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Correlation of Serum Glutamate Level and IL-1β on Encephalopathy and Sepsis in Premature Infant

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Abstract Premature birth worldwide still high, in United State was 1-5% and 15.5 in 100 live birth in Indonesia. Premature babies are vulnerable to critically illness especially infections and brain injury. Major problem in brain injury are placed at white matter, in term encephalopaty of prematurity. Ischemic condition will cause glutamate excitation. In many research before have proved that there is correlation between brain and serum glutamate. Intrauterine infection in premature babies have impact to brain with the excretion of pro inflammatory cytokine such as IL-1β. Sample was obtained from 72 premature babies (under 36 weeks GA) birth in June to August 2016 in Hasan Sadikin General Hospital Bandung. This was comparative analysis with cohort approach using logistic regression analysis to determine level of glutamate and IL-1β with minimal sample 63. All premature baby which met inclusion criteria were having blood examination according to algorithm. Median glutamate serum level in premature with encephalopathy higher (67.9µg/mL) than premature without encephalopathy (33.9µg/mL). Median level of IL-1B serum in premature encephalopathy and sepsis (8,67ρg/mL) were higher than premature without encephalopathy (12.3ρg/mL) as well as sepsis (1.7ρg/mL).It was found/revealed in this study that every increase in a unit of glutamate serum level of a premature neonate means 1.04 times risk to arise encephalopathy, while the increase of IL-1β level in the first 24 hours means 1.33 times risk to arise sepsis with 95% confidence interval..

Keywords: *brain injury, glutamate, premature, encephalopaty, hypoxia*

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1. Introduction

In 2010 the World Health Organization stated that premature births in Indonesia were 15.5 of 100 live births that placed Indonesia as the 9th of 184 countries with high premature births. [1] The number of premature births in Dr. Hasan Sadikin Hospital in 2014 and 2015 were 21-28% of the live births. [1,2,3] Short term morbidity referring to the premature births might be in the form of sepsis, hyaline membrane disease (HMD), bronchopulmonary dysplacia, necrotizing enterocolitis (NEC), patent ductus arteriosus (PDA), retinopathy of prematurity (ROP), periventricular and intraventricular hemorrgage PVIVH), and periventricular leucomalacia (PVL). The long term risk on premature are neurodevelopmental disorder. [4-9] The rate of the disorders is in the opposite rate of gestational age. The lower the gestational age, the higher the neurodevelopmental disorder rate is. [9] The premature baby are sensitive against brain disorders. About 25-50% of premature will have behavioral, social and or learning disorder. The most important problem in premature is the brain injury on the white matter (WM) area. [4-11] The abnormality of the WM area is called encephalopathy of prematurity. [12,13,14] A study by Kugelman et al revealed that compared to aterm neonates, the risk of developmental disorders might still frequently occur on neonates with 34-36 weeks gestational age (late preterm). [13]

Infection/inflammation and hypoxic-ischemia are potentially strengthen each other the disorders in physiologic process of the development of the neonate's brain. A trial on the brain of premature animal sample revealed that infetion and inflammation caused the disorder or the neuron and glia. [3,4,13-19] It was known that glutamate and IL-1B have an important role in the occurence of hypoxic-ischemia and infection/ inflammation that will cause clinical symptoms such as encephalopathy and sepsis. [4-10,11-19]

The aim of the study was to find out the relation of serum glutamate level and IL-1B with encephalopathy and sepsis as the results of hypoxia and inflammation on premature baby.

2. Methods

The study took place during May to August 2016. The subjects were premature baby birth with ≤36 weeks gestational age, birth weights <2000 grams born at Dr. Hasan Sadikin Hospital Bandung.

This was a comparative cohort analytic study, by examining the glutamate serum level and IL-1B at age 24 hours postnatal, then the occurence of sepsis and enephalopathy were recorded at age days-3. Other data

recorded were gestational age, birth weights, sex, complete clinical and laboratory examination at age 24 hours.

