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Correlation of serum levels of HIF-1α, SMAD3, and HDAC3 with the disease severity in stroke patients

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Abstract. – OBJECTIVE: Explore serum levels of hypoxia-inducible factor-1α (HIF-1α), signal transduction molecule 3 (SMAD3), and histone deacetylase (HDAC) and their correlation with the severity of the condition of stroke patients.

PATIENTS AND METHODS: Clinical records of 93 stroke patients and 93 healthy individuals were retrospectively analyzed. Serum levels of HIF-1α, SMAD3, and HDAC3 in patients with different disease degrees and lesion areas were compared between the two groups. Correlation between serum levels of HIF-1α, SMAD3, and HDAC3 and the severity and lesion area of the observation group were analyzed.

RESULTS: Serum levels of HIF-1α, SMAD3, and HDAC3 in the observation group were higher than those in the control group ($p<0.05$). Serum levels of HIF-1α, SMAD3, and HDAC3 in patients with moderate and severe disease were significantly higher than those in patients with mild disease and were the highest in patients with severe disease ($p<0.05$). Serum levels of HIF-1α, SMAD3, and HDAC3 in patients with moderate and large areas of cerebral infarction were significantly higher than those in patients with small areas of cerebral infarction and the highest in patients with large areas of cerebral infarction ($p<0.05$). Spearman correlation analysis showed that serum levels of HIF-1α, SMAD3, and HDAC3 significantly positively correlated with the severity of stroke and lesion area ($p<0.05$).

CONCLUSIONS: Serum levels of HIF-1α, SMAD3, and HDAC3 in stroke patients are highly expressed, and the increase positively correlates with the severity of the disease and the area of the lesion.

Key Words:
Stroke, HIF-1α, SMAD3, HDAC3, Disease severity.

Introduction

Stroke is an ischemic damage caused by impaired oxygen supply to the brain tissue[1], and is often accompanied by varying degrees of cognitive, behavioral, and sensory dysfunction in patients[1,2]. In recent years, with the gradual aging of the population and lifestyle changes, the incidence rate of stroke is on the rise[3,4]. Studies[5,6] show that timely assessment of the severity of stroke is crucial for guiding clinically targeted treatment and ensuring good disease outcomes. Therefore, identifying easily detectable and highly sensitive biomarkers has significant clinical value.

Hypoxic inducible factor-1α (HIF-1α) is an important factor in hypoxia response, regulating cell apoptosis, angiogenesis, and energy metabolism. Studies[7] show that HIF - activation during stroke may have undesirable vascular effects, promoting disease progression. The expression of signal transduction molecule 3 (SMAD3) is closely related to atherosclerosis, can participate in the pathological process of vascular diseases, and is up-regulated after cerebral ischemic stroke[8]. In addition, histone deacetylase (HDAC) plays an important role in cell division, cell death, and gene transcription regulation. HDAC3 can mediate non-histones or deacetylated histones, enhance cerebral ischemia-reperfusion tolerance, and promote the activation of microglia in damaged brain tissue[9].

This study retrospectively analyzed clinical data of stroke patients to explore the potential association of serum HIF-1α, SMAD3, and HDAC3 expression levels in stroke patients with the severity of the disease.

Patients and Methods

Clinical data from 93 stroke patients and 93 healthy individuals were retrospectively analyzed (Feb 2020 - Jun 2023, Affiliated Nanhua Hospital, Hunan, China) (Figure 1). The ethics

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committee of our hospital approved this study with the number 2023-KY-153.

Inclusion Criteria

- The observation group met the diagnostic criteria for stroke[10].
- The clinical data is complete and levels of HIF-1α, SMAD3, and HDAC3 were collected for all participants.

Exclusion Criteria

- Individuals with active infectious or inflammatory diseases.
- Women during lactation or pregnancy.
- Individuals with diseases of the immune system, blood system, and endocrine system.
- Individuals with benign or malignant tumors.
- Individuals with any other brain diseases.

Stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS) score[11]: Mild: 0-3 points; Moderate: 4-15 points; Severe: ≥16 points.

