



## Research article

Hypoxia-inducible factor-1 $\alpha$  in acute ischemic stroke: neuroprotection for better clinical outcomeLisda Amalia<sup>a,\*</sup>, Henny Anggraini Sadeli<sup>a</sup>, Ida Parwati<sup>b</sup>, Ahmad Rizal<sup>a</sup>, Ramdan Panigoro<sup>c</sup><sup>a</sup> Department of Neurology, Faculty of Medicine, Universitas Padjadjaran, Hasan Sadikin General Hospital Bandung, Indonesia<sup>b</sup> Department of Clinical Pathology Faculty of Medicine, Universitas Padjadjaran, Hasan Sadikin General Hospital Bandung, Indonesia<sup>c</sup> Department of Biomedical Science Faculty of Medicine, Universitas Padjadjaran, Indonesia

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## ABSTRACT

**Background:** Hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) is a transcription factor which maintains cellular homeostasis in response to hypoxia. It can trigger apoptosis while stimulating angiogenesis process and decrease neurological deficit after an ischemic stroke. Up until now, this protein complex has not been widely investigated especially in stroke patient.

**Objective:** Here, we examined the potential of HIF-1 $\alpha$  as a marker for neuroplasticity process after ischemic stroke.

**Methods:** Serum HIF-1 $\alpha$  were measured in acute ischemic stroke patients. National Institute of Health Stroke Scale (NIHSS) were assessed on the admission and discharge day (between days 7 and 14). Ischemic stroke divided into 2 groups: large vessel disease (LVD, n = 31) and small vessel disease (SVD, n = 27). Statistical significances were calculated with Spearman rank test.

**Results:** A total of 58 patients, 31 with large artery atherosclerosis LVD and 27 with small vessel disease (SVD) were included in this study. HIF-1 $\alpha$  level in LVD group was  $0.5225 \pm 0.2459$  ng/mL and in SVD group was  $0.3815 \pm 0.121$  ng/mL. HIF-1 $\alpha$  was higher ( $p = 0.004$ ) in LVD group than in SVD group. The initial NIHSS score in LVD group was  $15.46 \pm 2.61$  and discharge NIHSS score was  $13.31 \pm 3.449$ . Initial NIHSS score in SVD group was  $6.07 \pm 1.82$  and the discharge NIHSS was  $5.703 \pm 1.7055$ . In both SVD and LVD group, HIF-1 $\alpha$  were significantly correlated with initial NIHSS (both  $p < 0.001$ ) and discharge NIHSS ( $p < 0.0383$   $r = 0.94$ ,  $p < 0.001$ ,  $r = 0.93$ , respectively).

**Conclusions:** HIF-1 $\alpha$  has a strong correlation with NIHSS and it may be used as predictor in acute ischemic stroke outcome.

## 1. Introduction

Stroke is one of the leading causes of mortality, morbidity and severe disability in the world. Ischemic stroke is accounted for eighty percent of all strokes, via narrowing or blockage of arteries, leading to severe blood flow reduction. Prompt management is an important measure to minimize complications [1, 2].

A disproportion in the supply and oxygen demand in cerebral tissue promotes a sequence of biochemical and molecular events that leads to neuron cell death. Following a hypoxia condition, brain responses in many ways. One of the most intriguing ways is by producing pre-conditioning hypoxia protein which includes transcription factors Hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) [3, 4, 5]. Studies of HIF-1 $\alpha$  in

hypoxia was just started recently, although the factor itself has been found for more than 2 decades [4, 6, 7, 8].

Until now, research around HIF-1 $\alpha$  has some opposing results. In one hand, it acts as oxygen stability transcription regulator and a main stimulant of adaptive response via upregulation of several target genes. These genes are important in erythropoiesis, angiogenesis, glucose transport, and metabolism [7, 9, 10, 11]. In the other hand, some studies show the role HIF-1 $\alpha$  in the apoptosis cascade by stimulating pro-apoptotic molecules, namely Nix, IL-20 and BNIP30 which result in mitochondrial dysfunction and lead to neuronal cell death [7, 11, 12]. Therefore, a deeper understanding regarding the role of HIF-1 $\alpha$ , specifically in ischemic stroke, is required to improve recovery and brain repair process after stroke.

