

Association of Hypoxia-Inducible Factor 1- α and Vascular Endothelial Growth Factor with Acute Ischemic Stroke Outcome

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Citation

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Abstract

Background and Purpose

Ischemic stroke is a major cause of death worldwide. Ischemic precondition stimulates the production hypoxia-inducible factor (HIF-1 α). HIF-1 α regulates other genes to produce erythropoietin and vascular endothelial growth factor (VEGF) to increase oxygen delivery through induction of angiogenesis to improve survival and clinical outcome. This study aimed to measure the association HIF-1 α and VEGF level with clinical outcomes in acute ischemic stroke.

Methods

A cross-sectional study was conducted to 57 ischemic stroke patients in the neurology department of Dr. Hasan Sadikin General Hospital Bandung and Muhammadiyah Hospital Bandung from April to November 2018. HIF-1 α level was examined on admission and VEGF during 72 hours of hospitalization. Clinical outcomes were assessed using the National Institute Health Stroke Scale (NIHSS) on admission and 72 hours after onset, and Modified Rankin Scale (MRS) on day-30. The correlation between HIF-1 α and VEGF levels was analyzed using the Spearman rank test.

Results

There was a significant negative relationship between HIF-1 α level and NIHSS 24-hour after onset of ischemic stroke ($r = -0.331$, $p = 0.040$), day-7 after onset ($r = -0.342$, $p = 0.035$), and day-30 after onset ($r = -0.393$, $p = 0.018$). There was no significant relationship between VEGF levels within 72 hours after onset ($r = 0.202$, $p = 0.063$) and day-30 after onset ($r = 0.018$, $p = 0.83$).

Conclusion

Our study was the first to establish the association of HIF-1 α and stroke in human. The HIF-1 α level correlated with the clinical outcome in acute ischemic stroke patients, as measured by NIHSS.

INTRODUCTION

Stroke is a major cause of mortality and morbidity worldwide [1]. Ischemic stroke is the most common subtype of stroke (around eighty percent) [1–3]. It occurs as the arteries narrow or blocked, leading to a severe reduction in the blood flow or ischemia [4]. Ischemic stroke is a medical emergency and prompt treatment is crucial to minimize brain lesions and potential complications [5]. Following a hypoxic condition, brain responses it by promoting gene expressions that function in preconditioning hypoxia. One of the

transcription factors involved is Hypoxia-inducible factor-1 α (HIF-1 α) [6–8].

HIF-1 α is a transcription activator that plays an integral role to maintain homeostasis after a hypoxic insult [9]. A disparity in the oxygen supply and demand in the brain tissue initiate a complex biochemical and molecular events that cause neuronal death [10]. As a response, cerebral tissue stimulates several cellular mechanisms. One of the recently studied mechanism is the induction of HIF-1 α [11]. It is a

transcriptional regulator of oxygen stability and the main inducer of adaptive response by upregulating several target genes which are important in erythropoiesis, angiogenesis, glucose transport, and metabolism [9, 12].

In response to inadequate oxygen level, HIF-1 α starts to accumulate. The first few studies regarding the upregulation of HIF-1 α upregulation during hypoxia were conducted in mice and rats [13, 14]. HIF-1 α mRNA expression is first detected within thirty to sixty minutes after the onset of hypoxia [13]. This level can increase up to 15-17 times. HIF-1 α induction and activation of target gene transcription occur in the penumbra area after ischemia. The upregulation of HIF-1 α can be detected 7.5 hours after ischemia and remain constant up to 24 hours in the penumbra area [14]. Accumulation of HIF-1 α can appear up to ten days in wild type mice [8]. Activation of HIF-1 α will increase blood flow, oxygen, and nutrients to the penumbra area [15].

Loss of HIF-1 α function in neurons reduces post-hypoxic viability, while the loss of HIF-1 α function in astrocytes protects neurons from hypoxia-induced neuronal death. HIF-1 α has different functions on different cells depending on the protein encoded by genes in specific cell types. That is why, induction, accumulation under ischemic conditions on different brain cells will have a different effect [11].

