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Serum hypoxia-inducible factor 1 alpha levels as a prognostic factor in acute ischemic stroke

Esen Cicekli[a,*], Asli Aksoy Gundogdu[b], Dilcan Kotan[c], Asuman Deveci Ozkan[d]

[a] *Akyazı State Hospital, Department of Neurology, Sakarya, Türkiye*
[b] *Namik Kemal University, Faculty of Medicine, Department of Neurology, Tekirdag, Türkiye*
[c] *Sakarya University, Faculty of Medicine, Department of Neurology, Sakarya, Türkiye*
[d] *Sakarya University, Faculty of Medicine, Department of Medical Biology, Sakarya, Türkiye*

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Abstract

Aim: In order to survive, human cells must adapt to changes in the oxygen level of their environment. Many endogenous mechanisms operate in hypoxia. Hypoxia-inducible factor 1 (HIF-1) is a transcriptional protein involved in the head of the cascade that regulates the response of brain and other tissues to hypoxia. HIF-1 alpha (HIF-1α) is the main molecule that responds to hypoxia and is an important marker of cerebral hypoperfusion.

Materials and Methods: Thirty-nine acute stroke patients aged between 18-80 years and 38 control patients included in our study. We aimed to examine the serum HIF-1α levels in early period of acute ischemic stroke. We also aimed to evaluate its usefulness in determining tissue damage due to hypoxia and the prognosis of the disease.

Results: Serum HIF-1α levels were higher in acute ischemic stroke patients compared to the control group (p= 0.000). There was no significant correlation between infarct volumes, NHISS scores, blood pressure and HIF-1α values of the stroke patients.

Conclusion: HIF-1α could be an important biomarker in detecting cerebral hypoxia. It is possible to study this in human peripheral blood. Its usability in stroke may become widespread with studies on its therapeutic target.

Introduction

Ischemic damage to the brain occurring for various reasons can cause death or disability of varying degrees. As a result of ischemia, blood flow to the relevant tissue is reduced. When hypoxia develops, after a while, irreversible tissue damage occurs in the affected organ [1].

Human organs and cells must adapt to changes in oxygen levels to survive. Many endogenous molecules and mechanisms operate in hypoxia, especially hypoxia-inducible factors. Hypoxia-inducible factor 1 (HIF-1) is a transcriptional protein involved in the head of the cascade that regulates the response of brain and other tissues to hypoxia [2-5].

HIF-1 has a heterodimeric helix structure. It consists of α subunits that are activated in hypoxia and β subunits that are not affected by the oxygen level [3,6].

HIF-1 alpha (HIF-1α) is the main molecule that responds to hypoxia, and it is an important marker of cerebral hypoperfusion. It affects many genes that control adaptive mechanisms after hypoxia and regulates the transcription of these genes [7]. These genes encode molecules involved in events such as vasomotor control, angiogenesis, erythropoiesis, energy metabolism, and cell death [8]. In this way, it plays an important role in the development of cerebral ischemia by participating in many processes. It regulates neuroprotection, neurogenesis, migration of neuronal stem cells to the ischemic area, and proliferation into functional neurons [9].

HIF-1α is almost absent in the case of normoxemia. In case of hypoxia, its production is activated and regulates oxygen transport to the tissues. It has been shown that HIF-1α which rises within 12 hours after cerebral hypoperfusion, remains elevated for 56 days [10]. HIF-1α is known to play a role in many neurological diseases, including ischemic stroke, hypoxic-ischemic encephalopathy, and brain gliomas [11,12].

In recent years, it has been mentioned that it plays an important role not only in hypoxia, but also in the regulation of the inflammatory response [13].

*Corresponding author:
Email address: esencirit@gmail.com (Esen Cicekli)

In addition to its positive effects such as tissue protection and oxygenation, some studies have also reported harmful roles of HIF-1α, including severe inflammatory response, increased apoptosis, and post-stroke blood-brain barrier disruption [14,15]. In the light of this information, it was thought that HIF-1α increased after cerebral ischemia and its expression level was dependent on the duration of ischemia. It has been stated that this may partially explain the contradictory effects on the pathological process of stroke [16].

