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The Association between Asphyxia and Interleukin (IL)-6 and IL-1β Levels in Neonates

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Abstract

Background: Neonatal asphyxia is a respiratory failure during and just after birth. It can cause morbidity and mortality in neonates. Interleukin (IL)-6 and IL-1β are inflammatory cytokines produced by neuronal cells in early response to brain injury due to asphyxia. However, their role in neonatal asphyxia is remain elusive.

Objective: To determine the association between asphyxia and serumIL-6 and IL-1β levels in neonates.

Methods: Across-sectional study was conducted on neonates diagnosed with moderate to severe asphyxia who hospitalized at the Dr. Kariadi General Hospital Semarang Indonesia from December 2013 to May 2014. The subjects were examined for serum IL-6 and IL-1β. Blood samples were obtained from umbilical vein in the first 24 hours of life. Serum IL-6 and IL-1β levels were measured using immunoassay. Dependent variable were IL-6 and IL-1β level. Bivariate analysis was performed using chi-square test, for the assessment of the association between dependent and independent variables. A p-value of less than 0.05 was considered statistically significant.

Result: A total of 54 subjects were enrolled in this study. No significant difference between moderate and severe asphyxia neonatesin term of sex, birthweight, type of delivery, neonate's mother age, gestational age, and parity. Levels of IL-6 and IL-1β levels wereincreased significantly in both moderate and severe asphyxiagroups, and the levels were significant higher in the severe asphyxia than that of in the moderate, p=0.003 and p=0.007, respectively.

Conclusion: There was association between asphyxia and IL-6 and IL-1β levels in neonates.IL-6 and IL-1β levels were increased in neonates with moderate and severe asphyxia, with extend of increase was significant higher in the later.

Key-words: *Neonatal asphyxia; IL-6; andIL-1β*
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INTRODUCTION

Neonatal asphyxia is a condition of respiratory failure during and just after birth. It is a common cause of morbidity and mortality in neonate with an estimated incidence 1 to 6 per 1,000 baby births and about 4 million neonates around the world died from severe asphyxia each year.[1, 2]

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The imbalance between fetal and maternal blood supply causes oxygen supply disturbance and leads to hypoperfusion, autoregulation change, and hypoxia. Severe hypoxemia decreases adequate oxygen supply to several organs including brain, lung, heart, kidney, gastrointestinal tract, hematologic system, and muscle, and thus may cause organ damage or even death.[3-6]

Asphyxia can cause early inflammatory response and significant brain injury in neonates. White matter brain injury is the main cause of neurodevelopmental disorder and long-term disability. An animal experimental study

showed that cytokine plays a role in causing hypoxic-ischemic disorder,[7]including in response to brain injury.[8]

Pro-inflammatory cytokines increase during acute phase after hypoxia.[9] Interleukin (IL)-6 and IL-1β are pro-inflammatory cytokines produced by astroglia, microglia, and progenitor cells oligodendrocytes in central nervous system[10]and released in early response of cells injury and inflammation.[9]The involvement of IL-6 in a clinical study including preterm newborns in the cascade of cerebral injury during perinatal period may occurred with or without infection.[11]IL-6 level in serum and brain tissues in the hypoxic-ischemic neonatal rat was significantly increased by 6 hours, reached peak by 24 hours post injury and declining to the baseline level by 7 days. The study was conducted in rats model underwent left common carotid artery ligation followed by 8% O2 and 92% N2 exposure to become hypoxic-ischemic model.[12]The role of IL-6 in animal model in response to the ischemic-hypoxic after ischemic-hypoxic insult remains unclear. A study in animal model of myocardial infarction showed that IL-6 causes myocyte damage mediated by neutrophil.[13] On the other hand, the role of anti-inflammatory of IL-6 may have opposite effect of IL-1β. Recently, IL-6 was found having suppression effect on demyelination[14] and reducing toxicity of N-methyl-D-aspartic acid receptor and ischemic damage in vivo in animal.[15, 16]

The mechanism and inflammatory mediator which involve in this process remain unclear. Thus, in this study, we investigated the IL-6 and IL-1β levels in moderate and severe neonatal asphyxia, and found that their levels were increased with extend of increase was significant higher in severe asphyxia. This study examined serum IL-6 and IL-1b from umbilical cord of neonates in all gestational age, in one frame of study in early 24 hours of life. It therefore enables to identify early the severity of hypoxic-ischemic neonatal patients. The study can open possibility to use these biomarkers for evaluating the treatment and predict the outcome.