HIF-1 α upregulates the transcription factors of molecules with a protective property. Proteins such as erythropoietin, vascular endothelial growth factor (VEGF) and glucose transporter (GLUT) are produced by activation of HIF-1 α [16] have an important role in limiting the lesion of ischemia and angiogenesis so that apoptosis and necrosis can be reduced [17]. VEGF initiates the angiogenesis after ischemia [18]. The initiation of VEGF production is strongly influenced by HIF-1 α , while the production of HIF-1 α is influenced by the extent of brain lesions [16]. To date, studies regarding the relationship between HIF-1 α and stroke severity were conducted in animal models. Here, we aimed to measure the association HIF-1 α and VEGF level with clinical outcomes in acute ischemic stroke patients.

MATERIALS AND METHODS

Ethical clearance

This study was approved by the Research Ethics Committee of Hasan Sadikin General Hospital Bandung. All patients agreed to be included in the study were asked to signed informed-consent form.

Study period and locations

A cross-sectional study was performed from April 2018 to November 2018. The study was conducted in acute ischemic stroke patients (based on AHA/ASA criteria) that hospitalized in Neurology ward of Dr. Hasan Sadikin General Hospital Bandung and inpatient and outpatient clinic of Muhammadiyah Hospital Bandung.

Inclusions and exclusion criteria

All the patients that come to our clinic in the study period were offered to join this study, only patients who given their consent were enrolled. The inclusion criteria were a clinically acute ischemic stroke patients within 24 hours after onset, with a confirmation of non-contrast brain CT scan that shows no signs of bleeding, ages 18-70 years old, and not received anti-aggregation or neuroprotectant drug intervention on admission. The exclusion criteria were patient that has the following condition, a non-contrast brain CT scan showing extensive embolism (based on Bamford classification), acute myocardial infarction, acute kidney injury, acute limb ischemia, dementia, respiratory failure, head trauma, and any kind of malignancy. National Institute of Health Stroke Scale (NIHSS) were assessed on admission and seven days after onset of stroke, and Modified Rankin Scale (MRS) was examined on the 30th day during patients' follow-up visit.

Sample collection and ELISA

Patients' history and blood sample to examined HIF-1 α and other laboratory measure were obtained on admission before patients receive any medication. Blood sample (5ml) for HIF-1 α was taken during the first 24 hours and VEGF was taken during the first 72 hours of patients' admission at the same time with other routine blood works (vascular risk factors). All the blood samples were taken by trained nurse personnel and sent directly to the Department of Clinical Pathology Dr. Hasan Sadikin General Hospital Bandung for analysis. After centrifugation of blood sample, the serum was extracted then placed inside a cryotube. The sample was stored in a -20°C refrigerator if not directly used (maximal for 1 weeks) or in 4°C if measurement will be done in 2 hours.

The levels of HIF-1 α and VEGF blood serum HIF-1 α and VEGF were measured using ELISA. The enzyme linked immunosorbent assay (ELISA) was performed to detect the serum of subject using Hypoxia-inducible factor 1-alpha

(HIF-1 α) ELISA Kit (Elabsience). In brief, serum were incubated with Detection A solution at 37°C for 1h, washed for three times, incubated with Detection Reagent B working solution at 37°C for 45min, mixed with 90 μ l Substrate Solution followed by incubation at 37°C for 15min. Ang-1 levels were finally determined by measuring the absorbances at 450nm using a plate reader.

ELISA Kit for Human HIF-1 α (cat. no E-EL-H1277) and VEGF-A (Cat no E-EL-H0111) were procured from Elabsience (Houston, TX). The detection were done according to the manufacturer's guidelines using Elabsience Biotechnology Inc. (USA). The threshold according to the manufacturers are 0.10 ng/mL and 18.75 pg/mL for HIF1- α and VEGF, consecutively.

Statistical analysis

Statistical analysis was carried out using Statistical Product and Service Solution (SPSS) program for Windows version 25, (IBM, USA). Significant differences were determined using SPSS 25.0 software (IBM, USA). in the data are non-parametrics, Spearman Rank correlation analysis were performed for correlation, with p value < 0.05 was considered as significant.

RESULTS

Fifty-seven subjects were eligible for our study criteria (figure 1). The baseline characteristics of the subjects are displayed in table 1. Female subjects (52.6%) were more than male (47.4%). The most common risk factors were hypertension (n = 50), followed by hyperlipidaemia (n = 32), hyperuricemia (n = 26) and diabetes mellitus (n = 18).

