

**Angiogenesis in Ischemic Stroke Patients Aged 30 - 80 years  
at Gatot Subroto Army Central Hospital (RSPAD) Jakarta**

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**Background:** Angiogenesis, is one of the neurorepair process which plays an important role after ischemic stroke incident. vascular-endothelial growth factor (VEGF), Angiopoietin-1 (ANG1) and fibroblast growth factor (FGF) are the angiogenic factors involved in the process of angiogenesis. In this study we observed VEGF, ANG1 and FGF concentration to represent the angiogenesis process occurred in ischemic stroke patients with different onset time. **Methods:** This was a cross sectional study involving 63 ischemic stroke subjects aged 30 – 80 years old from The Central Hospital of the Army (RSPAD) Gatot Subroto Jakarta. Subjects were divided into 3 groups due to stroke onset time.: < than 7 days (Group A: 11 subjects), onset 7 – 30 days (Group B: 26 subjects) and > 30 days (Group C: 26 Subjects). VEGF, FGF and ANG1 serum levels were measured using multiplex method with luminex Magpix instrument. **Results:** VEGF, ANG1, and FGF were not significantly different between all groups. We did not find any significant correlation in all groups except FGF with ANG1 in group C. VEGF and ANG1 levels found to be highest in group B, and FGF levels in group A. **Conclusions:** VEGF, FGF and ANG1 increased soon after ischemic injury. FGF immediately increased in first week after onset and then decreased. VEGF and ANG1 levels reach their peak levels between 7-30 days after injury, showed that the optimal process of angiogenesis occurs in this period. There is no significant correlation between VEGF, FGF and ANG1 in all groups but we found a correlation between FGF and ANG1 in subjects with onset > 30 days after injury.

**Keywords:** Angiogenesis, ischemic, stroke, VEGF.

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## INTRODUCTION

Ischemic stroke is a rapid loss of brain function due to disruption of the blood supply to the brain caused by ischemia (lack of blood flow) due to blockage (thrombosis, arterial embolism).<sup>1</sup> In The prevalence rate of stroke in Indonesia increased from 8.3 per 1,000 in 2007 to 12.1 per 1,000 in 2013.<sup>2</sup>

Angiogenesis is the key factor for regeneration process in ischemic tissue. Angiogenic in stroke could increase blood flow, and decrease infark size.<sup>3</sup> Penumbra is an area with intense restoration and active angiogenesis.

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Microvascular endothelial cells generated by angiogenesis process play an important role in growth factors and chemokine secretion, which may support the survival of newly formed neurons.<sup>4</sup> Moreover, the number of new vessels formed in ischemic penumbra were correlated with a longer survival in ischemic stroke patients.<sup>5</sup>

Among 96 genes implicated in angiogenesis, 42 were significantly increased 1 hour after ischemic injury, while 13 genes increased after day twenty-one.<sup>6</sup> Some angiogenic growth factors increased after ischemic stroke including basic fibroblast growth factor (bFGF), Vascular endothelial growth factor (VEGF), angiopoietin1 (ANG1), fibroblast growth factor (FGF)<sup>7</sup>, and others.<sup>8-10</sup> They were regulated by hypoxia inducible factor (HIF).<sup>11</sup>

VEGF is the most potent angiogenic factor for neurovascularization in ischemic stroke which was secreted by endothelial cell and pericytes<sup>12</sup>, VEGF stimulates angiogenesis process in penumbra<sup>13</sup>, promotes vascular endothelial cells generation<sup>14</sup>, increases vascular permeability<sup>7</sup>, thus

shows a direct neuroprotective effect for neuronal cells<sup>15</sup>, stimulates axon growth from dorsal root ganglia (DRG), superior cervical ganglion (SCG), and primary cortical neurons.<sup>16</sup>

Both VEGF and ANG1 involved in angiogenesis process. VEGF stimulates angiogenesis whereas ANG1 suppresses leakage, inflammation and regression of microvessels.<sup>16</sup> ANG1 was expressed by pericytes and bind to Tie2 as receptor. Ang1 is needed as a vascular stabilizing factor and limits the angiogenesis response,<sup>17</sup> by tube formation, promoting monolayer integrity, and suppressing expression of inflammatory genes.<sup>16</sup> Ang1 also correlates with recruitment of newly formed immature neurons from Sub ventricle zone (SVZ) to penumbra.<sup>18</sup>