Stroke, especially ischemic stroke, is an important cause of mortality and morbidity and a serious financial burden all over the world. It has been studied for years on anti-stroke treatments and treatments to reduce post-stroke damage. Endogenous protection is an important mechanism of protection and recovery after cerebral ischemia and may be a potential treatment strategy in stroke. The HIF-1α signaling pathway plays an important role in endogenous protection [17]. HIF-1α promotes the expression of vascular endothelial growth factor (VEGF) to maintain homeostasis in hypoxic conditions [18].

During cerebral ischemia, HIF-1α is expressed in the chronic hypoxic area (penumbra) around the infarct area. Therefore, HIF-1α may become a new and valuable therapeutic target [19].

Ischemic stroke, a neurologic condition associated with hypoxia, is one of the most common diseases in the emergency department and its differential diagnosis can be difficult. In the early period of the disease, it may not be possible to comment on the prognosis. Various biomarkers have been studied for differential diagnosis, patient management, and clear interpretation of prognosis, and there are not enough human studies with HIF-1α. In our study, we aimed to evaluate the significance of serum HIF-1α levels in acute ischemic stroke patients and its relationship with the clinical and radiological parameters of the patients. We also aimed to evaluate its usefulness in determining tissue damage due to hypoxia and the prognosis of the disease.

Materials and Methods

Our study is a retrospective case-control study and simple probability sampling method was used. The sample size was calculated using the G.Power 3.1.9.7 program. The effect size was calculated using similar article data from PUPMED [20], and the sample size was 20 for both groups. We reduced the effect size by working with a larger sample size.

Serum samples, which were taken in previous studies and stored at -70 °C, were used for the study. The patient group included 39 patients between the ages of 18 and 80 who were admitted to the emergency department within the first 24 hours and diagnosed with acute ischemic stroke by neurological examination and neuroimaging methods. Patient files were examined retrospectively by the clinician. Demographic characteristics, cranial imaging results, stroke risk factors, infarct volumes, National Institutes of Health Stroke Scale (NIHSS) scores, and blood biochemistry results of all participants were recorded.

As a control group, 38 individuals who had no neurological symptoms or signs, no chronic disease, and whose serum samples were stored at -70 °C were included. Individuals with a history of hemorrhagic stroke, transient ischemic attack, myocardial infarction, liver and kidney diseases, malignancy, tumor, systemic infection, head trauma, using oral contraceptives, anticoagulants or chemotherapeutic agents were excluded from the study.

This study was approved by the Sakarya University Faculty of Medicine Ethics Committee and informed consent was obtained from all individuals.

Table 1. Demographic data of stroke and control group.

	Stroke group (n=39)	Control group (n=38)	p value
Gender (%)			
Male	17 (43.6%)	15 (39.5%)	P=0.714
Female	22 (56.4%)	23(60.5%)	
Age			
Med.±SS	66.18±10.034	53.97±23.052	
Min-maks	48-84	18-92	**P=0.024**
Median	65.5	53.5	

Collection and preparation of blood samples

Venous blood samples taken from all individuals in tubes that do not contain any anticoagulant in approximately 10 cc amount, were centrifuged for 5 minutes at 5000 rpm/min and their serums were separated. For the measurement of HIF-1α, the serums were kept in a deep freezer at -70°C. HIF-1α levels were studied with the BioTekEon ELx50 Brand USA ELISA device using the MyBio Source USA brand commercial ELISA kit in our biochemistry laboratory.

Statistical analysis

Statistical analyzes were performed using spss version 21. The conformity of the variables to the normal distribution was examined using visual (histogram and probability graphs) and analytical methods (kolmogorov-simirnov test and shapiro-wilk test). Descriptive analyzes were given using mean and standard deviation for normally distributed variables, median and interquartile range for non-normally distributed variables, and frequency tables for nominal variables. Comparisons between groups were made with Student's t test for normally distributed variables and with Mann Whitney U test for non-normally distributed variables. The relationship of normally distributed variables was analyzed by Pearson correlation analysis, and the relationship of non-normally distributed continuous variables was examined by Spearman's rank correlation test. The incidence of categorical variables was given using cross tables. The chi-square test was used to determine whether there was a difference between the groups in terms of frequencies. Cases with a P value below 0.05 were considered statistically significant.