Figure 1

Enrollment of the study subjects.

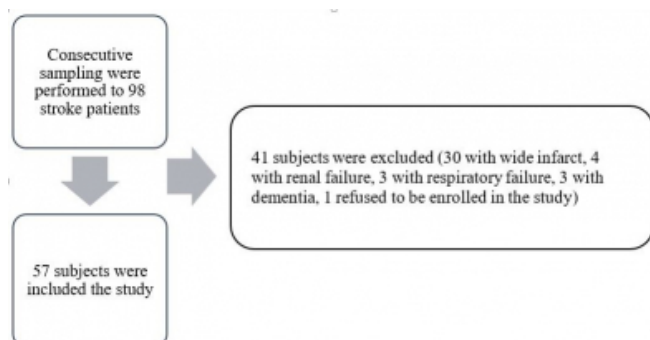


Table 1

Baseline Characteristics of the Subjects

Variables	n (%)	Mean \pm SD	Median (Min-max)
Age (years)		54 \pm 8	
Gender			
Male	27 (47,4)		
Female	30 (52,6)		
Risk Factors* :			
Hypertension	50 (39,6)	14,5 \pm 1,4	
Diabetes	18 (14,2)	43,1 \pm 4,1	
Hyperlipidaemia	32 (25,3)		
Hyperuricemia	26(20,6)		
Laboratory Results:			
Haemoglobin (gr/dL)			
Haematocrit (%)		142 \pm 64	9050 (7000 – 15630)
Leukocyte (/mm ³)			5,10 (4,11 – 5,70)
Erythrocyte (/mm ³)			273 (206 – 538)
Thrombocyte (/mm ³)			136,5 (68 – 332)
Random Plasma Glucose (mg/dl)			209 (112 – 386)
Cholesterol total (mg/dl)			41 (24 – 65)
HDL-cholesterol (mg/dl)			
LDL-cholesterol (mg/dl)			
Triglyceride (mg/dl)			135 (49 – 459)
Fasting Plasma Glucose (mg/dl)			126 (82 – 313)
Two-hour Plasma Glucose (mg/dl)			156 (92 – 362,0)
Uric Acid (mg/dl)			5,6 (2,6 – 13,9)
HbA1c (%)			6,5 (5,1 – 10,2)

SD: standard deviation; Min: minimum; Max: maximum; *in one subjects can be found more than one risk factor; HDL-cholesterol: high density lipoprotein cholesterol; LDL-cholesterol: low density lipoprotein cholesterol.

The distribution of HIF-1 α , VEGF, NIHSS, and MRS levels can be seen in table 2.

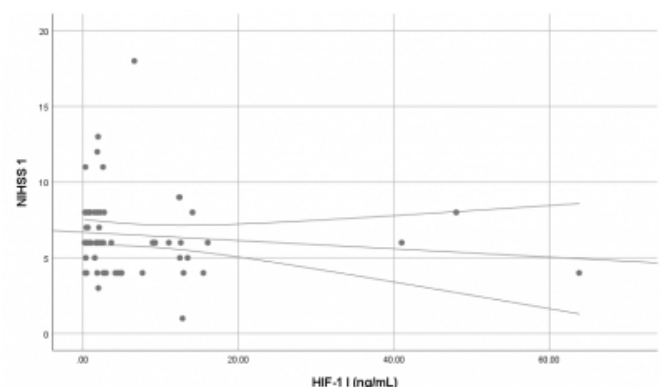
Table 2

HIF-1 α , VEGF, NIHSS, and MRS

Variables	Median (Min-Max)
HIF-1 α (ng/mL)	2.60 (0.24 – 63.80)
VEGF (pg/mL)	296.3 (45.8 – 91.8)
NIHSS 1 (24 hours)	6 (1 – 18)
NIHSS 2 (7 days)	3 (1 – 12)
MRS	1 (0 – 2)

Figure 2

Scatter plot of HIF-1 α and NIHSS 1



Analysis of the relationship between HIF-1 α and VEGF levels with the degree of neurological deficit 24-hours after onset (NIHSS 1) can be seen in table 3. There was a weak negative correlation with a between HIF-1 α and NIHSS 24-

hours after onset ($r = -0.331$, $p = 0.040$), this indicates that the higher HIF-1 α , the lower the NIHSS. When assessing the degree of neurological deficit based on the NIHSS, the higher the NIHSS value, the more severe the degree of neurological deficits. This will result in poorer clinical outcomes. The scatter plots of HIF-1 α and NIHSS 1 showed in figure 2.

