

Clinical Research

Serum vascular endothelial growth factor as a predictor of clinical outcomes in anterior circulation ischemic stroke

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ABSTRAK

Latar belakang: Respons inflamasi pada fase akut stroke iskemik akan memicu proses neuroplastisitas dan menentukan luaran klinis. Angiogenesis dan neurogenesis diinduksi oleh ekspresi vascular endothelial growth factor (VEGF) pada fase akut stroke. Tujuan penelitian ini adalah untuk mengetahui hubungan antara kadar VEGF serum pada fase akut stroke dengan luaran klinis.

Metode: Penelitian ini menggunakan desain kohort longitudinal terhadap 64 pasien stroke iskemik sistem anterior dan baru pertama kali mengalami stroke, dibuktikan dengan diffusion-weighted magnetic resonance imaging (DWI) kepala. Pemeriksaan kadar VEGF serum dilakukan pada 72 jam dan 7 hari pasca-stroke dan dinilai luaran klinis pada 30 hari pasca-stroke menggunakan skor National Institutes of Health Stroke Scales (NIHSS). Analisis data menggunakan uji T, uji Spearman, dan regresi logistik.

Hasil: Kadar VEGF jam ke-72 dan hari ke-7 yaitu $5,84 \pm 0,736$ ng/mL and $5,797 \pm 0,96$ ng/mL ($p > 0,05$). Kadar VEGF 72 jam pasca-stroke yang tinggi dapat digunakan untuk memprediksi derajat klinis yang buruk pada 30 hari pasca-stroke (OR = 6,5; 95% CI = 1,15-36,61; $p = 0,034$). Subjek yang mengalami peningkatan kadar VEGF pada 7 hari dibandingkan 72 jam pasca-stroke menunjukkan derajat klinis hari ke-30 yang cenderung lebih ringan. (Skor NIHSS = $1,33 \pm 1,22$ vs $3 \pm 3,78$; $p = 0,232$).

Kesimpulan: Kadar VEGF pada fase akut stroke iskemik menggambarkan derajat kerusakan otak, dinamika kenaikan kadar VEGF pasca-stroke berkaitan dengan perbaikan luaran klinis.

Keywords: angiogenesis, clinical outcomes, ischemic stroke, VEGF

pISSN: 0853-1773 • eISSN: 2252-8083 • <http://dx.doi.org/10.13181/mji.v24i2.1196> • Med J Indones. 2015;24:109-14

• Received 31 Jan 2015 • Accepted 19 Jun 2015

ABSTRACT

Background: Inflammatory response in the acute phase of ischemic stroke will trigger the process of neuroplasticity and determine the clinical outcomes. Angiogenesis and neurogenesis are induced by expression of vascular endothelial growth factor (VEGF) in the acute phase of stroke. The purpose of this study was to determine the association between VEGF serum level in acute phase of stroke with the clinical outcomes.

Methods: This longitudinal cohort study was conducted on 64 patients suffering from first-attack of anterior circulation blockage as evidenced by cephalic diffusion-weighted magnetic resonance imaging (DWI). VEGF serum level was measured at 72 hours and 7 days after stroke and the clinical outcomes were assessed on day 30 post-stroke using the National Institutes of Health Stroke Scale (NIHSS).

Results: VEGF level at hour-72 and on day-7 were 5.84 ± 0.736 ng/mL and 5.797 ± 0.96 ng/mL, respectively ($p > 0.05$). High VEGF levels at hour-72 can be used to predict poor clinical outcome 30 days after stroke (OR = 6.5; 95% CI = 1.15-36.61; $p = 0.034$). Subjects who have increasing levels of VEGF on day-7 compared to hour-72 tend to have better clinical outcomes on day-30. (NIHSS score = 1.33 ± 1.22 vs 3 ± 3.78 ; $p = 0.232$).

Conclusion: VEGF levels in the acute phase of ischemic stroke reflect the degree of brain damage, the dynamic of the increase in VEGF levels after a stroke was associated with better clinical outcomes.

