pair case control study, aimed to find out whether the increase of IL-1ß, IL-6 and decrease of IL-1Ra are the risk factors of memory impairment in patient with partial complex epilepsy.

Sample of the study were 60 complex partial epilepsy patients who fulfilled the inclusion and exclusion criterions. They were recruited from the reachable representative population in Neurology out patient clinic of Sanglah General Hospital and Private practice office for 14 months. They were selected as the sample after they agreed to participate in this study. They were asked for doing some tests such as story recall memory test and mini mental status test for the patients who had been suffering from epilepsy for over 6-8 months without an episode of seizure.

The sample were classified into 2 groups: 30 complex partial epilepsy patients with memory impairment as the case group, and 30 complex partial epilepsy patients without memory impairment as the control group. These groups were matched according to age, length of suffering epilepsy, type of AED and education.

The level of IL-1ß, IL-6 and IL-1Ra were measured in each group. Serum was taken from blood samples after they were left to clot in room temperature for about 30-45 minutes and centrifugation in 3000 RPM/15 minute. The level of IL-1ß was examined by ELISA sandwich (pg/ml). The cut-off point for the highest IL-1ß was determined by using ROC curve since there has not been a confirmed standard level for IL-1 ß yet. The level above the cut-off point was categorized as high and below the cut-off point was categorized as low. According to ROC curve the cut-off point for IL-1ß is 0.63 pg/ml (sensitivity is of 93% and specificity is of 83%). Research material is 15 cc of blood samples which were taken from cubital vein. The samples were left in room temperature until they clotted (30-45 minutes) and to be centrifuged in 3000 RPM/15 minute. The blood serum was taken and kept in 9 cups. The cups were labeled, 3 of them for IL-1ß examination, 3 more for IL-6 examination, and the last 3 for IL-1Ra examination using ELISA sandwich method (pg/ml).

Data obtained were statistically analyzed by comparative study. The normal dispersion data was tested by t-independent test, abnormal dispersion data was tested by Mann-Whitney U test. McNemar test was done to analyze the odd ratio of each non-dependent variable. To analyze the odd ratio of all the

variables simultaneously, Logistic Regression test of Multivariate test was conducted. All statistic analyze was performed in SPSS for windows program.

RESULTS

Base line data were listed in Table 1. As can be seen in Table 1 age, length of suffering epilepsy and education were normally distributed (p>0.05).

Table 1 Base line Data of Subject

Characteristic	Groups		t	p
	Case	Control		
Age (years)	23.43±8.18	21.93±7.44	0.743	0.460
Length of epilepsy (month)	7.00±0.91	7.07±0.94	0.278	0.782
IL-1β (pg/mL)	2.74±4.36	0.42±0.18	2.91	0.007
IL-6 (pg/mL)	5.89±6.32	2.34±1.80	2.96	0.006
IL-1Ra (pg/mL)	440.85±154.9	413.28±106.8	430	0.767

The level of IL-1ß, IL-6 are significantly different between case and control group (p<0.05), and no significant difference between case and control in the level of IL-Ra (p>0.05).

Chi-square test was applied to analyze the significance of increased IL-1ß, IL-6, and IL-1Ra as the risk factors of memory impairment, as seen in Table 2. The cut-off point level of IL-1ß is 0.63 according to the ROC curve (sensitivity of 93% and specificity of 83%), and categorized as high level if the level is ≥ 0.63.

Table 2 The risk of high level of IL-6 to the incidence of memory impairment

Parameter		Groups		OR	CI 95%	p*
		case	control			
IL-1β	High	25	2	70	12.46-393.36	0.001
	Low	5	28			
IL-6	High	16	6	4.57	1.45-14.39	0.007
	Low	14	24			
IL-1Ra	High	20	22	0.73	0.24-2.21	0.573
	Low	10	28			

*significance at p < 0.05

Table 2 shows that the increase of IL-1ß ≥ 0.63 (high IL-1ß) might be able to increase the incidence of memory impairment 70 times higher in partial complex epilepsy patient (OR = 70; CI 95%: 12.46 – 393.36); p = 0.001).

