

Comparison of Mean VEGF-A Expression Between Acute Ischemic Stroke Patients and Non-Ischemic Stroke Subjects

Ismail Setyopranoto¹, Ahmad Hamim Sadewa², Samekto Wibowo¹, I Putu Eka Widyadharna^{3*}

¹Department of Neurology, Faculty of Medicine, Universitas Gadjah Mada and Dr Sardjito General Hospital, Yogyakarta, Indonesia; ²Department of Biochemistry, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia; ³Department of Neurology, Faculty of Medicine, Udayana University and Sanglah General Hospital, Bali, Indonesia

Abstract

Citation: Setyopranoto I, Sadewa AH, Wibowo S, Widyadharna IPE. Comparison of Mean VEGF-A Expression Between Acute Ischemic Stroke Patients and Non-Ischemic Stroke Subjects. Open Access Maced J Med Sci. <https://doi.org/10.3889/oamjms.2019.175>

Keywords: Acute ischemic stroke; Hypoxia; Expression; VEGF-A

***Correspondence:** I Putu Eka Widyadharna. Department of Neurology, Faculty of Medicine, Udayana University and Sanglah General Hospital, Bali, Indonesia. E-mail: eka.widyadharna@unud.ac.id

Received: 13-Jan-2019; **Revised:** 17-Feb-2019; **Accepted:** 18-Feb-2019; **Online first:** 13-Mar-2019

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

BACKGROUND: Glucose and oxygen supply to neurons are disrupted during acute ischemic stroke, resulting in hypoxia. This event, in turn, activates the transcription of hypoxia-inducible factor (HIF-1), which is responsible for activating genes responsible for angiogenesis, including vascular endothelial growth factor (VEGF). VEGF and their receptor systems exert complex mechanisms of angiogenesis, including the stimulator, inhibitors, angiogenic and modulator. VEGF-A is the primary regulator of angiogenesis, both during physiological and pathological conditions. Nevertheless, the role of VEGF on the prognosis of hypoxia remains controversial.

AIM: The purpose of this study was to address if there is any difference between the mean expression of VEGF-A between acute ischemic patients and non-ischemic stroke subjects.

METHODS: This was an observational study with a cross-sectional design, the population in this research is the acute ischemic stroke patients and non-ischemic stroke subjects, which were admitted on Emergency Room and later treated in the Stroke Unit, Dr Sardjito General Hospital, Yogyakarta, Indonesia. Subjects were recruited using the purposive method, yielding a total of 64 subjects on both groups. Diagnosis of acute ischemic stroke was established using a head CT scan. Patients who meet the inclusion criteria and willing to participate in the study were asked to provide informed consent. Laboratory analysis was conducted during the first 24 hours after being treated at Stroke Unit, Dr Sardjito General Hospital, Yogyakarta, Indonesia, with venous blood was withdrawn VEGF-A levels between acute ischemic stroke and non-ischemic stroke subjects were subsequently compared. Categorical variables (including gender) were tested using either chi-square or Fisher exact test. Interval data was examined using student t-test if data distribution was normal.

RESULTS: As many as 35 acute ischemic stroke and 35 non-ischemic stroke patients were included in the study, among whom were 18 men (51.43%) and 17 women (48.57%) among stroke patients and 21 (60%) men and 14 (40%) women among subjects without stroke. The average of the subject's age on stroke and non-ischemic stroke group was 58.51 and 48.57 years old. VEGF-A levels were significantly higher in the non-stroke group (561.77 ± 377.92) compared with stroke group (397.78 ± 181.53) with $p = 0.02$.

CONCLUSION: expression of VEGF-A in acute ischemic stroke group was lower when compared with the non-stroke group.

Introduction

VEGF-A is the primary regulator of angiogenesis, both during physiological and pathological conditions, including those related to malignancy or hypoxia of any cause including acute ischemic stroke [1]. Hypoxia triggers smooth muscle cell proliferation and endothelial cells to form new blood vessels so that it will improve the function of oxygenation. Hypoxia activates several important

genes and signal pathways under the condition above [2].

As a result of hypoxia, the gene will be controlled by the transcription of Hypoxia-Inducible Factor 1 (HIF-1). HIF-1 will activate genes responsible for angiogenesis, for example, Vascular Endothelial Growth Factor (VEGF) [3]. Oxygen functions as a regulator of VEGF production, hypoxia causes an increase in VEGF levels and returns to the baseline level within 24 hours, and then the cell returns to normoxia condition [4]. VEGF levels will be reduced in

hyperoxia in vitro and in vivo [5]. Nevertheless, the role of VEGF in hypoxia remains controversial, considering several studies showing different results.

This study aims to prove that there are differences in the mean expression of the Vascular Endothelial Growth Factor-A (VEGF-A) between acute ischemic stroke patients with non-stroke patients.