Table 3
Bivariate analysis between HIF-1 α and NIHSS 1

Variables	NIHSS 1	
	R coefficient	P value
HIF-1 α (ng/mL)	-0.331	0.040*

*statistically significant

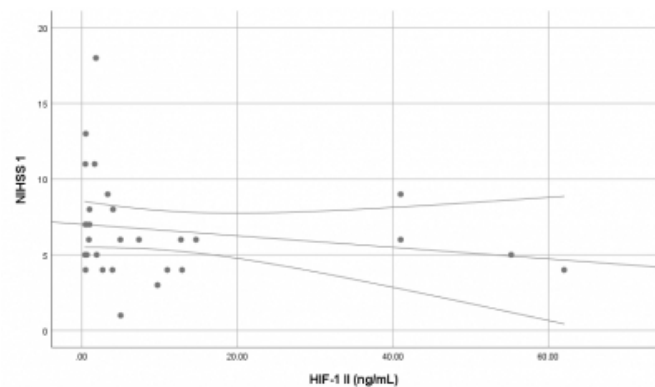
Analysis of the relationship between HIF-1 α and VEGF levels with the degree of neurological deficit based on day-7 NIHSS (NIHSS 2) can be seen in table 4. We found a weak negative correlation between HIF-1 α and NIHSS 7 days after stroke onset ($r = -0.342$, $p = 0.035$), meaning that the higher HIF-1 α , the lower the NIHSS marked by lower degrees of neurological deficits in patients with acute ischemic stroke.

Table 4
Bivariate analysis between HIF-1 α and NIHSS 2

Variables	NIHSS 1	
	R coefficient	P value
HIF-1 α (ng/mL)	-0.342	0.035*
VEGF (pg/mL)	0.205	0.063

*statistically significant

Figure 3
Scatter plot HIF-1 α and NIHSS 2



Analysis of the relationship between HIF-1 α and VEGF levels with the degree of neurological deficit based on MRS 30 days after onset of stroke is shown in table 5. We identified a weak negative correlation between HIF-1 α and MRS 30 days after onset ($r = -0.393$, $p = 0.018$), this shows

that the higher HIF-1 α , the lower the MRS 30 days. HIF-1 α may be used to determine long-term improvements in the clinical outcome based on the MRS scale. The scatter plots of HIF-1 α and NIHSS 1 showed in figure 3.

Table 5
Correlation between HIF-1 α , VEGF and MRS

Variables	NIHSS 1	
	R coefficient	P value
HIF-1 α (ng/mL)	-0.393	0.018*
VEGF (pg/mL)	0.122	0.183

*statistically significant

DISCUSSION
Fifty-seven subjects met the inclusion and exclusion criteria in our study. We found significant negative correlations between HIF-1 α and NIHSS at 24-hours after ischemic stroke onset ($r = -0.331$, $p = 0.040$), HIF-1 α and NIHSS stroke on day-7 after onset ($r = -0.342$, $p = 0.035$) and HIF-1 α and MRS on day 30 ($r = -0.393$, $p = 0.018$). These results showed that the higher the HIF-1 α , the lower the NIHSS. The higher the HIF-1 α , the lower the MRS. It was in accordance with our preliminary study which showed a parallel conclusion (unpublished work). Our study indicated that HIF-1 α may be used as a positive prognostic marker in acute ischemic stroke.

During cerebral ischemia, HIF-1 can induce VEGF expression up to 30-fold as damaged tissue attempts to increase oxygen delivery by producing VEGF to induce angiogenesis. This process is characterized by an increase in the number of microvasculatures in the infarct area Not only VEGF expression but other survival factors for angiogenesis such as angiopoietin-2 and insulin growth factor (IGF)-2 and its receptors are induced by HIF-1 α [19].To date, studies regarding the relationship of HIF-1 α and ischemic stroke were all conducted in animal model and intervention study in animal which is giving Xenon preconditioning in middle cerebral occlusion and renal injury rat model showed a good outcome [24,25,26].