Results

The demographic characteristics of 39 patients with acute ischemic stroke and 38 healthy individuals included in our

Table 2. Serum HIF-1α levels of stroke and control patients

Stroke/Control		n	Average	Standard Deviation	Min	Max	Median	Change Range	Interquartile range	z	p
HIF	Control	38	0.168	0.144	0.01	0.763	0.129	0.753	0.145	-4.407	**0.000**
	Stroke	39	0.285	0.109	0.081	0.507	0.28	0.426	0.167		

HIF- 1 α: Hypoxia inducible factor 1 alpha.

Table 3. Distribution of patients with and without hypertension, diabetes mellitus, previous stroke and mean HIF-1α values.

		Average	n	Standard Deviation	Min	Max	Change Range	Median	Interquartile range		p
Hypertension	Yes	0.276	28	0.100	0.081	0.507	0.426	0.286	0.143	**-0.809**	**0.423**
	No	0.307	11	0.131	0.113	0.497	0.384	0.280	0.241		
Diabetes Mellitus	Yes	0.291	10	0.126	0.117	0.507	0.390	0.266	0.211	**0.201**	**0.842**
	No	0.283	29	0.105	0.081	0.497	0.416	0.298	0.153		
Stroke history	Yes	0.244	10	0.114	0.081	0.415	0.334	0.256	0.208	**1.376**	**0.177**
	No	0.299	29	0.105	0.113	0.507	0.394	0.298	0.164		

HIF-1α: Hypoxia inducible factor- 1 alpha.

Table 4. Admission (1) and discharge (2) NIHSS values, systolic and diastolic blood pressure, and infarct volume mean-minimum-maximum values of patients in the stroke group.

Stroke Group	n	Average	Standard Deviation	Min	Max	Median	Change Range	Interquartile range
NIHSS 1	39	10.23	9.166	0	31	7	31	13
NIHSS 2	39	8.23	10.035	0	35	5	35	8
Systolic blood pressure	39	171.03	29.54	120	250	170	13	50
Diastolic blood pressure	39	89.49	14.5	70	150	90	80	20
Infarct volume	39	48.582.86	79.952.73	43.506	318.661.46	11694.7	318.617.95	60301.85

HIF- 1 α: Hypoxia inducible factor 1 alpha.

Table 5. Mean HIF-1α values of stroke patients with cardioembolic stroke and other etiology.

TOAST	Average	n	Standard Deviation	Minimum	Maximum	Change Range	Median	Interquartile range	t	p
Cardioembolic	0.296	13	0.110	0.092	0.497	0.405	0.301	0.164	0.129	0.899
Other	0.291	14	0.107	0.113	0.415	0.302	0.307	0.217		

HIF-1α: Hypoxia inducible factor- 1 alpha.

study are summarized in Table 1.

Serum HIF-1α levels were found to be statistically significantly higher in acute ischemic stroke patients compared to the control group (p= 0.000) (Table 2).

There was no significant difference between the genders in terms of HIF-1α levels in both groups (p=0.343, p=0.362). Again, no significant correlation was found between HIF-1α levels and age in both groups (p=0.681, p=0.744). The mean HIF-1α values of patients with and without hypertension, diabetes mellitus, and previous stroke history in the stroke group are shown in Table 3. There was no significant difference between the groups.

Admission (1) and discharge (2) NIHSS values, systolic and diastolic blood pressure and infarct volume mean-minimum-maximum values of patients in the stroke group are shown in Table 4. There was no significant correlation between the blood pressure values measured at admission and HIF-1α values of stroke patients (p=0.14, r=0.241). No significant correlation was found between HIF-1α values and NIHSS values measured at admission and discharge of stroke patients (p= 0.557, r=0.097). There was no significant correlation between infarct volumes and HIF-1α values of the patients (p=0.578, r=0.092).

When the patients were grouped as cardioembolic stroke and other etiologies, no significant difference was found between the two groups in terms of HIF values (Table 5).

There was no significant difference between the HIF-1α mean values of the groups with normal and high C- reactive protein (CRP), triglyceride, total cholesterol, and HbA1c values at the time of admission (Table 6).