Encephalopathy is defined when there was one or more abnormal symptoms in at least three of six following categories: level of consciousness, spontaneous activity, posture, response against sound, primitive reflexec (sucking or Moro), and autonomic neural system (pupil reflex, heart beat, or respiration). [11,20,21,22] The diagnosis of sepsis was based on Tolner scoring; value score >10 means sepsis. [23-28]

The relation of glutamate serum level and IL-1B towards sepsis and encephalopathy was analysed by doublelogistic regression. Mean statistical test was based on p-value <0.05. The minimum sampel amout needed was 63 premature neonates, based on 5% significancy level, power test 90%, and mean clinical correlation 0.4. Serum glutamate examination using ELISA method with Microplate Reader Biorad model 680 (Labor Diagnostika Nord GmbH & Co.KG, Nordhorn, Germany). IL-1B examination using Quantikine ELISA Human (R&D System, Inc., Minneapolis, USA).

3. Results

During the study there were 112 premature births, 80 of which fulfilled the inclusion and exclusion criteria of the study, the parents of 8 neonates refused to be included in the study so we had 72 subjects.

The subjects characteristics is shown in Table 1.

The total of male and female patients were almost similar. The birth weight rates of 72 subjects were 1487.5 grams and gestational age were 28-36 weeks.

Table 2 shown the correlation between glutamate and IL-1β serum with subject.

Table 1. Characteristics of the subjects (n=72)

Characteristics	Statistical		
	average (SD)	Median	Rentang
1. Sex:			
Male31 (43%)			
Female 41 (57%)			
2. Birth weight (gram)	1487,5 (262,4)	1505,0	850 – 1900
3. Gestational age (week)	31,6 (1,1)	32	28 – 36

Table 2. Correlation of glutamate serum level and IL-1β with subjects characteristics

Correlation with	Glutamate		IL-1β	
	r	*p*	r	*p*
1. Sex	0,012	0,920	-0,071	0,553
2. Gestational age (week)	-0,463	<0,001	-0,276	0,019
3. Birth weight (gram)	-0,483	<0,001	-0,111	0,354
4. Asphyxia	0,219	0,065	0,216	0,068

Note: r = Correlation of Spearman rank coefficient, except sex and asphyxia using Biserial

Point correlation

Table 2 shows that sex has no significant relation with glutamate level and IL-1B; while festational age has negative correlation with glutamate level and IL-1B. Birth weight has significant correlation only with glutamate level, the higher the birth weight, the lower the glutamate level is.

Table 3 shows the median glutamate serum level is statistically significantly different in premature with clinical symptoms of encephalopathy compared to without encephalopathy, while those with and without clinical symptoms of sepsis are not different, but neonates with sepsis showed higher glutamate level compared to those without sepsis. Neonates with clinical symptoms of sepsis or encephalopathy had higher median compared to those without sepsis and encephalopathy, and the difference was statistically significant (p<0.005).

Table 3. The relation of glutamate level with encephalopathy and sepsis

	Glutamate (µg/mL)			IL-1β (ρg/mL)		
	Median	**Rate**	***p**)**	**Median**	**Rate**	***p**)**
1. Sepsis :						
Yes (n = 25)	62	9,6-166,5	0,279	8,67	0,87-21,0	< 0,001
No (n = 47)	39,1	14,7-162		1,4	0,38-19,01	
2.Encephalopathy:						
Yes(n = 34)	67,9	9,6-166,5	< 0,001	8,67	0,77-21,0	0,046
No (n = 38)	33,9	14,7-78		2,3	0,38-18,50	

Note: * = based on Mann-Whitney.

Table 4. The double logistic regression analysis of glutamate level, IL=1B, and other confounding with clinical outcomes of premature neonates

Variable	coefisien B	SE (B)	P	OR_{adj} (CI 95%)
I. Correlation with ensephalopaty:				
Glutamate	0,041	0,015	0,005	1,04 (1,01-1,07)
IL-1β	0,050	0,058	0,385	1,05 (0,94-1,18)
Gestational age(<32 mg)	0,484	0,899	0,590	1,62 (0,28-9,45)
Birth weight (< 1500 g)	0,891	0,682	0,191	2,44 (0,64-9,28)
Asphyxia (+)	0,309	0,604	0,609	1,36 (0,42-4,45)
II. Correlation with sepsis :				
Glutamate	0,007	0,011	0,520	1,01 (0,98-1,03)
IL-1β	0,286	0,073	<0,001	1,33 (1,15-1,54)
Usia gestasi (<32 mg)	3,206	1,163	0,006	24,69 (2,53-241,14)
BBL (<1500 g)	1,550	0,808	0,055	4,71 (0,97-22,95)
Asphyxiia (+)	0,963	0,716	0,179	2,62 (0,64-10,67)

Based on the results mentioned above (on those results), to find out the relation of glutamate and IL-1β levels with encephalopathy and sepsis, we used double logistic regression by including gestational age variability, birth weight and asphyxia as confounding factor because the bivariable analysis resulted p-value <0.25, that will shown in Table 4.