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Stroke is the third leading cause of death in developing countries among population above 60 years old, after heart disease and cancer. Approximately, more than 80% cases of stroke is ischemic and the rest is hemorrhagic.^{1,2} In Indonesia, stroke is the first leading cause of death.³ It is not just an elderly disease, but also affects young people. Stroke causes both physical and psychological burden for patients, families, and communities. The impact of this medical issue economically results in the decline of productivity and economic capacity of communities and nations.¹⁻⁴

Mechanism of brain injury after ischemic stroke is a complex process and is concomitant with inflammatory process that involves neuronal cells, glial cells, endothelial cells, extracellular matrix, and peripheral leukocytes.⁵⁻⁷ Post-stroke outcome is not solely determined by pro- and anti-inflammatory cytokines, but also by neurotrophic factors released in acute stroke as a result of neuronal injury. After cerebral ischemia, neuron plasticity is once again triggered on surviving cells to compensate the dead neurons and support the function of the damaged ones. The mechanisms underlying neuronal plasticity are dendrite reorganization, axonal sprouting and activation of endogenous pluripotent cells which are able to differentiate into neurons. Angiogenesis and arteriogenesis are the major processes of vascular plasticity. After an ischemic attack, angiogenesis is necessary to provide new capillary and increase blood flow to regenerate the suffering cells.⁸

Vascular endothelial growth factor (VEGF) is a vascular permeability factor, a major protein that induces angiogenesis in both normal and pathological conditions. VEGF binds to tyrosine kinase receptors, vascular endothelial growth factor receptor-1 (VEGFR-1) and VEGFR-2 / kinase insert domain receptor (KDR) / fetal liver kinase-1 (Flk-1), and mediates intracellular signals for cell growth and cell survival.⁸ In the brain, VEGF is produced primarily by neurons, vascular cells, and astrocytes. Following ischemic stroke, hypoxic condition induces VEGF expression followed by fibroblast growth factor (FGF), angiopoietins, transforming growth factor beta (TGF β), platelet-derived growth factor (PDGF) and tissue-plasminogen activator (tPA).^{9,10}

Previous studies reported that the VEGF serum

level in acute phase of stroke (within 24 hours onset of symptoms) is associated with infarct volume and a better prognosis in three months. Plasma VEGF levels increase immediately after the onset of ischemic stroke in all types of strokes up to 90 days.¹¹

Although it seems to be constructive in long term, this response may contribute to deleterious edema formation during the acute phase of ischemic stroke. Several studies showed that VEGF contain an unfavorable effect towards outcome. High VEGF in acute ischemic stroke correlates with cerebral micro-bleeding.¹² Early post ischemic administration of recombinant VEGF to ischemic rats significantly increased blood brain barrier leakage, hemorrhagic transformation and ischemic lesions.¹³

This study aimed to explore the role of VEGF in the pathomechanism of acute ischemic stroke and its role in predicting clinical outcomes.

METHODS

This longitudinal cohort study was conducted from August to December 2014, in Siloam Hospital, Karawaci, West Java. Patients with acute onset (<72 hours) of anterior circulation ischemic stroke, were closely observed for one month after the initial stroke. Patients with history of previous stroke and thrombolytic therapy, decreased liver and kidney functions, congestive heart failure, acute myocardial infarction, and pneumonia were excluded from the study. Magnetic resonance imaging (MRI) was performed at admission to confirm the ischemic stroke and to assess cerebral infarction volume. Formula $ABC/2$ (mm³) was used to measure infarct volume (A:length; B:width; C:number of slice).¹⁴

VEGF serum levels were measured with human VEGF immunoassay Quantikine® (R&D Systems, Inc, USA) within 72 hours and on day 7 after stroke. This assay employed the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for VEGF has been pre-coated onto a microplate. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color develops in proportion to the amount of VEGF bound in the initial step. The color development was stopped and the

intensity of the color was measured at 450 nm wavelength.