Discussion

As a result of the study, serum HIF-1α levels were significantly higher in acute stroke patients than in the control

Table 6. Mean HIF-1α values of stroke patients with high and normal CRP, triglyceride, total cholesterol, and HbA1c $\leq$6 and >6.

		n	Average HIF	Standard Deviation	Min	Max	Change Range	Median	Interquartile range	t	p
CRP	Normal	13	0.298	0.108	0.092	0.497	0.405	0.298	0.175	**0.552**	**0.585**
	High	26	0.278	0.111	0.081	0.507	0.426	0.270	0.185		
Triglyceride	Normal	29	0.288	0.10	0.092	0.497	0.405	0.298	0.166	**0.312**	**0.757**
	High	10	0.275	0.14	0.081	0.507	0.426	0.257	0.235		
Total cholesterol	Normal	23	0.287	0.097	0.117	0.507	0.390	0.280	0.160	**0.132**	**0.896**
	High	16	0.282	0.127	0.081	0.497	0.416	0.291	0.212		
HbA1c	HbA1c$\leq$6	19	0.260	0.086	0.081	0.401	0.320	0.280	0.114	**-1.410**	**0.168**
	HbA1c>6	20	0.308	0.125	0.113	0.507	0.394	0.311	0.220		

HIF-1α: Hypoxia inducible factor- 1 alpha. CRP: C reactive protein HbA1c: Hemoglobine A1c.

group. We can say that in case of acute cerebral ischemia, HIF protein increases, and this can also be detected in peripheral blood. Detecting HIF protein, an indicator of ischemia in serum is an easier and more cost-effective method than ischemic tissue examinations.

In previous studies, it was determined that HIF-1α is mainly released from penumbra tissue in stroke. This can be interpreted as indicating the presence of brain tissue that can be salvaged. Treatment methods targeting HIF-1α will be promising in the future in terms of protecting healthy brain cells and treating damaged neurons in events such as ischemia and trauma [21,22]. Studies on the effects of drugs, natural products and supplements on HIF-1α and their role in preventing ischemia are continuing.

Despite the significant difference in HIF-1α levels between the stroke and control groups in our study, no significant difference was found when examining the relationship between infarct volumes, admission and discharge NIHS scores and HIF-1α levels of stroke patients. There are studies indicating that patients' NIHSS scores or infarct volumes have a significant relationship with HIF levels [23,24]. This can be interpreted as HIF protein increasing as the amount of ischemic area or damaged tissue increases. In our study, HIF was studied in serum samples taken from patients in the acute period. The duration and severity of hypoxia are important in HIF-1α activation. High level of HIF-1α protein expression was also measured in the chronic phase of cerebral hypoperfusion [10]. In a study, cerebral HIF-1 α expression was evaluated in cases who died due to acute hypoxia. As a result, this molecule was not considered sufficient for the diagnosis of cerebral hypoxia [18]. Based on these studies, we can say that the results we obtained may be due to the patients being in the early stages of ischemia.

We found that there was no significant correlation between HIF-1α and the blood HbA1c levels of the patients. It has been stated that diabetic tissues are hypoxic and adaptive mechanisms against diabetes are impaired due to HIF-1α signaling disorder in diabetes. Emphasizing both the protective and pathological role of HIF-1α in diabetes, it was said that studies are still needed on the mechanism to be targeted to prevent complications [25]. In the future, new studies can be planned on how beta cells respond to hypoxia and how there is a relationship between blood sugar fluctuations and HIF-1α in diabetic stroke cases.

In our study, no statistically significant relationship was found between CRP, which is an acute phase reactant and an inflammatory marker, and HIF-1α. The duration of hypoxia is important in HIF-1α activation, and we explained that it is not associated with CRP as it is not a determinant in the pathophysiological mechanism.

Limitations

The limitations of our study can be stated as being retrospective, being conducted in a single center and with a small number of cases and working with serum samples taken from patients in the acute period. The usability of HIF as a prognostic factor in ischemic stroke will be further clarified with new multicenter, large case series studies that will also evaluate the chronic phase of the patients.

Conclusion

In conclusion, HIF-1α is an important biomarker in detecting cerebral hypoxia and it is possible to detect it in peripheral blood. It can be concluded that HIF-1α may be a biomarker that will provide information about the clinical course and prognosis of patients during and after ischemia. Its availability in stroke may become widespread with studies on its therapeutic target.

Ethical approval

This study was approved by the Sakarya University Faculty of Medicine Ethics Committee (21.04.2017/92).

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