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CORRELATION OF NITRIC OXIDE AND ABSOLUTE NEUTROPHIL COUNT TO CLINICAL OUTCOME AMONG ISCHEMIC STROKE PATIENTS

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ABSTRACT

Nitric Oxide (NO) has a dual role as neuroprotector and neurotoxin in the pathophysiology of brain ischemia. Patients with acute ischemic stroke commonly have increased leukocyte count when admitted to the hospital. Patients with acute ischemic stroke and high leukocyte count often have poor clinical outcomes. The aim of this study was to determine the correlation of NO levels and Absolute Neutrophil Count (ANC) with patients with the outcome of acute ischemic stroke patients. The study was a longitudinal prospective, conducted from June to October 2017, sampling was performed three times a day on day 1,3,7. Nitric oxide and ANC were measured using Chemwell analyzer and SYSMEX XN-1000, respectively with total twenty-one patients participated in the study. Fourteen males (66.67%), 7 females (33.33%), ANOVA test showed no difference in absolute neutrophil counts on day 1,3,7 (p=0.001) and Kruskall-Wallis test. There was no difference between the levels of NO days 1,3,7 (p=0.716). Spearman's correlation test results showed there was no correlation between absolute neutrophil count and outcomes in acute ischemic stroke (p=0.001) and no nitric oxide correlation with the outcome (p > 0.05). The ANC is a possible clinical outcome in acute ischemic stroke patients. Therefore, it is recommended for the clinician to observe the ANC as a potential prognosis in acute ischemic stroke patients and perform more specific nitric oxide examinations such as eNOS, nNOS, or iNOS of ischemic stroke patients

Key words: Absolute neutrophil count, ischemic stroke, nitric oxide

INTRODUCTION

Stroke is a serious neurological disease and the cause of disability with there are 15 million people diagnosed with stroke every year worldwide.[1] Five million people die and 5 million are permanently disabled.[2] In the United States, stroke ranks third as the cause of death after cardiovascular disease.[3] In Indonesia, the national stroke in Indonesia data show the highest mortality rate of 15.4% as the cause.[4]

Data in Indonesia also show a tendency of increased stroke cases in terms of death, incident, or disability. The mortality rate by age is 15.9% (age 45- 54 years), 26.8% (age 55 -64 years) and 23.5% (age ≥ 65 years).[5]

Ischemic stroke is a heterogeneous syndrome that occurs due to several mechanisms of disease caused by tissue damage resulting from disturbances in the blood circulation of the brain.[6] Ischemic stroke occupies 85-87% of all stroke cases.[1]

Nitric Oxide (NO) has a dual role as neuroprotection and neurotoxin in the pathophysiology of brain ischemia. Nitric Oxide Synthase (NOS) consists of three isoforms, namely neuronal NOS (nNOS), inducible NOS (iNOS) and endoses NOS (eNOS).[7] Nitric oxide neurotoxicity and neuroprotection as double roles, result in debate and conflict in stroke studies.[8]

Study by Rajeswar showed that serum NO levels were significantly higher in stroke patients compared with controls (P <0.001) indicating a state of oxidative stress.[9] Similar results were also shown from a study by Varyani *et al.* who found elevated levels of NO in patients with ischemic stroke compared with controls (p <0.005).[2] Contrastinaly a study by Abdullah showed that there was a significant decrease of NO levels within the first 24 hours of acute ischemic stroke patients compared with controls (p <0.001). The decrease of NO levels in this study was possibly related to cerebral blood circulation regulation in acute cerebral ischemia or may also be associated with peroxynitrite metabolism due to oxidative processes.[6]

Patients with acute ischemic stroke will undergo increased leukocytes count when admitted to the hospital. Patients with acute ischemic stroke and high leukocyte count commonly have poor clinical outcomes. Knowledge of the prognosis of stroke is necessary for the rehabilitation of the patient. Thus, it

is necessary to recognize the role and contribution of leukocyte numbers in acute ischemic stroke populations as indicators of severity and predictors of clinical outcome outcomes after acute ischemic stroke.[10]

A study by Wu showed a positive correlation between the number of leukocytes, neutrophils and long-term ischemic stroke.[11] The number of leukocytes and neutrophils had a similar ability to predict the occurrence of long-term ischemic stroke among Taiwanese people. It was recommended that both leukocyte and neutrophil counts be considered as risk factors for long-term ischemic stroke.[11]

In a study by Rafsanjan it was stated that high leukocyte values at admission did not correlate with prognosis in the mortality of acute ischemic stroke patients. Prognostic deterioration of ischemic stroke may be increased due to other frequent risk factors such as hypertension, hyperglycemia and dyslipidemia.[12] Based on this study aimed to determine the correlation of NO and ANC with the outcome of patients with acute ischemic stroke.