Clinical outcomes were assessed at hour-72, day-7 and up to day-30 after stroke using National Institutes of Health Stroke Scales (NIHSS). The severity of stroke was evaluated with NIHSS and they were categorized as mild (0-3), moderate (4-15) and severe (16-42). Ethical clearance was obtained from Mochtar Riady Institute for Nanotechnology Ethics Committee Number 072/MRIN-EC/08/2014.

Data were analyzed with SPSS 20.0. T-test was used to compare NIHSS scores on day 30 in group with increased or decreased VEGF levels. Spearman correlation test was used to evaluate the correlation between VEGF levels with NIHSS score. Logistic regression analysis was used to predict 30 days outcome after stroke.

RESULTS

In total, 64 patients aged 37 - 89 year old (mean of 58.8 ± 11.3 years) met the inclusion criteria of this study, consisted of 46 (62.0%) males and 18 (29.0%) females. The largest group of age was 46.8% in the range of 50 - 59 years old. About 93.8% of participants had history of hypertension, while 76.7% of them also had lacunar anterior circulation infarct (LACI). About 59.4 % of subjects had a moderate NIHSS score (4-15) on admission with an average score of 5.2 ± 3.7 . On day 30, we found 89.5% of subjects had a mild NIHSS score (0-3). There were only 16 patients evaluated for VEGF serum level on day-7 after stroke (Table 1).

Mean VEGF serum level at hour-72 was 5.84 ± 0.736 ng/mL, and on day-7 was 5.797 ± 0.96 ng/mL ($p > 0.05$). VEGF serum level at hour-72 had a significant correlation with NIHSS score on admission ($r = 0.264$; $p = 0.035$) (Table 2).

There was no significant correlation between VEGF level at hour-72 with NIHSS score on day-30 ($r = 0.156$; $p = 0.217$). VEGF levels on day-7 (5.797 ± 0.96 ng/mL, $n = 16$) was significantly associated with 30 days clinical severity with a value of $r = 0.518$ and $p = 0.040$ (Table 2).

The changes in VEGF levels at hour-72 and on

day-7 can be categorized as "increased" and "decreased" (Figure 1). T-test was then used to see the difference in NIHSS score on day-7 and -30. In this category, the group with increasing VEGF serum level ($n = 9$) tended to have milder clinical severity than the group with decreased VEGF level ($n = 7$). NIHSS score in both groups were 1.33 ± 1.22 and 3.001 ± 3.78 , respectively; $p = 0.232$) (Figure 2).

This study also explored correlation between infarct volume, Bamford classification, and clinical outcomes 30 days after stroke. There was positive correlation between infarct volume

Table 1. Characteristic of patients, VEGF serum levels, and NIHSS score

| Characteristics | n | Value |
|--|----|--------------------|
| Gender | | |
| Male | 46 | 71.88 % |
| Female | 18 | 28.13% |
| Bamford classification | | |
| LACI* | 49 | 76.56% |
| Non-LACI | 15 | 23.43% |
| Age (mean \pm SD ; years) | 64 | 58.78 ± 11.31 |
| BMI [§] (mean \pm SD) | 64 | 25.11 ± 3.88 |
| MAP [#] (mean \pm SD ; mmHg) | 64 | 111.26 ± 14.28 |
| Infarct volume* (logmean \pm SD; mm ³) | 64 | 1.05 ± 1.77 |
| VEGF level hour-72 (logmean \pm SD; ng/mL) | 64 | 5.84 ± 0.73 |
| VEGF level day-7 (logmean \pm SD ng/mL) | 16 | 5.79 ± 0.96 |
| NIHSS [‡] hour-72 (mean \pm SD) | 64 | 5.2 ± 3.74 |
| NIHSS day-7 (mean \pm SD) | 64 | 3.55 ± 3.22 |
| NIHSS day-30 (mean \pm SD) | 64 | 1.76 ± 2.28 |

* LACI: Lacunar anterior circulation infarct

MAP : Mean arterial pressure

§ BMI: Body mass index

* Infarct volume measured using formula $ABC/2$ mm³ (A=length; B=width; C=number of slice) 14,20