FGF is one of the angiogenesis major regulator which was upregulated due to hypoxia. FGF mRNA increases until 72 hours after ischemic.<sup>19</sup> FGF also involved in neurogenesis, by enhancing neuronal sprouting, synapse formation, progenitor cell proliferation and differentiation,<sup>20</sup> FGF pathway also regulates the expression of SDF1 $\alpha$ .<sup>21</sup>

Angiogenesis is an important process in neurorepair after ischemic stroke, where the process is related to neurogenesis and neurorepair process is related to the time of onset.<sup>22,23</sup> In this study we observed VEGF, ANG1 and FGF concentration to represent the angiogenesis process occurred in ischemic stroke patients with different onset time.

## MATERIALS AND METHODS

This was a cross sectional study with 30 – 80 years old ischemic stroke subjects from The Central Hospital of the Army (RSPAD) Gatot Subroto Jakarta. Diagnosis of ischemic stroke was made using clinical examination and MRI by neurologist.

## Subjects

Subjects were divided into 3 groups due to stroke onset time. Patients with onset less than 7 days were defined as Group A, Patients with onset 7 – 30 days defined as Group B, and patient with onset more than 30 days defined as Group C. Subject with the history or with carcinoma, hematoma subdural, or other carcinoma, global ischemia, could not conduct MRI, blood clotting disorders, seizures, were excluded

## Specimen collection

Blood were collected intravenously, serum was separated, stored frozen (at -21°C) and tested for biomarkers. VEGF (R&D reagent no cat LXSAHM-03), FGF and ANG1 (R&D reagent no Cat FCSTM02-02) concentrations were measured using multiplex method with luminex Magpix instrument.

## Statistical analysis

Statistical analysis was performed for data distribution normality was analyzed using Shapiro-Wilk, and t-test analysis or Mann-Whitney performed to observe the difference between each groups. The correlation of VEGF, FGF, and ANG1 were analyzed by Spearman or pearson test.

## RESULT

63 ischemic stroke patients involved in this study, which divided into 3 groups of: 11 subjects for group A, 26 subjects for group B and 26 subjects for group C. Characteristics of our subjects (age, body mass index (BMI), systole blood pressure (SBP), diastole blood pressure (DBP) and the results of Shapiro-Wilk normality test data were shown in Table 1.

Table 1. General Characteristics of subjects and the normality test (n=63)

Variable	Min	Max	Mean $\pm$ SD	Median	p*
Age (Year)	38	76	57.17 + 9.479	56	0.463*
BMI (Kg/m <sup>2</sup> )	18.25	36.71	25.4754 + 3.7798	24.977	0.034
SBP (mmHg)	57	159	88.222 + 16.0206	90	0
DBP (mmHg)	100	220	145.762 + 25.014	140	0.001
VEGF (pg/mL)	10.5	459.02	81.204 + 66.62	66.62	0
FGF (pg/mL)	10.62	110.01	55.1675 + 17.9863	56.378	0
ANG1 (pg/mL)	8528	134,000.00	52082 + 17850.9	51,513.00	0

\*Distribution assessment with Shapiro-Wilk test. Significant at p>0.05

### Differentiation VEGF, FGF, and ANG1 concentration on all three groups

Table 2 showed that the levels of VEGF, FGF, and ANG1 were not significantly different between all groups. However, VEGF and ANG1

levels were higher in group B than the other groups (shown in Figure 1). whereas FGF levels of group A is higher compared to other groups.

There is no significant difference between the all groups for age, BMI, and Blood Pressure.

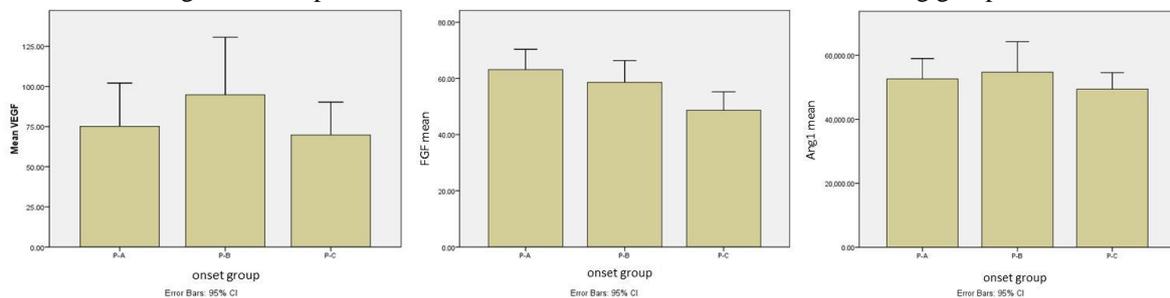
Table 2. Comparison of biomarkers among groups