The logictic regression analysis in Table 4 showed that from the five variables analyzed, only the glutamate level had significant relation with encephalopathy (p=0.005) OR = 1.04 means by every change in one unit of glutamate level, the risk for encephalopathy arised 1.04 times. The result of double logistic regression analysis were two significant variables for IL-1β and gestational age (p<0.05). OR = 1.33 means by every cgange in one unit IL-1β level, the risk for sepsis arise 1.33 times, and gestational age (≥32 weeks), with OR = 24.69 means the neonate with ≥32 weeks gestational age has 34-69 times risk to have sepsis compared to prematures with <32 weeks gestational age.

4. Discussion

Premature neonates are sensitive towards brain injury. [4-9] Pervious study revealed that the lower the gestational age, the higher the risk for prenatal hypoxia and inflammation that might increase infection and brain injury. [4-9] Similarly, in this study we found that gestational age and birth weight had negative correlation that means, the lower the gestational age, and the lower the birth weight, the glutamate serum level and IL-1β will be higher.

Glutamate is the mostly found amino acid in the brain and becomes the main exitation transmitter. [11-19] In hypoxic-ischemic condition, the ATP decreases and depolarisation of the membrane, exitotoxicity of glutamate, were binded by the receptor and reach the cells through the receptor. At the same time the CBF decreases, resulted in anaerobic glycolysis and lactate production that caused metabolic acidosis and increase of intracellular calcium concentration. This process will increase the extracellular glutamate receptor that will cause exitotoxic process. [16,17,18,19,29-41] A study by Campos et al on amimal samples using Magneting Resonance Imaging (MRI) revealed that the decrease of glutamate level in the blood was significantly related with the decrease of glutamate level in the brain. [33]

Similarly with previous studies, in this study we found that the neonate that was diagnosed as encephalopathy at the third day had highr glutamate serum level (67.9µg/mL) than premature neonate without encephalopathy (33.9µg/mL). The previous study found the glutamate concentration in the extracellular fluid (ECF) about 0.3/0.5-2 µmol/L. The glutamate was diffused into the blood through electrochemical gradient and reach the plasma in 40 µmol/L. [15,17,18,29] Campos etal, in a study on animal samples using MRI found the decrease of blood glutamate level was in accordance (was similar) with the decrease of brain glutamate level and was significantly related with neurological improvement. [33] Other study found that the increase of serum glutamate had a relation with the increase of brain glutamate that caused glutamate exitatory and might cause brain injury in the neonate. [42-51]

Previous study revealed that asphyxia/hypoxia might increase exitotoxic glutamate level as well as induce the inflammation reaction on the fetus that will stimulate the cytokin forming. [8,42,43,44,45] Accordingly, this study revealed a higher median of IL-1B serum level in prematures diagnosed as encephalopathy and or sepsis on the third day (8,67ρg/mL) compared with neonates without encephalopathy (12.3ρg/mL) as well as sepsis (1.7ρg/mL). Inflammation and ischemia may have a role in the process of brain injury. Infection/inflammation and hypoxic-ischemia are potential to strengthen each other in making disorders in the physiologic process of brain development of the fetus that might cause injury of the white matter. A trial on the brain of premature animal sample found that infection and inflammation caused disorders of the neuron and glia. [4-10,39] A previous study had found that the increase of proinflammation cytokin in the amnotic liquid and blood of infected neonate would be the cause of infection and cerebral palsy. [3,4,36,37,38,39] In the process of inflammation, cytokin has an important effect/role in destructing neurological cells through apoptosis process. [42,43,44,45]

It was found/revealed in this study that every increase in a unit of glutamate serum level of a premature neonate means 1.04 times risk to arise encephalopathy, while the increase of IL-1β level in the first 24 hours means 1.33 times risk to arise sepsis. This was only a preliminary study with a hope have to other following studies with cohort MRI examination study. The limitations of this study was the asphyxia based on APGAR score not from pH Blood Gas Analysed.

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