‡ NIHSS : National Institute of Health Stroke Scale

Table 2. Correlation between VEGF level and NIHSS score

| | n | R | p |
|-------------------------------------|----|-------|-------|
| hour-72 VEGF level vs NIHSS hour-72 | 64 | 0.264 | 0.035 |
| hour-72 VEGF level vs NIHSS day-30 | 64 | 0.156 | 0.217 |
| Day-7 VEGF level vs NIHSS day-30 | 16 | 0.518 | 0.040 |

and NIHSS score 30 days ($r = 0.362$; $p = 0.005$). There was also correlation between Bamford classification and NIHSS score 30 days after stroke. Multivariate analysis logistic regression showed that VEGF was the strongest clinical predictor on day 30 after stroke, compared to the infarct volume and Bamford classification with an OR = 6.5 and $p = 0.034$ (CI = 1.15-36.61) (Table 3).

DISCUSSION

VEGF showed a dramatic increase in serum of stroke patients with highest expression occurred on day 7, and it remained significantly elevated at 14 days after stroke, even after 3 months post-stroke.^{11,15}

Lee, et al¹⁶ reported a relationship between elevated serum level of VEGF and infarct volume, and the highest level of VEGF was found in large vessel disease (LVD) stroke. Moreover, he also found that the high VEGF serum level in 24 hours post-stroke was related with more favorable prognosis in three months follow-up. This study seems to show different result, as the high VEGF serum taken in

acute phase of stroke was related with more severe prognosis ($r = 0.264$; $p = 0.035$). This could be explained by result of Matsuo's study.¹¹ They found that the VEGF serum level in acute ischemic stroke was higher than control, this elevation continued up to three months after onset. It is interesting that VEGF level varied in subtype stroke according to TOAST (Trial of Org 10172 in acute Stroke Treatment) classification of ischemic stroke type. There was a positive correlation between VEGF level and clinical presentation within three months after onset on cardioembolic infarct subtype and lacunar infarct. On the other hand, a negative correlation was found in atherothrombotic infarct subtype. In this study, VEGF serum levels taken within 72 hours post-stroke were positively correlated with early clinical presentation within 72 hours after onset ($r = 0.264$ and $p = 0.035$), and also have positive correlation with 30 days outcome ($r = 0.15$ and $p = 0.217$). Whereas VEGF serum level on day-7 after ischemic stroke had a positive correlation with 30 days outcome ($r = 0.518$ and $p = 0.04$). Referring to Matsuo study,¹¹ the positive correlation between VEGF and 30 days clinical outcome, might be caused by lacunar infarct (LACI) happened in majority of the study subjects. (Bamford classification).