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Moreover, to date, all HIF-1 $\alpha$  studies related to ischemic stroke by far were done only in animal model. Our study was the first study to determine the association between HIF-1 $\alpha$ , the severity of stroke ischemia, and its outcome in patients.

## 2. Methods

This was a cross-sectional study on acute ischemic stroke patients.

### 2.1. Clinical and imaging evaluation

Subjects were clinically diagnosed by two senior consultants, and then divided into two groups: large vessel disease (LVD) and small vessel disease (SVD). National Institute of Health Stroke Scale (NIHSS) was assessed in the admission and before discharge. The other inclusion criterion was onset less than 24 h. Exclusion criteria were patients with chronic renal failure, acute myocardial infarction, severe respiratory failure, severe anemia, dementia, acute limb ischemia and history of brain injury. Stroke imaging confirmation was non-contrast brain computerized tomography (CT) scan.

### 2.2. HIF-1 $\alpha$ measurement

Blood sample was collected less than 24 h from onset of stroke. The serum was separated by allowing blood to clot for 30 min and then centrifuging 2500 RPM for 15 min. It was stored in -20 °C – -80 °C if not directly processed. Enzyme linked immuno-sorbent assay was done according to manufacturer protocol (cat no E-EL-H1277, Elabscience Biotechnology Inc.). The result was rounded to the closest 1 decimal value.

### 2.3. Statistics

Statistical calculations were done using IBM SPSS 22.0 (IBM Corp, Armonk, NY). Spearman rank-test was utilized to examine relationship. P value below 0.05 is considered significant. R coefficient was calculated to establish the correlation.

### Ethical approval

This study is approved by Dr. Hasan Sadikin General Hospital Bandung Human Research Ethic Committee. This study had complied with all relevant ethical regulations (including The Declaration of Helsinki). Written consent was obtained from all patients.

## 3. Results

A total of 58 subjects were enrolled in this study, i.e. 31 subjects with LVD and 27 subjects with SVD). The mean age was  $59.52 \pm 11.52$  years. Laboratory data of risk factor including blood glucose, uric acid, renal and lipid profile showed in [Table 1](#).

Control group was enrolled with matched age, and sex. However, the HIF-1 $\alpha$  level in this healthy population, was fall below the detection limit of the ELISA kit.

HIF-1 $\alpha$  level in LVD group was  $0.5225 \pm 0.2459$  ng/mL (mean  $\pm$  SD) and SVD group was  $0.3815 \pm 0.121$  ng/mL. The level of HIF-1 $\alpha$  was significantly higher ( $p = 0.004$ ) in the LVD group than in the SVD group ([Table 2](#) and [Figure 1](#)).

Clinical features in LVD or SVD patients were in accordance with the size of ischemic lesions identified from our non-contrast brain CT results (supplementary data). Wide ischemic changes were found in the LVD group.

The initial NIHSS score in LVD group was  $15.46 \pm 2.61$  (mean  $\pm$  SD) and discharge NIHSS score was  $13.31 \pm 3.449$ . Initial NIHSS score in SVD group was  $6.07 \pm 1.82$  and the discharge NIHSS was  $5.703 \pm 1.7055$  ([Table 3](#)). The higher the HIF-1  $\alpha$  the less NIHSS changes is showed in [Figure 2](#).

[Table 4](#) showed the correlation between HIF-1 $\alpha$  level and NIHSS in both groups. Here, we identified a strong correlation between the two variables in both groups.