The lesion area was defined as follows: intracranial scanning was performed using Philips 1.5T magnetic resonance imaging (MRI) equipment (Amsterdam, Netherlands). Patients were placed in a supine position, heads first, and the examination sequence included routine sequence, susceptibility-weighted imaging (SWI), and diffusion-weighted imaging (DWI). The following modalities were used: conventional sagittal T1-weighted imaging (T1WI), TE 21.8 ms, TR 1750 ms; transverse position T2WI TE 107.3 ms, TR 3 598 ms; cross-section fluid-attenuated inverse recovery (FLAIR), TR 8,400 ms, TE 87 ms, recording the patient's lesion status. DWI transverse position, TR 6,000 ms, TE 73.5 ms. SWI scan TR 37.4 ms, TE 22.9 ms, FOV 24 cm x 24 cm, image matrix 416 x 320, layer thickness 2 mm, spacing 0 cm, collected once, reversed by 20°. Two diffusion gradient fields were enabled

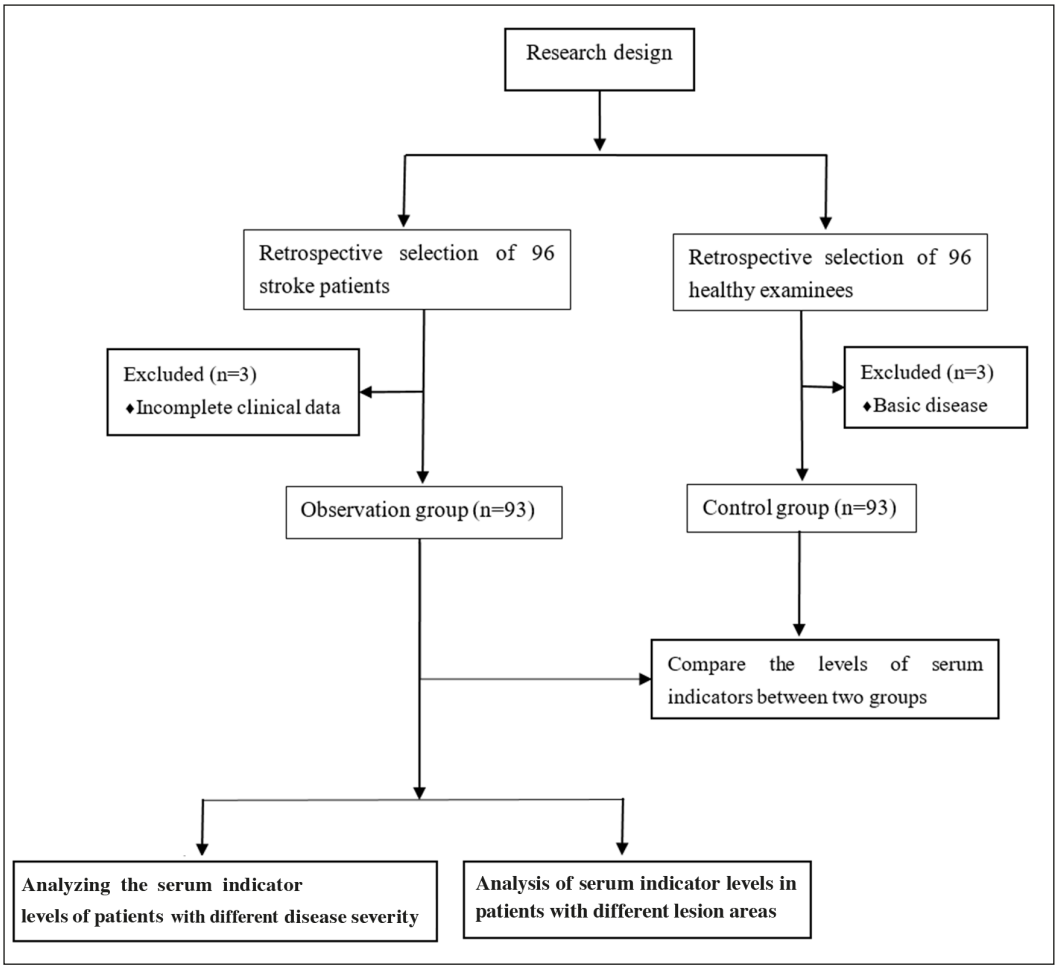

Figure 1. Flow diagram.

(b1=0, b2=1,000 s/mm^2) during the inspection, the signal intensity was observed on the DWI image, and the apparent diffusion coefficient (ADC) value was measured on the apparent diffusion coefficient image, taking the average value. Small infarct area: <5 cm; medium infarct area: 5-10 cm; large infarct area: >10 cm.

Observation Indicators

Serum levels of HIF-1α, SMAD3, and HDAC3 were detected in the serum extracted from 4 ml of fasting venous blood using enzyme-linked immunosorbent assay. The reagent kit was purchased from Wuhan Biotech Co., Ltd. (Wuhan, China).

Statistical Analysis

All data analysis was conducted according to a predefined statistical analysis plan. The data analysis was conducted using SPSS version 26.0 software (IBM Corp., Armonk, NY, USA). The normal distribution measurement data were represented as mean±standard deviation (SD), and *t*-test was used to compare two independent samples between groups. For repeated measurement data, analysis of variance for repeated measurements (ANOVA) was used for intra-group comparison of different disease degrees and infarct sizes. The counting data were represented by the number of use cases using the Chi-square test. Spearman analysis was used to investigate the correlation between serum levels of HIF-1α, SMAD3, and HDAC3 with the severity and lesion area of stroke; $p<0.05$ indicated a statistically significant difference.