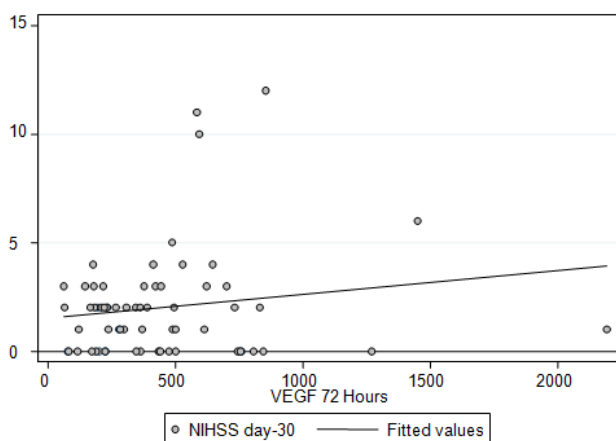


Figure 1. VEGF level at 72 hours compared to NIHSS score 30 days after stroke

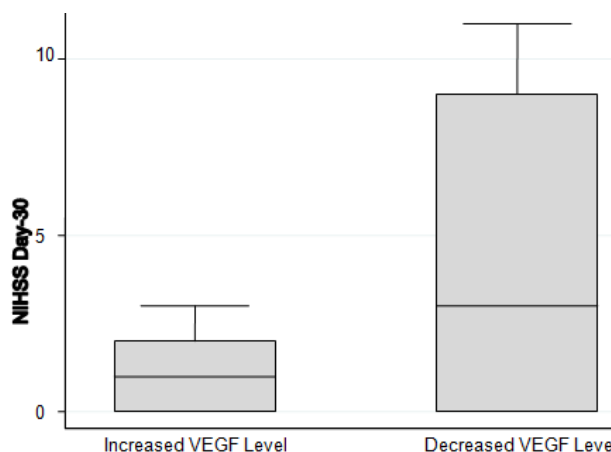


Figure 2. NIHSS scores at day 30 after onset in group with increasing (n = 9) and decreasing (n = 7) VEGF level

Table 3. Logistic regression to predict 30 days outcome after stroke

| Variables | OR | SE | z | p | (95% CI) |
|------------------------|------|------|------|-------|--------------|
| Infarct volume | 5.97 | 1.08 | 1.65 | 0.099 | 0.02 - 1.39 |
| Bamford classification | 1.16 | 0.99 | 0.16 | 0.874 | 0.12 - 5.97 |
| VEGF | 6.5 | 0.88 | 2.12 | 0.034 | 1.15 - 36.61 |

After acute stroke, VEGF expression appears within six hours in response to hypoxia along the ischemic area.¹⁷ *In vivo* study shows that introducing intravenous Vascular Endothelial Growth Factor-A (VEGF-A) two days post-stroke would induce angiogenesis in penumbra and help neuron recovery due to ischemic process.¹⁸ Intraventricular VEGF antibody had shown negative impact in increasing infarct volume in post focal cerebral ischemia mice.¹⁹ Topical and intramuscular VEGF had also shown that infarct volume and edema was significantly decreased in medial cerebral artery occluded mice. This revealed that VEGF has a neuroprotective effect.

This study revealed that subjects whose levels of VEGF on day-7 increased compared to levels at hour-72 led to the better NIHSS score at 30 days after stroke, and subjects with VEGF level on day-7 decreased compared to the level at hour-72 led to worse clinical outcomes (see Figure 2). Although this is not statistically significant, this phenomenon supports earlier statement that VEGF was believed to play a key role in angiogenesis and neurogenesis after stroke.

It could also be explained that VEGF serum level in acute phase reflected the brain response to hypoxia degree and neuronal damage, whereas VEGF dynamic change showed VEGF role as neuroprotector through angiogenesis and neurogenesis processes. We deduced that the clinical outcomes did not only rely on high VEGF serum level in early phase of stroke, but also on its dynamic in acute and after acute-phase of stroke.

We evaluated correlation between infarct volume and Bamford classification with clinical outcomes. Infarct volume measured at hour-72 post-stroke had significant correlation with clinical outcomes at day-30 ($r = 0.382$; $p = 0.005$). This is similar to other previous studies.²⁰ Bamford classification also had correlation with clinical outcomes at day 30 after stroke ($r = 0.243$; $p = 0.053$). In this study we found that VEGF was the strongest clinical predictor on day-30, compared to the infarct volume and Bamford classification with an OR = 6.5 and $p = 0.034$ (95% CI = 1.15-36.61). (Table 3)

Limitations of this study was the small sample size, and there were 48 patients not measured VEGF level on day-7 and no measurement of VEGF level on day-30.

In conclusion, VEGF levels in the acute phase of ischemic stroke can predict the degree of brain damage. The dynamics of the increase in VEGF levels after stroke correlated with improved clinical outcomes. Further research on neuroprotective role of VEGF in post-stroke recovery may open up opportunities in the management of post-ischemic stroke patients.

Acknowledgment

We would like to thank Anyeliria Sutanto and Nora Tu for collecting data over the study period, also to Veli Sungono and Pricilla Gunawan for statistic analysis. We thank Prodia Laboratory for the support in this study. This study received financial support from Pelita Harapan University.

Conflict of interest

The authors affirm no conflict of interest in this study.

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