MATERIAL AND METHODS

This was analytic observational study with a cross-sectional design. Fifty-four neonates with moderate to severe asphyxia who hospitalized in the Dr. Kariadi Hospital from December 2013 to May 2014 were enrolled the study. The inclusion criteria for the study sample were neonates with moderate and severe asphyxia and the parents agreed the informed consent. Patients with sepsis or infection, suffered from major congenital disorders, congenital heart disease, anemia, and their mother suffered from infection were excluded from the study. Subjects were selected with consecutive sampling. This study received ethical approval from the Ethic Committee of Faculty of Medicine Universitas Diponegoro/ Dr. Kariadi Hospital, and conformed to the principles outlined in the Declaration of Helsinki.[17]All legal guardians of the subjects have provided written consent to participate in the study.

The subjects were examined for serum IL-6 and IL-1β. Blood samples were obtained from umbilical vein in the first 24 hours of life. Serum IL-6 and IL-1β levels were measured using ELISA in GAKI Laboratory of Universitas Diponegoro University. ELISA kits used for IL-6 and IL-1b examination were commercial kits purchased from Elabscience (Texas, USA).

All study variables were described based on their measurement scale. Categorical data were described using absolute and relative frequencies (percentage), while numerical data were described using mean and standard deviation. To assess the association between dependent and independent variables, bivariate analysis using Chi-square test was conducted.P-value < 0.05 was considered as statistically significant. Mann Whitney U test was used as alternative test when the conditions for applying Chi-Square test cannot be fulfilled.

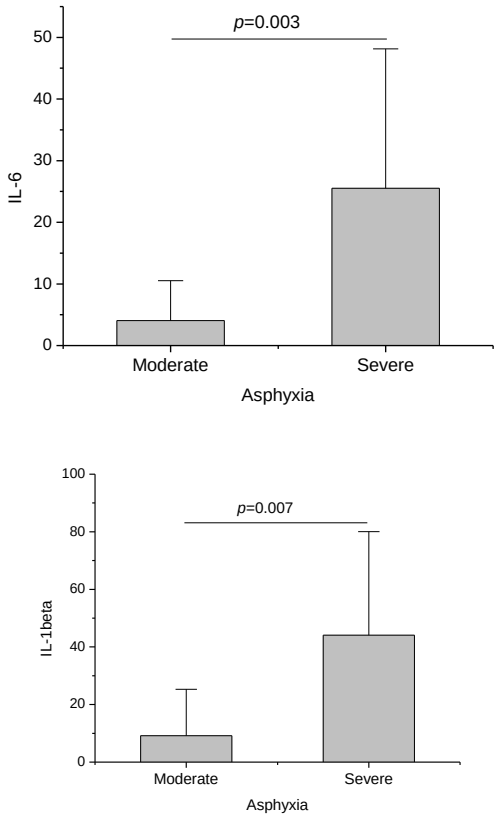

Figure 1. Levels of IL-6 (upper panel) and IL-1β (lower panel) (pg/mL) in the moderate and severe asphyxia neonates. Mann Whitney test was used for statistical analysis.

RESULTS

A total of 54 patients were enrolled in this study. Subject characteristics were showed in Table 1. There were no significant differences between moderate and severe asphyxia neonatesin sex, birthweight,type of delivery, mother age, gestational age, and parity.

Table 2 showed that IL-6 level in severe asphyxia was significantly higher than moderate asphyxia (p<0.001; PR=24.0 (95%CI 4.448 – 129.49). The mean of IL-6 was also significant higher in the severe asphyxia (p=0.003, Figure 1A). The cutoff point between normal and abnormal values of IL-6 was 10.2 pg/mL.[18]The mean of IL-1βwas higher significantly in the severe asphyxia than that of the moderate (p=0.0s07, Figure 1B). Table 3 showed that IL-1βlevel in severe asphyxia was also higher significantly (p=0.003; PR=5.786 (95%CI 1.706–19.621). The cutoff point between normal and abnormal values of IL-1β was 11.7 pg/mL.[19]

Table 1. Subject Characteristics

Variables	Grade of asphyxia				*p*	PR (CI 95%)
	Severe (20)		Moderate (34)			
	n	%	N	%		
Sex						
Male	13	65	13	38.2	0.057€	3.0 (0.950–9.475)
Female	7	35	21	61.8		
Birth weight						
< 2500 gram	8	40	19	55.9		
≥ 2500 gram	12	60	15	44.1	0.260€	0.526 (0.171 – 1.616)
Delivery						
Non-spontaneous	13	65	21	61.8	0.812€	1.150 (0.364 – 3.631)
Spontaneous	7	35	13	38.2		
Mother's age						
Risky age	6	30	10	29.4	0.964€	1.029 (0.307 – 3.442)
Un risky age	14	70	24	70.6		
Gestasional age						
Aterm	9	45	15	44.1	0.950€	1.036 (0.341 – 3.148)
Preterm/posterm	11	55	19	55.9		
Parity						
Primiparity	15	75	25	73.5	0.905€	1.080 (0.304 – 3.834)
Multiparity	5	25	9	26.5		