In the LVD group, the level of HIF-1 $\alpha$  was significantly correlated with initial NIHSS ( $p < 0.001$ ) and discharge NIHSS ( $p < 0.001$ ). In the SVD group we identified a similar result, the level of HIF-1 $\alpha$  significantly correlated with initial NIHSS ( $p < 0.001$ ) and discharge NIHSS ( $p = 0.0383$ ).

## 4. Discussion

Our study includes 58 acute ischemic stroke subjects that grouped into LVD and SVD. Our study is the first time in human. We identified association between HIF-1 $\alpha$  level and the subtypes of ischemic stroke.

Due to stroke, HIF-1 $\alpha$  starts to accumulate as a response to inadequate oxygen level. Early hypoxia studies of HIF-1 $\alpha$  regulation were done in rodents [13]. HIF-1 $\alpha$  mRNA expression after hypoxia could be detected within first thirty to sixty minutes [14] and may be increased to 17 folds. HIF-1 $\alpha$  induction and transcription activation of target gene is occurred in penumbra. This will lead to increase blood flow, oxygenation, and nutrients transport to the penumbra area [15]. The regulation of HIF-1 $\alpha$ , in penumbra, is rising until 7.5 h after onset of ischemia and plateaued for 24 h [13]. The HIF-1 $\alpha$  accumulation, in rodents, may persist up to ten days [9].

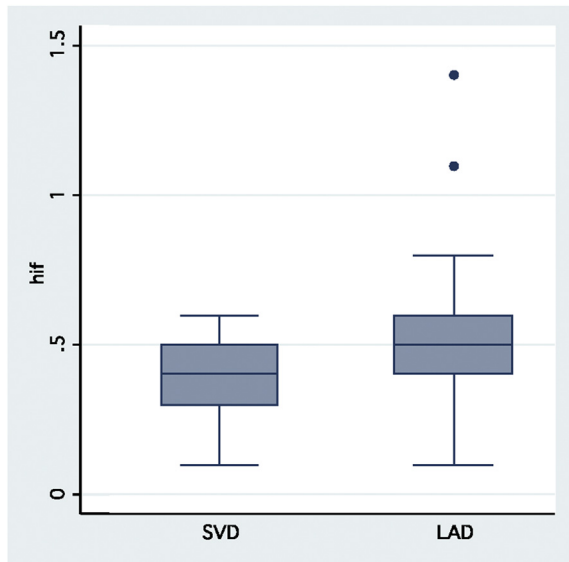
The level of HIF-1 $\alpha$  ([Table 2](#) and [Figure 1](#)) was significantly higher in the LVD group than in the SVD group ( $p = 0.004$ ). This result was in line

**Table 1.** Demographic and laboratory data.

No	Variable	Mean	SD	Min	Max
<i>Demographic</i>					
1	Age (year)	59.53	11.52	31	91
<i>Risk factor</i>					
2	Cholesterol (mg/dL)	200.65	45.07	83	299
3	Triglyceride (mg/dL)	123.15	60.13	49	366
4	HDL (mg/dL)	46.63	16.99	17	99
5	LDL (mg/dL)	141.09	38.36	60	224
6	Random Blood Glucose (mg/dL)	141.17	60.02	77	363
7	Urea (mg/dL)	37.52	31.16	13	267
8	Creatine (mg/dL)	0.93	0.32	0.38	2.4
9	Uric acid (mg/dL)	6.23	2.13	2.1	12.4
10	HbA1c (%)	7.61	2.58	4.5	13.3

**Table 2.** HIF-1 $\alpha$  level in LVD and SVD groups.

HIF-1 $\alpha$	Mean	SD	Min	Max	p
Large vessel disease	0.5225	0.2459	0.1	1.4	0.004
Small vessel disease	0.3815	0.121	0.1	0.6	

**Fig. 1.** HIF-1 $\alpha$  level in SVD and LVD groups.

with physiology and previous studies conducted in animal model of cerebral ischemia [3].

