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Prognostic value of Sirtuin1 in acute ischemic stroke and its correlation with functional outcomes

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Abstract

Background: The blood–brain barrier is impaired in patients with stroke. The release of protein markers such as Sirtuin1 (SIRTI) into circulation may be useful to assess the prognosis of patients with cerebrovascular disease. In this study, we investigated the predictive value of SIRT1 levels in acute ischemic stroke (AIS) patients.

Methods: In all, 101 AIS patients and 38 healthy controls were enrolled, and blood samples were collected within 72 hours of stroke onset. SIRT1 was analyzed using a commercially available enzyme-linked immunosorbent assay kit. On admission, neurological status was assessed by the standardized National Institutes of Health Stroke Scale (NIHSS). Functional outcomes were measured 1 year after admission using the modified Rankin scale.

Results: Compared with the control group, SIRT1 was significantly increased in the AIS group (0.63 ± 0.75 vs 0.48 ± 0.80 ng/mL; $P \leq 0.05$). However, there was no significant correlation between SIRT1 and NIHSS score at admission ($r = -0.01$, $P = .920$). In addition, with an unadjusted odds ratio of 0.862 (95% confidence interval 0.495–1.502), SIRT1 was not significantly correlated with functional outcomes.

Conclusions: Serum concentrations of SIRT1 have no significant predictive value for favorable functional outcome after acute stroke in our study.

Abbreviations: AIS = acute ischemic stroke, AUC = area under the curve, BBB = blood–brain barrier, CI = confidence interval, ELISA = enzyme-linked immunosorbent assay, HDL = high-density lipoprotein, HRP = horseradish peroxidase, hs-CRP = high-sensitivity C-reactive protein, LDL = low-density lipoprotein, mRS = modified Rankin scale, Nampt = nicotinamide phosphoribosyl transferase, NIHSS = National Institutes of Health Stroke Scale, OGD = oxygen and glucose deprivation, ROC = receiver-operating characteristic, ROS = reactive oxygen species, SIRTI = Sirtuin1, SIRT3 = Sirtuin3.

Keywords: acute ischemic stroke, biomarker, prognosis, SIRT1

1. Introduction

There are 2.5 million new stroke cases and 7.5 million stroke survivors each year in China.[1] Ischemic stroke usually occurs when the blood supply to the brain is interrupted, and accounts for the majority of stroke cases.[2] Ischemic stroke is still a major burden in modern society. The early prediction of short-term prognosis after an ischemic stroke is essential for planning acute and rehabilitation strategies on the first day of hospital care.[3]

Sirtuins are nicotinamide adenine dinucleotide-dependent deacetylases with a wide range of metabolic and pressure-resistant properties. Among them, the most widely studied sirtuin is sirtuin1 (SIRT1), which is expressed at high levels in the brain than in other organs.[4] Studies have shown that the SIRT1–sirtuin3 (SIRT3) axis is an important regulator of the blood–brain barrier (BBB) and can be a target of treatment through its regulation of mitochondrial reactive oxygen species (ROS), which are produced after an ischemic stroke.[5]

Sirtuin1 is also thought to have a neuroprotective effect on stress in cell culture.[6] Based on these findings, we hypothesized that SIRT1 may be a useful marker in stroke patients. However, its clinical value is still unknown. This study aimed to investigate the potential prognostic value of SIRT1 in Chinese acute ischemic stroke (AIS) patients and its correlation with functional outcomes.

2. Materials and methods

2.1. Patients

In the Department of Neurology at the affiliated hospital, we enrolled 101 consecutive patients with AIS defined according to the World Health Organization criteria within 72 hours of the onset of stroke symptoms. There were 60 male and 41 female patients. For all patients, it was their first stroke, and they were treated according to the guidelines of the American Heart

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Association. In all, 38 healthy controls with no history of stroke were enrolled at the same time from the Department of Physical Examination. At the time of study enrollment, we excluded participants with cerebral hemorrhage, brain surgery or brain trauma, malignant tumors, other-system end-stage disease, and age <60 years. The study was approved by the ethical committee of the First Affiliated Hospital of Chongqing Medical University and was conducted in accordance with ethical principles. All subjects provided written informed consent before inclusion in the study.

2.2. Method

Blood samples were collected at 8:00 am on the second day of admission. After centrifuging, serum samples were separated, aliquoted, and frozen at −80°C until assayed. Serum SIRT1 levels were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits for SIRT1 (Cusabio, Wuhan, China) according to the manufacturer's instructions. This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for SIRT1 has been precoated onto a microplate. Standards and samples are pipetted into the wells, and any SIRT1 present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for SIRT1 is added to the wells. After washing, avidin-conjugated horseradish peroxidase (HRP) is added to the wells. After a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of SIRT1 bound in the initial step. The color development is stopped and the intensity of the color is measured. A standard curve was constructed from the standards provided by the manufacturer. The sensitivity of the assay was 0.039 ng/mL. Standard laboratory methods were used to measure other biomarkers such as low-density lipoprotein (LDL), high-density lipoprotein (HDL), high-sensitivity C-reactive protein (hs-CRP), and leukocytes. Institutes of Health Stroke Scale (NIHSS) score (scores range from 0 to 42, with greater scores indicating increasing severity) was assessed at the time of admission. Based on the modified Rankin scale (mRS), which was blinded to SIRT1 levels, functional outcomes after 1 year were obtained. The favorable functional outcome of stroke patients defined as mRS scores of 0 to 2 points. The results were assessed by 2 trained researchers who telephoned patients, or, if possible, conducted structured follow-up telephone interviews with relatives.