€*Pearson Chi Square test*

DISCUSSION

This was a cross sectional study investigated IL-6 and IL-1β level in neonatal asphyxia. According to the ICD-10 categories, neonatal asphyxia is classified by clinical signs and a 1-minute APGAR score category. When the 1-minute APGAR score is 0-3, it is classified as severe asphyxia. While mild and moderate asphyxia is classified when the 1-minute APGAR score is 4-7.[1]This study showed that IL-6 and IL-1β level significantly increased in both moderate and severe asphyxia. Asphyxia affords to activate immune system because of hypoxic-ischemic injury.[20] IL-6 and IL-1β are proinflammatory cytokine released in acute phase of inflammatory response in response to hypoxic-ischemic brain injury.[21-23]

There were potential markers as predictive factors of abnormal outcome in asphyxia. The markers were IL-6, IL-1β, and neuron specific enolase (NSE).[24]Asphyxia can cause cells damage if reoxygenation isn't supplied properly.[25] In animal study, asphyxia reduced survival rate.[26, 27] IL-6 and IL-1β were negative predictor for outcome in asphyxia.[12, 19]

Our study showed that IL-6 level significantly increased in both moderate to severe asphyxia. A study reported that level of serum IL-6 elevated significantly in neonates suffered from asphyxia and developed to hypoxic-ischemic encephalopathy (HIE) compared to normal group. IL-6 level was predictor for bad outcome in HIE.[28]

Measurement of IL-6 level in umbilical cord neonates suffered from asphyxia could predict negative outcome of asphyxia. Median value of IL-6 level in neonates with asphyxia that developed to be HIE increased 376-fold higher than normal group. The value increased 5,5-fold higher in neonates with asphyxia who didn't suffer from HIE than normal group.[9, 18]

IL-6 level significantly increased in first day of neonatal asphyxia compared to control group and associated with severity of asphyxia. The elevation of IL-6 level indicated that this cytokine was potential inflammatory marker of asphyxia. IL-6 level decreased in third day of asphyxia.[29]IL-6 level was associated with the time of handling sample. Elevation of IL-6 level in median value of 17,5 hours after asphyxia and the level decreased in median value of 36 hours.[30]

IL-1β level in our study increased in both moderate and severe asphyxia. IL-1β level was associated with stage of asphyxia.[31] Level of IL-1β in severe asphyxia significantly higher than moderate asphyxia.[32] IL-1β is cytokine released by mononuclear cells and macrophages in response to infection and tissue injury. IL-1β level in neonates with asphyxia significantly increased in the first life after injury compared to control group and it was associated with poor prognosis.[28]

IL-1β level increased during acute phase of inflammation (1-12 hours) after hypoxic-ischemic injury. An animal study showed that proinflammatory cytokines induced brain damage. IL-1 injection led to neuronal

Tabel 2. IL-6 level in moderate and severe asphyxia

Grade of Asphyxia	IL-6 (pg/mL)				*p*	PR (CI 95%)
	> 10.2		≤ 10.2			
	n	%	n	%		
Severe	12	85.7	8	20	0.000*€	24.0 (4.448 – 129.49)
Moderate	2	14.3	32	80		

*p<0.05, € Pearson Chi Squarec

Tabel 3. IL-1β level in moderate and severe asphyxia

Grade of asphyxia	IL-1β (pg/mL)				*p*	PR (CI 95%)
	>11.7		≤11.7			
	n	%	n	%		
Severe	12	63.2	8	22.9	0.003*€	5.786 (1.706 – 19.621)
Moderate	7	36.8	27	77.1		

*p<0.05, € Pearson Chi Square

death and delayed myelinization in mice babies. A review in Italia showed that IL-1β, TNF-α, and neuron specific enolase (NSE) were potential biomarker of brain damage in neonatal asphyxia.[28, 33]

Asphyxia led to *poly (ADP-ribose) polymerase-1 (PARP-1)* overactivity causing energy demand elevation, metabolic failure and decreasing NAD^+, activation of NF-kB signaling pathway subunit p65, increased IL-1β and TNF-α, and other proinflammatory mediators so and finally it induced cells death in mesencephalon of mice with asphyxia. PARP-1 activity increased immediately after asphyxia. It reached maximum level during 1-8 hours after insult.[34]Elevation of IL-1β, IL-6, and TNF-α in liquor cerebrospinal (LCS) of neonates with asphyxia who developed to be HIE. IL-1β level in LCS was associated with poor outcome in neonates with asphyxia.[35]

CONCLUSION

There is association between asphyxia and IL-6 and IL-1β levels in neonates.IL-6 and IL-1β levels increase in neonates with moderate and severe asphyxia, with extend of increase is significantly higher in the later.

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