We then analyzed the brain CT. From non-contrast brain CT scans, we identified a relation between HIF-1 $\alpha$  concentration to the size of ischemic lesions (supplementary data). Wide ischemic changes were found in the LVD group. This finding showed the extent of neuronal brain damage after ischemic stroke.

This result was in accordance to previous studies which suggest that a wide brain damage is associated with an increase of HIF-1 $\alpha$  production. This increase is initiated in the preconditioning-hypoxia sequence induced by neuronal damage [7, 8, 9, 10, 11, 12].

HIF-1 $\alpha$  level, as the master regulator of the cellular response to hypoxia, is tightly controlled through synthesis and degradation. HIF-1 $\alpha$  protein accumulation during hypoxia is a result from inhibition of its oxygen-dependent degradation by von Hippel Lindau protein (pVHL) pathway. However, recent data described a distinctive new pVHL or oxygen-independent mechanisms for HIF-1 $\alpha$  degradation. Despite the global inhibition of protein translation during hypoxia, a constitutive translation of HIF-1 $\alpha$  is still occurred [16].

Other study mentioned that normal population also have some levels of. He et al, in 2016 garnered a normal healthy population age over 18, with no major disease, including heart, liver, lung, kidney and other vital organ disease and not in menstrual period for female. In that study, HIF-

1 $\alpha$  activity median level was 274.92 pg/mL, with a higher concentration on non small cell lung carcinoma patient 297.7 pg/mL [17].

For control, we had already examined HIF-1 $\alpha$  in healthy, age, sex matched subject, however HIF-1 $\alpha$  were too low to be detected by our ELISA system. Although, given the median HIF-1 $\alpha$  concentration that showed by He et al, a common ELISA assay like ours may be able to detect the concentration of normal population.

In a study by Cai et al, they compared HIF-1 $\beta$  and PGC-1 $\beta$  of breast cancer and a healthy patient, however in the body text, the study are comparing HIF-1 $\alpha$  and PGC-1 $\alpha$  of breast cancer and a benign breast tumor. This was a decent publication, nonetheless confusing, since they were different protein and different type of subject sample, and of course refer to different entity [18].

A wide brain damage also correlates with worsening of neurological deficit in acute ischemic stroke patients [4]. In this study we found that in LVD group initial NIHSS score was  $15.46 \pm 2.61$  and discharge score was  $13.31 \pm 3.449$ . In SVD group initial NIHSS score was  $6.07 \pm 1.82$  and the discharge score was  $5.703 \pm 1.7055$  (Table 3).

We then established an association between HIF-1 $\alpha$  and NIHSS (Table 4). There were strong correlations between HIF-1 $\alpha$  and on admission and discharge NIHSS in both groups. In previous rodent studies, HIF-1 $\alpha$  was activated in a biphasic manner lasting up to ten days after a hypoxic stimulation [3]. It stimulates pro-angiogenic factors [4], promotes neuronal viability [7], and ameliorates brain injury [11]. We examined the HIF-1 $\alpha$  level only at admission, but as it significantly correlated with the NIHSS, we may suggest that HIF-1 $\alpha$  level would decrease on discharge day and is associated with the improvement of clinical outcome, as occurs in mice model. Future study examining serial HIF-1 $\alpha$  level needs to be performed to prove this hypothesis.

Our finding is in accordance to Xiu et al. They reported that HIF-1 $\alpha$  increased as early as 1 h after acute ischemic stroke and may be used as marker of severity of neuronal damage and recovery in brain ischemic [19].

Lee et al mentioned the feasibility of HIF-1 $\alpha$  as therapeutic target in chronic disease conditions to effectively manage or delay the progression of disease (heart, lung, liver and kidney disease). Clinical trials on targeting HIF-1 $\alpha$  are being done [20].