METHODS

The study was a longitudinal prospective, conducted from June to October 2017, sampling was conducted three times on day 1,3,7. The population in the study were all patients with acute ischemic stroke at the Adam Malik Hospital Medan who met inclusion and exclusion criteria to be the subjects of the study. Inclusion criteria were all acute ischemic stroke patients confirmed by CT-Scan examination of the head and signed the informed consent to participate in the study. Exclusion criteria were diabetes mellitus, acute coronary syndrome, infection, chronic renal failure, and neoplasm. Nitric oxide levels and absolute neutrophil counts were examined using Chemwell analyzer and SYSMEX XN-1000 respectively.

This study was approved by the research Ethical commitee of Sumatra Utara University (No. 310/TGL/KEPK FK USU-RSUP HAM/2017).

RESULT AND DISCUSSION

This study involved 21 patients consisting of 14 males (66.67%) and 7 females (33.33%) with acute ischemic stroke, with the characteristics shown in Table 1. The similar was found in Abdullah's research where the number of males were more 27 (51%) than females 26 (49%). Stroke was more common in males than females due to female estrogen that provides protection in the process of atherosclerosis. However, in this study, the female patients with age of over 50 years suggesting an equal risk of stroke between females and males after menopause.[6]

In this study, the mean age of patients with acute ischemic stroke was 60.76±9.90 years. Abdullah's study found that the mean age of patients with acute ischemic stroke was 64.5±15.8 where the increased incidence and mortality resulting from stroke increased with age. Mean systolic and diastolic blood pressure among subjects were 174±27.68 mmHg and 97±11.46 mmHg respectively. Suggesting a potential of hypertension to be a major risk factor for ischemic and hemorrhagic stroke. This is due to its ability because to rupture vessels or constriction blood vessels of the brain. Rupture of the brain blood vessels will result in cerebral hemorrhage, while blood vessels constriction in the brain will disrupt blood flow to the brain that eventually leads to the death of brain cells.[13]

Table 1. Acute ischemic stroke patients characteristic

Variable	
Age (mean ± SD)	60.76 (9.90)
Gender	
Male	14 (66.67%)
Female	7 (33.33%)
Blood pressure (mean±SD)	
Systole	174(27.68) mmHg
Diastole	97(11.46) mmHg
NIHSS	
Recovery	15
Without recovery	6
mRS	
Recovery	17
Without recovery	4

In Table 2 the mean of NO levels on day 1, 3 and 7 was decreased with mean NO days 1 (2010,24±1973,13) pg/mL, on day 3 (1875,10±1927,28) pg/mL, and at day 7 (1749,37±1812,76) pg/mL. However, there was no significant difference between NO levels on day 1, 3, and 7 (p=0.716). In was contradictive to the theory suggesting that cerebral ischemia in Middle Cerebral Artery Occlusion (MCAo) causes increased production of NO 20-fold for 30 minutes primarily through increased calcium uptake and nNOS activation. After that, NO in the brain tissue will decrease and unable to be detected in 7 days showing a long NO deficiency in the ischemic brain. If reperfusion occurs, NO concentrations may rise transiently by 50% for 30 mins.[14]

Table 2. Difference of NO and absolute neutrophil count in acute ischemic stroke patients on day 1, 3, and 7

Variable	Day (mean ± SD)			*p*
	1	3	7	
Nitric oxide**	2010,24±1973,13	1875,10±1927,28	1749,37±1812,76	0.716
Absolute neutrophil count*	10,58±2.93	8.18±2.02	6.77±1.83	0.001***

* ANOVA test **Kruskal-Wallis test *** Significant P < 0.05

Table 3. Nitric oxide and absolute neutrophil counts with outcome (NIHSS and hospitalization) in acute ischemic stroke day 1 and 7

Variable	Day	NIHSS				Hospitalization			
		Day 1		Day 7		Day 1		Day 7	
		R	*p*	r	*p*	R	*p*	r	*p*
Nitric oxide	Day 1	0.232	0.156	0.058	0.401	0.032	0.445	0.108	0.320
	Day 7	0.211	0.179	0.033	0.444	0.039	0.433	0.111	0.316
Absolute neutrophil	Day 1	0.841	0.000*	0.751	0.001*	0.635	0.001*	0.739	0.001*
	Day 7	0.386	0.042*	0.419	0.029*	0.170	0.230	0.493	0.012*

* Correlation test using Spearman's test rho significant if p< 0.05

In acute ischemic stroke, the increased levels of NO metabolites was correlated with the severity of brain cell damage. Although a decrease in cerebral blood flow in the ischemic central region was a major factor responsible for necrotic damage, other factors involved were metabolic degrees, capillary density, amino acid excitotoxicity and possible differences in local NOS activity.[8]