Material and Methods

This was an analytical observational study. The inclusion criteria in this study were acute ischemic stroke patients both male and female, of 35 to 70 years old, with a maximum 5-day duration between the time of the attack and hospital admission, (2) stroke onset was defined as the last time the patient was known by others in a healthy condition and had not shown any signs of neurological deficit, (3) the presence of one of the neurologic deficits are decreased consciousness, hemiparesis, seventh and/or eighth cranial nerve palsy, dysarthria, aphasia, or hemianopsia, (4) proven acute ischemic stroke with head CT scan.

The eligible criteria in this study were: (1) intracerebral haemorrhage patients or the space-occupying process for other reasons, (2) any history of previous strokes.

Non-ischemic stroke groups including non-infectious low back pain patients who were treated in the Neurological Ward or general check-up patients who are known to have no degenerative abnormalities that have vascular influences, such as hypertension, diabetes mellitus or dyslipidemia, taken in the same period, both male and female.

Subjects were recruited by a consecutive method, in which every new ischemic stroke patient who came through an Emergency Department and treated at the Stroke Unit Dr Sardjito General Hospital, who fulfilled the inclusion criteria and willing to participate in the study after being explained with the risks and benefits. All participants included in the study were asked for informed consent.

These steps must be carried out until the specified sample size reaches the number of samples. The participants above identity and characteristics were then recorded in the case form.

History taking and physical examination of all included subjects were performed by a neurology resident and those who were suspected of having acute ischemic stroke underwent additional head CT scan.

Laboratory analysis was carried out once, at 24 hours after being treated at the Stroke Unit Dr Sardjito General Hospital, and blood was withdrawn

from a vein, then VEGF-A levels were examined by ELISA method.

Comparative analysis was made on subjects with acute ischemic stroke with non-stroke subjects concerning VEGF-A levels. The level of significance used is 0.05 or 95% confidence interval ($p < 0.05$), with 80% power. All statistical analyses were performed using SPSS for Windows version 20.

Results

This study began in August to December 2011. A total of 11 subjects were not included in this study, among whom 8 of them refused to participate in the study and three subjects were excluded because of acute renal insufficiency. Seventy subjects, therefore, were included in the study, i.e., every 35 subjects with acute ischemic stroke and non-ischemic stroke cases. The basic characteristics of the research subject were obtained through descriptive analysis.

Of the total subjects in the stroke group based on sex, 18 (51.437%) subjects were of males, and 17 (48.57%) subjects were of females. Subject comparison based on sex were then considered homogenous. In contrast, in the non-ischemic stroke group, there were more female than male subjects (21 [60%] vs 14 [40%]).

Table 1: Basic Characteristics of Research Subjects

	Stroke Groups	Non-Stroke Groups	p
Male	18 (51.43%)	14 (40.00%)	-
Female	17 (48.57%)	21 (60.00%)	-
Age (years)	58.51 ± 9.89	48.57 ± 11.36	-
VEGF-A level	397.78 ± 181.53	561.77 ± 377.92	0.02

Furthermore, based on the subjects' age, the average stroke group was 58.51 years old, higher than the non-ischemic stroke group with an average of 48.57 years old. While VEGF-A levels were significantly higher in the non-ischemic stroke group, i.e. 561.77 ± 377.92, compared to the stroke group (397.78 ± 181.53) with $p = 0.02$.

Discussion

VEGF-A levels in the stroke group were lower than that of non-ischemic stroke group. This result is different from other studies, in which VEGF-A levels were found at higher levels. For instance, in cases of malignancy, an increase in VEGF-A expression can be observed in lung adenocarcinoma, six colorectal carcinomas, pancreatic carcinomas, breast carcinoma, and ovarian carcinoma [7], [8], [9], [10].

There are several explanations as to why in the stroke group there was a lower VEGF-A level compared with the non-ischemic stroke group. Stroke is caused by several potential risk factors associated with a decrease in VEGF levels, for example, elderly, diabetes mellitus and also the possibility of early complications of kidney failure, although the results of laboratory tests have not shown signs of kidney failure. Low serum albumin levels are often found in elderly patients. Hypoalbuminemia was reported in 19% of stroke patients [11].

Furthermore, albumin significantly reduced VEGF expression during hypoxia [12].

Hypoalbuminemia is associated with endothelial dysfunction in all causes of death in patients with chronic kidney disease and cardiovascular mortality in terminal kidney disease patients. However, it is not yet clear whether endothelial dysfunction is a direct result of a decrease in albumin levels or due to other factors, such as chronic inflammation and dyslipidemia, and indeed it is often found in conditions associated with hypoalbuminemia [12].

In contrast to previous *in vitro* studies, in this study, we found that a decrease in VEGF-A levels is a response to hypoxia. An explanation might be related to the study of Lerman et al., in mice, by which the production of VEGF by fibroblasts in diabetic conditions-because of the presence of glucose intolerance-did not increase the regulation of VEGF under hypoxic conditions [14]. Hypoxia causes glucose intolerance *in vivo*, so in this study, VEGF production may be influenced by glucose intolerance, because stroke patients may have risk factors for diabetes mellitus [15].