Drug or molecule which activates HIF-1 $\alpha$  *in vitro* and *in vivo* was proved to own a neuroprotective effect in mice cerebral ischemia model [3, 9, 10]. Therefore in the long run, development of molecule regulator for HIF-1 $\alpha$  stability and signaling in human may serve as a marker and a therapeutic option to enhance recovery and repair after ischemic stroke.

In the future, analysis of CT perfusion and Magnetic Resonance Imaging Diffusion Weighted Imaging to differentiate infarct volume from infarct core with will be a pivotal aspect, which in the meantime is beyond the scope in our research.

**Table 3.** NIHSS during admission and discharge time points.

NIHSS	Mean	SD	Min	Max
Large vessel disease				
On admission	16.06	2.67	11	21
On discharge	15.13	3.15	6	20
Small vessel disease				
On admission	6.07	1.82	4	9
On discharge	5.73	1.71	4	9

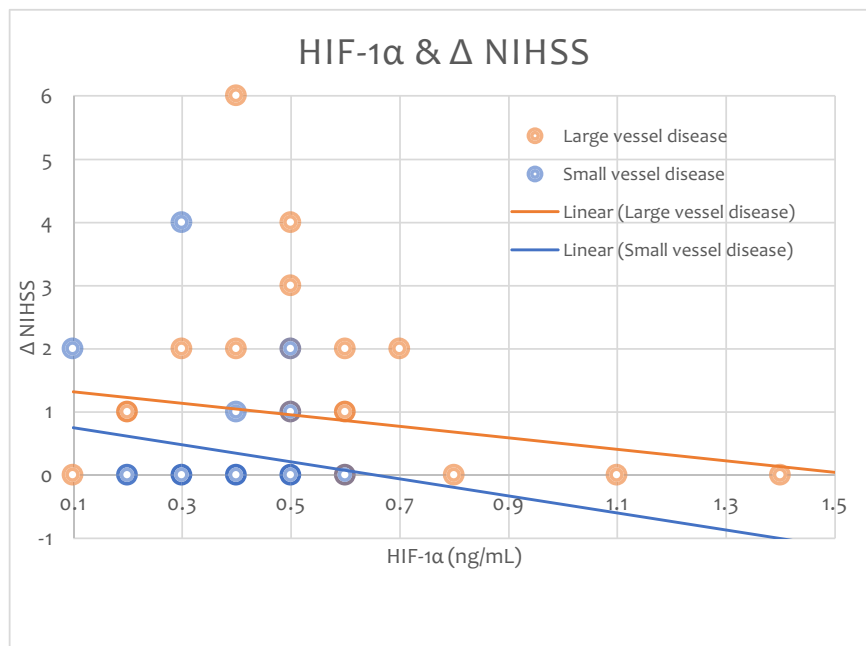


Fig. 2. HIF-1α level in SVD and LVD groups and NIHSS changes.

Table 4. Correlation of HIF-1α level and NIHSS.

HIF-1α	NIHSS on admission (mean ± SD)	NIHSS on discharge (mean ± SD)	p	r
Large Vessel Disease	16.06 ± 2.67	15.13 ± 3.16	<0.001*	0.9299
Small Vessel Disease	6.07 ± 1.82	5.7 ± 1.7	<0.001*	0.9394

\* statistically significant.

From this study we conclude that HIF-1α may be used to predict the clinical outcome in acute ischemic stroke patients. A further study to establish its precise predictive value is warranted.

5. Conclusion

HIF-1α level was associated with severity of ischemic lesion, large vessel disease had a higher level of HIF-1α compared with small vessel disease. HIF-1α had a strong correlation with NIHSS, both on admission and patients' discharge. From our findings, we can conclude that HIF-1α may serve as a predictor for clinical outcome in acute ischemic stroke patients, the higher HIF-1α the worse the outcome be.

Availability of data and material

The full data showed in supplementary material.

Declarations

Author contribution statement

L. Amalia: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

H. Sadeli, A. Rizal and R. Panigoro: Analyzed and interpreted the data.

I. Parwati: Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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