The collateral blood vessel is the first defense by the ischemic tissue to provide an alternative pathway for blood flow to the tissue. Collateral blood vessel supply in the brain circulation involves intracranial and extracranial blood vessels. An adequate collateral system will help reduce stroke severity and is a major target in the treatment of

stroke [17, 20].

In this study there was no significant correlation between VEGF and NIHSS levels on day-7 after the onset of strokes ($r = 0.205$, $p = 0.603$) and between VEGF and 30-day MRS ($r = 0.122$, $p = 0.183$). This was not in accordance with the previous study which showed that VEGF had a positive correlation with the degree of neurological deficits [18]. This may be due to several reasons. First, the difference in the extent of brain lesions. A study conducted by Matsuo et al., found increased VEGF levels in infarction stroke with four subtypes: atherothrombotic stroke; lacunar stroke; cardioembolic stroke; and another type of stroke. The highest increase in VEGF was found on the third day after onset and in the cardioembolic type. The study correlates the increase in VEGF levels with the degree of neurological deficits and a significant relationship was found in the cardioembolic stroke group, where increased VEGF levels correlated with poor neurological deficits in its group. It can be seen that VEGF formation is strongly influenced by the extent of lesions in the brain [18]. In our study, subject inclusion was restricted to non-extensive brain lesions (based on Bamford classification and head CT scan), this may contribute to the difference of result between our study and the study by Matsuo et al.,. Second, our study was observational. We did not control for the effects of drug administration such as anti-platelet aggregation or neuroprotectant. Although the blood collection for HIF-1 α was performed before definitive therapy was given, the blood collection for VEGF was performed during the therapy. Thus the NIHSS value may have been affected by the medication, not only by VEGF elevation. Third, experimental study of exogenous VEGF in animal model conducted by Fukino et al., showed that genetics play an important role in the spontaneous collateral formation after brain ischemia [21]. In our study, the race of the subjects had been narrowed down to two races (Sundanese and Javanese), but the study did not examine and consider the role of other genetic factors that may affect the angiogenesis process by VEGF after cerebral ischemia.

Although our study did not establish a correlation between VEGF and NIHSS, we would like to emphasize caution to future VEGF study, specifically clinical trials with VEGF as a therapeutic modality in acute phase ischemic stroke [17, 22, 23]. Under certain conditions, VEGF can induce cerebral edema in the experimental animal model [23]. VEGF injected intraventricularly after occlusion of the medial cerebral

artery at high doses results in the worsening of neurological deficits marked by infarct-volume expansion in the animal model. The expansion of the infarct volume is due to an increase in cerebral vascular permeability caused by the induction of angiogenesis and the opening of the collateral system by VEGF after cerebral ischemia. In addition, VEGF is known as Vascular Permeability Factor (VPF) which is a specific mitogen for endothelial cells. VEGF plays a role in the cardiovascular system for myofibroblasts formation, where VEGF contributes to tissue remodeling in the infarcted area. In the process of atherosclerosis, uptake of LDL oxidized by macrophages will initiate foam cell formation in atherosclerotic lesions. This will increase the production of VEGF by monocytes and macrophages. VEGF also increases atherosclerosis by increasing vascular permeability to LDL [24]. This should be put into consideration when designing a study with VEGF administration.

This study has several limitations. Many factors influence the VEGF levels such as the administration of statin drug that also function in blood vessel remodeling. The drug could be a confounding factor when establishing the effect of VEGF in angiogenesis. Further research with a larger sample population, controlling for confounding factors, and providing similar treatment that affects the levels of HIF-1 α and VEGF needs to be conducted.

CONCLUSIONS

Our study showed a relationship between HIF-1 α level and the degree of neurological deficits in acute ischemic stroke. There was no relationship between VEGF levels and the degree of neurological deficits of acute ischemic stroke.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical Clearance

This study was approved by the Research Ethics Committee of Hasan Sadikin General Hospital Bandung and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All patients agreed to be included in the study were asked to signed informed-consent form prior to their inclusion in the study.

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