Results

There was no significant difference in the general information such as age, gender, body mass index, and education level between the two groups ($p>0.05$) (Table I).

Serum levels of HIF-1α, SMAD3, and HDAC3 in the observation group were higher than those in the control group ($p<0.05$) (Table II).

Among 93 stroke patients, the severity of the disease was mild in 29 cases, moderate in 33 cases, and severe in 31 cases. Serum levels of HIF-1α, SMAD3, and HDAC3 in moderate and severe patients were higher than those in mild patients, and the highest in severe patients ($p<0.05$) (Table III).

Of 93 stroke patients, 27 had small area infarcts, 36 had medium, and 30 had large area infarcts. Serum levels of HIF-1α, SMAD3, and HDAC3 in patients with moderate and large area infarction were higher than those in patients with small area cerebral infarction, and the levels were the highest in patients with large area infarction ($p<0.05$) (Table IV).

There was a significant positive correlation between serum levels of HIF-1α, SMAD3, and HDAC3 and the severity and lesion area of stroke ($p<0.05$) (Table V).

Discussion

The results of this study showed that serum levels of HIF-1α, SMAD3, and HDAC3 in stroke patients

Table I. Comparison of baseline data between two groups.

Group	Gender (male/female)	Age (years)	BMI (kg/m^2)	Education level	
				Below high school	High school and above
Observation group	60/33	57.49±9.70	23.93±3.33	62 (66.67)	31 (33.33)
Control group	56/37	58.95±11.13	24.26±3.31	57 (61.29)	36 (38.71)
χ^2/t	0.367	-0.948	-0.682	0.583	
p	0.545	0.344	0.496	0.445	

BMI: body mass index.

Table II. Comparison of serum indicator levels between the observation group and the control group.

Group	n	HIF-1α (ug/ml)	SMAD3 (ng/ml)	HDAC3 (ng/ml)
Observation group	93	219.48±34.59	45.65±11.09	26.71±7.45
Control group	93	125.13±25.04	31.65±6.17	15.09±3.72
t		21.310	10.639	13.453
p		<0.001	<0.001	<0.001

Hypoxia-inducible factor-1α (HIF-1α), signal transduction molecule 3 (SMAD3), histone deacetylase (HDAC).

Table III. Comparison of serum indicator levels in patients with different degrees of illness in the observation group.

Disease severity	n	HIF-1α (ug/ml)	SMAD3 (ng/ml)	HDAC3 (ng/ml)
Mild	29	198.72±22.22	36.72±7.84	20.59±4.20
Moderate	33	214.89±29.60[a]	44.18±8.08[a]	25.82±5.20[a]
Severe	31	243.81±34.96[ab]	55.55±8.34[ab]	33.39±6.46[ab]
F		18.073	41.356	43.011
p		<0.001	<0.001	<0.001

Compared with mild patients, [a]$p<0.05$; Compared with moderate patients, [b]$p<0.05$. Hypoxia-inducible factor-1α (HIF-1α), signal transduction molecule 3 (SMAD3), histone deacetylase (HDAC).

Table IV. Comparison of serum indicator levels in patients with different lesion areas in the observation group.

Lesion area	n	HIF-1α (ug/ml)	SMAD3 (ng/ml)	HDAC3 (ng/ml)
Small area infarcts	27	197.67±22.58	37.07±7.84	20.52±4.29
Medium area infarcts	36	215.69±28.91	43.19±8.54	25.33±5.31
Large area infarcts	30	243.67±35.54	56.30±7.34	33.93±5.79
F		17.576	44.178	49.243
p		<0.001	<0.001	<0.001

Compared with small area infarction, [a]$p<0.05$; Compared with mid-size infarction, [b]$p<0.05$. Hypoxia-inducible factor-1α (HIF-1α), signal transduction molecule 3 (SMAD3), histone deacetylase (HDAC).

Table V. Correlation of serum indicators with disease severity and lesion area.

	HIF-1α		SMAD3		HDAC3	
Indicators	*r*	*p*	*r*	*p*	*r*	*p*
Disease severity	0.508	<0.001	0.684	<0.001	0.701	<0.001
Lesion area	0.505	<0.001	0.687	<0.001	0.721	<0.001

Hypoxia-inducible factor-1α (HIF-1α), signal transduction molecule 3 (SMAD3), histone deacetylase (HDAC).

are higher than those of unaffected individuals, and this increase positively correlates with the severity of the stroke and the size of the infarct lesion.