There is a study on changes in VEGF levels in the circulation which are likely to be influenced by glucose transporters, on the blood-brain barrier [16] [17]. Besides, VEGF also mediates the induction of endothelial fenestration, thus increasing the transport of small molecules such as sucrose and fluorescein to penetrate the blood-brain barrier [18], [19]. Based on these mechanisms on the blood-brain barrier, a decrease in VEGF after acute hypoxia can reduce glucose transfer across the blood-brain barrier which ultimately is neuroprotective.

Furthermore, a decrease in VEGF-A levels in the stroke group compared with the non-ischemic stroke group might be partly explained by the fact that astroglial cells are the most abundant cells in the central nervous system and play an important role in the pathology of brain tissue. Although never proven directly, astrocytes are thought to have a neuroprotective role in protecting neurons from oxidative stress during a stroke. The hypothesis is based on the ability of astrocytes to act as a buffer, to transport and metabolise amino acids, glucose, and other important molecules, and also improve antioxidant regulation and free radical scavenger in

the area of ischemia. Reactive gliosis is the response of the astrocytes at the time of central nervous system injury, including cerebral ischemia, and reactive astrocytes undergo changes in morphology and expression in various molecules [20].

Reported by Bareto et al., that although there is extensive cell proliferation, especially in microglia and neutrophils/monocytes at one week after the stroke, however, there are some adult astrocytes that re-enter into the cell cycle, and this is concentrated around the infarct area [21].

Astrocytes are known to be more resistant to oxidative stress than neurons and act as neuroprotectors through their ability to take potassium and glutamate and release mitogenic factors. But in response to injury, astrocytes will pull off its endfeet from the blood vessels, which will result in increased permeability, as well as due to proliferation, it will cause scars on the glial cells [22].

Hypoxia/ischemia causes activation of adaptive mechanisms and changes in gene expression in injured areas to counteract the pathological progression caused by inducing transcription of Hypoxia-Inducible Factor (HIF-1). The primary target of HIF-1 is a gene that plays a cytoprotective role, VEGF. Astrocytes can secrete VEGF under physiological conditions, and then hypoxia will induce both mRNA and protein [23].

Other factors also cause a decrease in VEGF expression due to hypoxia, namely Glia Cell-Derived Conditioned Medium (CM) [24]. Furthermore, it was explained that hypoxia which causes astrocytes could inhibit an increase in VEGF mRNA expression Conditioned Medium (CM) or C6 CM glioma cells. During normoxia, CM astrocytes and C6 CM glioma cells do not change the expression of VEGF mRNA. Thus hypoxia which causes an increase in VEGF protein will be inhibited by CM glia cells.

Studies are documenting the potential of VEGF therapy to repair peripheral neuropathy in diabetes [25]. Other studies reported that intramuscular gene transfer with VEGF-bound plasmids was able to improve motor and sensory function in a rabbit model who had peripheral ischemic neuropathy [26]. Besides, VEGF administration therapy in post-stroke patients can reduce the extent of cerebral infarction because VEGF will stimulate angiogenesis and neurogenesis in areas near the penumbra [27], [28]. The administration of specific antibodies to block the function of VEGF in injured animal models apparently can increase the size of the lesion and reduce angiogenic activity and astroglia in the striatum [29].

There are also other studies that support the role of VEGF in controlling damage from brain injury, for example, the administration of VEGF to spinal cord contusions can improve cellular levels, and the administration of VEGF significantly increases nerve

regeneration when the Matrigel implant is inserted into sciatic nerve injury [30], [31], [32].

Based on these findings, for example in models of stroke, diabetic neuropathy, and spinal cord injury, VEGF therapy significantly increases cellular and functional improvement, so it is reasonable that when a cellular injury occurs due to hypoxia, it appears that there is a decrease of VEGF expression. Another possibility of decreasing VEGF-A expression is the activity of Glia Cell-Derived Conditioned Medium (CM), both CM astrocytes and C6 CM glioma cells because in acute stroke there will be an increase in proliferation of glial cells, including astrocytes.

Another theory that VEGF will increase its expression in the acute phase of brain injury so that it can affect endothelial permeability which results in cerebral oedema, for example in patients with acute stroke, is still controversial, and further research is needed with a focus on VEGF receptor activity that might answer the controversy.

The limitations of this study were: (1) the activity of glial cells during hypoxic conditions is difficult to control, (2) the maximum time taken for taking blood in patients is 6 hours from the time of onset, and (3) the number of other variables that can affect VEGF-A expression in acute ischemic stroke group.

It was concluded that the expression of VEGF-A in the acute ischemic stroke group was lower than in the non-ischemic stroke group.

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