The cut-off point level of IL-6 is 2.87 according to the ROC curve (sensitivity of 80% and specificity of 53%), and categorized as high level if the level is ≥ 2.87. The risk of high level of IL-6 to the incidence of memory impairment

Table 2 shows that the increased level of IL-6 ≥ 2.87 (high IL-6) might be able to increase the incidence of memory impairment in complex partial epilepsy patient 5 time higher (OR=4.57; CI 95%: 1.45-14.39; p = 0.007)

The risk of low IL-1Ra level to the incidence of memory impairment was analyzed by applying Chi-square test. The result shows in Table 2, IL-1Ra cut-off point is 471.00 (sensitivity of 73% and spesifisity of 63%) applied according to the ROC curve. Low level of IL-1Ra is 471.00. Table 2 shows that IL-1Ra ≤ 471.00 (low IL-1Ra) does not increase the risk of incidence of memory impairment in complex partial epilepsy patient with OR is 0.73 s higher (RO= 0.727; CI 95% : 0.24-2.21;0.573).

Bivariate analysis revealed two variables which are IL-1ß and IL-6, significantly related to the incidence of memory impairment in partial complex epilepsy. Furthemore these two variables were analysed in depth to figure out which of them affect more to the memory impairment. The analyses was conducted as follow : risk variables are ; X1 = IL-1ß, and X2 = IL-6. The analysis was performed simultaneously using logictic regression of multivariate analysis, the Backward Wald method, a selection method of dependent and independent variables of memory impairment. The result is shown in Table 3.

Table 3 Multivariate analysis

Risk factor	OR	CI 95 %		p
		Low limit	Upper limit	
IL-1β	63.86	10.24	398.40	0.001
IL-6	1.28	0.21	7.91	0.791

Table 3 reveals that only one variable is significant to increase the incidence of memory impairment. This risk factor is IL-1ß (OR = 63.86: CI 95%: 10.24 – 398.40, p = 0.001). The multivariate analysis revealed that in causing memory impairment among partial complex epilepsy patient, IL-1ß is more important than IL-6 .

DISCUSSION

Mean of IL-1ß level between case and control groups were different significantly (case 2.74 ± 4.36 pg/ml, control 0.42 ± 0.18 pg/ml, p = 0.007), with McNemar test OR is 70 (CI 95%: 12.46 – 3393.46, p = 0.001). The meaning of this result is that of increased IL-1ß level is the risk factor of memory impairment in partial complex epilepsy and individuals with high level of IL-1ß have the risk to become memory impaired 70 times higher compared with low level of IL-1ß individuals. The research result supports the theory of high level of IL-1ß will impair the memory prosessing. Other investigator, Bruno Conti (2008) in his immunohistochemistry study on mice showed that there was 50 – 100 fold increase of IL-1ß level in first 90 minutes of seizure and kept maintained at this level for a long time.[9] An animal study by Annamaria & Baram (2007), obtain similar result that IL-1ß played significant role in creating cognitive disfunction in provoked seizure animals.[7,10-12] High level of IL-1ß is obtained as the risk factor of memory impairment,

hence the result of this study might be able to be applied on epilepsy patient to prevent and detect earlier the possibility of following memory impairment, by examining the level of IL-1ß in partial complex epilepsy patients serum.

This study shows that mean of IL-6 level between case and control was significantly different (case 5.89 ± 6.32 pg/ml, control 2.34 ± 1.80 pg/ml, p = 0.006). McNemar test revealed OR = 4.57 (CI 955: 1.45 – 14.39; p = 0.007). This result indicates that high level of IL-6 is the risk factor for memory impairment in complex partial epilepsy patient. This will increase the risk nearly 5 fold compared with the low level of IL-6. The result also supports the theory that high level of IL-6 will impair the memory process. Giovanna et al (2000), according to his study on mice, said t IL-6 provoked seizure in previously healthy mice. In accordance with this result, examining the level of IL-6 might be used as a marker to detect and prevent memory impairment.[13]

The level of IL-1Ra in partial complex epilepsy patients with memory impairment were higher compared with without memory impairment (case 519.81 ± 262.64 pg/ml, control 413.28 ± 106.85 pg/ml, p = 0.125)but the result was not statistically significant with p = 0.05. The result indicated that Low level of IL-1Ra is not the risk factor of memory impairment and failed to support the theory. The reason of this might be because the samples of research were not in seizure when their blood sample were taken. In non seizure state, the expression of IL-1Ra can not be used as the marker. IL-1ß and IL-6 remained high during non seizure state because the degenerative process was not ceased.

CONCLUSION

High level of IL-1ß and IL-6 were determined as risk factors of memory impairment in complex partial epilepsy patients, high level of IL-1ß increase the risk to 70 times higher. Complex partial epilepsy patients with high level of IL-6 increase the risk to impair memory nearly 5 fold higher. Low level of IL-1Ra in epilepsy patient does not risk the patient to have memory imparment.

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