Jin et al[12] showed that levels of HIF-1α in early ischemic patients were significantly increased, which is consistent with our results. HIF-1α not only promotes angiogenesis and accelerates the process of nerve repair, but its high expression state can lead to cell apoptosis. HIF-1α is also a widely distributed oxygen regulatory subunit in mammalian organisms. Under hypoxic conditions, the rate of HIF-1α hydrolysis decreases, leading to its excessive accumulation and subsequent adaptive response to hypoxic environments[13,14]. Ni et al[15] investigated the role of HIF-1α in ischemic cerebrovascular disease and found that HIF-1α can upregulate the expression of vascular endothelial growth factor, promote metabolic adaptation to hypoxia, and increase oxygen transport between tissues. Jiang et al[16] also showed that in normal brain tissue, the expression of HIF-1α is mainly inhibited by chromatin silencing signal regulatory factor (SIRT3). However, hypoxia and ischemia lead to a decrease in SIRT3 levels, resulting in a significant increase in HIF-1α transcription levels. This, in turn, can stimulate downstream vascular endothelial growth factor expression, enhance blood-brain barrier permeability, and exacerbate brain tissue damage[17,18].

HDAC is an important medium for modifying non-histone targets after translation using acetylation[19,20]. Research shows that HDAC3 can limit the inflammatory response of immune cells under multiple stressors[21]. Yuan et al[22] reported that the inhibitory effect of HDAC3 can enhance myelin regeneration and growth, and improve functional recovery after peripheral nerve damage. HDAC3 and its inhibitors can significantly affect vascular function, plaque stability, lipid accumulation, and inflammation during the progression of atherosclerosis[22]. The study by Yao et al[23] shows that HDAC3 is highly expressed in patients with stroke and post-stroke vascular cognitive impairment, which

is consistent with the results and conclusions of this study, indicating that measuring HDAC3 levels can be effectively used to evaluate stroke and vascular cognitive impairment[23].

SMAD3 also plays an important role in vascular diseases. Animal studies by Wang et al[24] have confirmed that SMAD3 is activated in macrophages and can respond to transforming growth factor-β (TGF-β) homologous stimuli. Additionally, through independent TGF-β activity, SMAD3 is rapidly activated after phagocytosis in cells expressing phosphatidylserine, which has a pro-inflammatory function and can stimulate cell proliferation and survival[24]. Jianjun et al[25] found that the level of SMAD3 in patients with cerebral infarction is abnormally high compared to normal levels. Its high expression status can accelerate disease progression and affect good disease outcomes, which is consistent with the conclusions of this study[24,25].

This study also investigated the levels of serum HIF-1α, SMAD3, and HDAC3 levels in patients with different severity of stroke. Our results showed a significant positive correlation between the NIHSS score and the size of the lesion area in stroke patients. Other studies[16,22-24,26] confirm that HIF-1α, SMAD3, and HDAC3 can participate in the pathological progression of stroke. We may speculate that this association may be explained by a positive correlation between the neurological function status, infarct size, and the degree of cerebral tissue hypoxia and ischemia in stroke patients. After hypoxic-ischemic damage occurs in brain tissue, the mitochondrial metabolism of macrophages in the brain tissue is disrupted. The abnormally high expression of HIF-1α, SMAD3, and HDAC3 can promote the release of pro-inflammatory factors such as tumor necrosis factor-α and interleukin-6 by macrophages, exacerbating inflammatory damage to brain tissue cells and hindering the prognosis[27,28]. Meanwhile, it is expected that this study can provide new ideas and practical references for the assessment of stroke patients' condition. Guide clinical implementation of targeted interventions to ensure the effectiveness and correspondence of patient treatment, and avoid overtreatment or improper treatment.

Limitations

Our study has some limitations. Firstly, this is a single-center retrospective study, and patient selection is prone to selection bias. Secondly, neither group was randomly assigned, and baseline information may be imbalanced and biased, which is also one of the shortcomings of our retrospective study. Thirdly, although we attempted to minimize confounding factors in this study, there may be unmeasurable variables and residual confounding factors in the results. Finally, higher-quality research will continue to be conducted in the future to validate the content of this conclusion.

Conclusions

Serum levels of HIF-1α, SMAD3, and HDAC3 in stroke patients are elevated, and the increase is positively correlated with the severity of the disease and the size of the infarct area.

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Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' Contributions

XL conceived and designed the study. SY, JL, SW, WT and YT collected the data and performed the analysis. XL was involved in the writing of the manuscript. All authors have read and approved the final manuscript.

Ethics Approval

The Ethics Committee of The Affiliated Nanhua Hospital, Hengyang Medical School, approved this study with the number 2023-KY-153.

Conflict of Interest

The authors declare that they have no competing interests.

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