Variable	Subject onset Group (mean + SD)			Independent t-test/Mann Whitney*		
	Group A (n=11)	Group B (n=26)	Group C (n=26)	A vs B	B vs C	A vs C
Age (Year)	59.45 + 11.076	57.58 + 9.729	55.81 + 8.644	0.585	0.585	0.311
BMI (Kg/m <sup>2</sup> )	27.5408 + 5.32925	25.5953 + 3.13765	24.4818 + 3.36269	0.166	0.068	0.068
SBP (mmHg)	96.727 + 25.8615	89.038 + 14.8391	83.808 + 9.798	0.712	0.177	0.177
DBP (mmHg)	140.182 + 29.2398	146.462 + 27.6134	147.423 + 20.7406	0.411	0.185	0.409
VEGF	72.0973 + 43.2549	94.7815 + 88.7381	71.4792 + 52.0209	0.73	0.503	0.503
FGF	63.4926 + 11.9017	58.5782 + 19.2293	48.2346 + 16.8314	0.37	0.059	0.059
ANG1	52590 + 10480.3	54692 + 23575.1	49256 + 13230.2	0.925	0.315	0.453

\* Independent t-test (using underline), and Mann Whitney result

\*\*Significant at p < 0.05

Figure 1. Comparison VEGF mean, FGF mean and ANG1 mean among groups.



### Correlation between VEGF, FGF and ANG1

We found no significant correlation between VEGF, FGF and ANG1 in each group, but we found that FGF correlated with ANG1 in C group.

Table 3. Correlation analysis Between VEGF, FGF and ANG1

Group		VEGF (r)	FGF (r)	ANG1 (r)
A	VEGF	1	-0.384	0.147
	FGF	-0.384	1	-0.049
	ANG1	0.147	-0.049	1
B	VEGF	1	0.327	0.022
	FGF	0.327	1	0.048
	ANG1	0.022	0.048	1
C	VEGF	1	0.129	0.292
	FGF	0.129	1	.489**
	ANG1	0.292	.489**	1

### DISCUSSIONS

Condition of ischemic stroke, in addition to the response of brain injury such as hypoxia, loss of energy inflammation, also activate the neurovascular response as vascular remodeling, angiogenesis, and neurogenesis.<sup>22</sup>

Our study showed no significant difference of VEGF level between all groups, but. Graph 1. showed that group B has the highest level of VEGF among all groups, followed by Group A then Group C, this results in line with slevin, et all, which state

that the angiogenesis process occurred after ischemic stroke reached its peak levels between 7 – 14 days after onset.<sup>24</sup> Newly formed thin-walled vessels and more prominent collaterals have been observed between 7 and 30 days after cortical ischemia in rats, together with a corresponding increase in blood flow.<sup>16</sup> ANG1 levels showed similar behavior as VEGF with slighter changes and tend to be stable. ANG1 mRNA expression was essentially did not altered in the first two hours, and increased between 48 hours to one week after injury, then tail off slowly.<sup>16</sup> Different with FGF level which showed its highest in A group, followed by another two consecutively. This showed that FGF was necessary in early onset of ischemia for angiogenesis.

ANG1 inhibit the intense of new vessel sprouting and reserve stabilization of vascular and FGF stimulate vascular growth, in this research which have significant correlation between FGF and ANG1 on more than 30 days' injury. Probably because Angiogenesis process decreased after 14 days.<sup>16,19,24</sup>

### CONCLUSIONS

Angiogenesis is the first step for endogenous neurorepair after ischemic stroke, some angiogenic growth factors include VEGF, FGF and ANG1 were increased after this injury. FGF immediately increased in first week after onset and then decreased. VEGF and ANG1 levels reach their peak levels between 7-30 days after injury, showed that the optimal process of angiogenesis occurs in this

period. There is no significant correlation between VEGF, FGF and ANG1 in all groups but we found a correlation between FGF and ANG1 in subjects with onset more than 30 days after injury.

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