In this study, the average number of absolute neutrophils on day 1, 3 and 7 was decreased with the average number of absolute neutrophils on day 1 (10.58±2.93)x103/μL, on day 3 (8.18±2.02)x103/μL and day 7 (6.77±1.83)x103/mL was respectively. The statistical test results showed a significant difference of day-1,3,7 (p=0.001). A similar finding was found in the Dangoran study showing that the mean absolute neutrophil count of peripheral blood on day 7 was lower than that of admission day, and there was a significant difference between the absolute neutrophils count of peripheral blood on admission day and that of day 7 (p=0.03). This was consistent with the theory suggesting that in the acute phase there is inflammation leading to leukocyte movements that are affected by the cytokines TNF α and IL-1, especially the neutrophils leading to the ischemic region. After the acute period passed the inflammatory reaction gradually disappeared and was marked by a decrease proinflammatory cytokines levels.[15]

In Table 3, there was no significant correlation between NO with NIHSS and hospitalization in acute ischemic stroke patients (p> 0.05), showing similar results to Rajeshwar's study where serum NO levels were found to be significantly higher in stroke patients than in controls showing a oxidative stress state. The increased serum levels of NO stroke patients showed an acceleration in the process of atherosclerosis, and showed no significant correlation between NO levels and outcomes in stroke patients.[9]

In this study there was a significant correlation between absolute neutrophils and with NIHSS on day 1 (r=0.841, p=0.000), absolute neutrophils with NIHSS on day 7 (r=0.751, p=0.001), absolute neutrophils day 7 with NIHSS day 1 (r=0.386, p=0.042), absolute neutrophils and with NIHSS on day 7 (r=0.419, p=0.029), absolute neutrophils and with hospitalization on day 1 (r=0.635, p=0.001), absolute neutrophils day 1 with 7 days of hospitalization (r=0.739, p=0.001) and 7 days absolute neutrophil 7 of hospitalization day 7 (r=0.493, p=0.012). Therefore, it can be concluded that the absolute neutrophil count can be used to predict outcome in acute ischemic stroke patients. This is similar to a study by Gofir's showing that the leukocyte numbers have a correlation to the clinical outcome and length of treatment among acute ischemic stroke patients where the acute phase response is a common phenomenon that follows acute ischemic stroke and is mediated by elevated

levels of cytokines such as interleukins and Tumors Necrosis Factor (TNF). The release of interleukins and the increased cellular activity play an important role in increasing rates of leukocytes.[10] In Guven's study, high leukocyte and neutrophil counts were associated with vascular disease and can be a predictor of stroke severity.[16]

Neutrophils are the earliest blood leukocytes found in the brain parenchyma. Neutrophils begin to increase on the first day in the damaged brain tissue, reach its peak level on the third day and decrease until day 6, before replaced by mononuclear cells. Clinical studies confirmed that neutrophils accumulated intensively in areas of human cerebral infarction in the early ischemic phase, and this accumulation was correlated with the severity of brain tissue damage and poor neurological outcomes after ischemic stroke.[17]

The number of leukocytes counts at admission is an independent risk factor for poor prognosis in ischemic stroke patients as well as neutrophils who play the same role based on NIHSS score and hospitalization scores within 12 and 72 hours of stroke onset, supported by many studies showing a potential role of leukocytes as a marker of acute or chronic inflammation. Elevated number of leukocyte is an independent risk factor for atherosclerotic disease. Phagocytic leukocytes trigger a series of reactions that cause damage to blood vessels and the formation of atherosclerosis. Many studies have shown that leukocytes and neutrophils were independent risk factors for acute ischemic stroke and acute myocardial infarction.[18]

This result was consistent with the pathophysiology of acute ischemic stroke in the event of ischemic tissue that neutrophils attached and migrated through the endothelium of the brain microvascular. In the extravascular state, neutrophils produced free radicals, released proteolytic enzymes, stimulated the release of neutrophils leading to the further movement of neutrophils and other leukocytes. High number of neutrophils during reperfusion, intravascular neutrophils, and tissue accumulation are components of the inflammatory response.[19]

The strength of this research was by a longitudinal prospective method where NO levels and absolute neutrophil count were measured serially so as to give the accuracy of the results obtained. While the weakness of this study was that specific nitric oxide examination (nNOS, iNOS, eNOS) were not performed.

CONCLUSIONS AND SUGGESTIONS

In this study, there was a significant correlation between absolute neutrophil count and clinical outcome but no significant correlation between NO and clinical outcomes in acute ischemic stroke patients.

This study showed that the absolute number of neutrophils could be an outcome in acute ischemic stroke patients. Therefore, it was recommended that the clinicians consider the absolute number of neutrophils as a prognosis of acute ischemic stroke patients and perform more specific NO examinations such as eNOS, nNOS, or iNOS in acute ischemic stroke patients.

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