2.3. Statistical analysis

Statistical analyses were performed using a commercially available software package (IBM SPSS version 21.0, Armonk, NY), and statistical significance was set at $P<.05$. The categorical and continuous results were presented as absolute numbers with percentages (%) and mean ± SD values, and were compared using chi-square tests and nonparametric Mann–Whitney U tests. Correlations were determined using Spearman critical value rankings. We used logistic regression models to investigate the relationship between SIRT1 and functional outcomes. Results were represented as adjusted odds ratios (ORs) with the corresponding 95% confidence interval (CIs). We explored whether serum SIRT1 could be used to diagnose AIS using receiver-operating characteristic (ROC) curves, and calculated the area under the curve (AUC) to assess the statistical significance of the test.

3. Results

Table 1 presents the clinical characteristics of subjects. There were no significant differences in age or sex between the AIS group (60 males and 41 females, mean 73.83 ± 9.52 years) and control group (16 males and 22 females, mean age 71.16 ± 7.99 years). Serum SIRT1 levels were significantly higher ($P \leq .05$) in the AIS group compared with controls (0.63 ± 0.75 vs 0.48 ± 0.80 ng/mL). SIRT1 levels in stroke patients were not significantly correlated with NIHSS scores at the time of admission ($r=-0.01$, $P=.920$; Fig. 1). The ROC curve of serum SIRT1 activity for AIS patients is shown in Fig. 2, and the AUC for AIS patients was 0.752. Based on the ROC, the maximum sensitivity and specificity of serum SIRT1 activity for diagnosing AIS were 47.5% and 76.3%, respectively, with the AUC at 0.615 (95% CI 0.511–0.719), when choosing the optimal cut-off point (0.45 ng/mL).

In the 41 patients with an unfavorable functional outcome, serum SIRT1 levels were higher than those with a favorable outcome (0.58 ± 0.70 vs 0.66 ± 0.79 ng/mL; $P=.224$). The clinical characteristics of AIS patients with different functional outcomes are shown in Table 2. Table 3 shows the results from binary logistic regression analyses, which calculated the correlations between SIRT1 levels and NIHSS score, hs-CRP, age, and other risk factors. With an unadjusted OR of 0.862 (95% CI 0.495–1.502), SIRT1 was not significantly correlated with a favorable functional outcome. In contrast, age, NIHSS score, smoking history, and hs-CRP were all significant predictors of outcome (Table 3).

Table 1

Clinical characteristics of stroke patients and healthy controls.

Variable (SD/%)	Stroke patients (101)	Control (38)	P	Variable (SD/%)	Stroke patients (101)	Control (38)	P
Age (year)	73.83 ± 9.52	71.16 ± 7.99	.167	hs-CRP (mg/L)	4.40 ± 5.13	2.41 ± 3.15	.004
Sex, male	60 (59.4%)	16 (42.1%)	.270	Leucocytes	7.67 ± 2.84	6.19 ± 2.28	.002
TC (mmol/L)	4.48 ± 0.95	4.11 ± 0.5	.025	Smoking history	38 (37.6%)	13 (34.2%)	.002
Cholesterol (mmol/L)	1.57 ± 1.24	1.33 ± 0.64	.574	Alcohol consumption	33 (32.7%)	6 (15.8%)	.000
LDL (mmol/L)	2.84 ± 0.85	2.55 ± 0.71	.068	Hypertension	68 (67.3%)	21 (55.3%)	.001
HDL (mmol/L)	1.25 ± 0.31	1.35 ± 0.46	.470	Diabetes mellitus	28 (27.7%)	8 (21.1%)	.000
SIRT1	0.62 ± 0.75	0.48 ± 0.80	.037	Hypercholesterolemia	29 (28.7%)	12 (31.6%)	.000
Admission NIHSS	7.59 ± 6.50	—	—	CHD	18 (17.8%)	6 (15.8%)	.000
HbA1c (%)	7.03 ± 2.16	6.20 ± 1.03	.066	Atrial fibrillation	26 (25.7%)	0 (0.0%)	.000

CHD = coronary heart disease, HbA1c = glycated hemoglobin, HDL = high-density lipoprotein, hs-CRP = high-sensitivity C-reactive protein, LDL = low-density lipoprotein, NIHSS = National Institutes of Health Stroke Scale, SD = standard deviation, TC = total cholesterol.

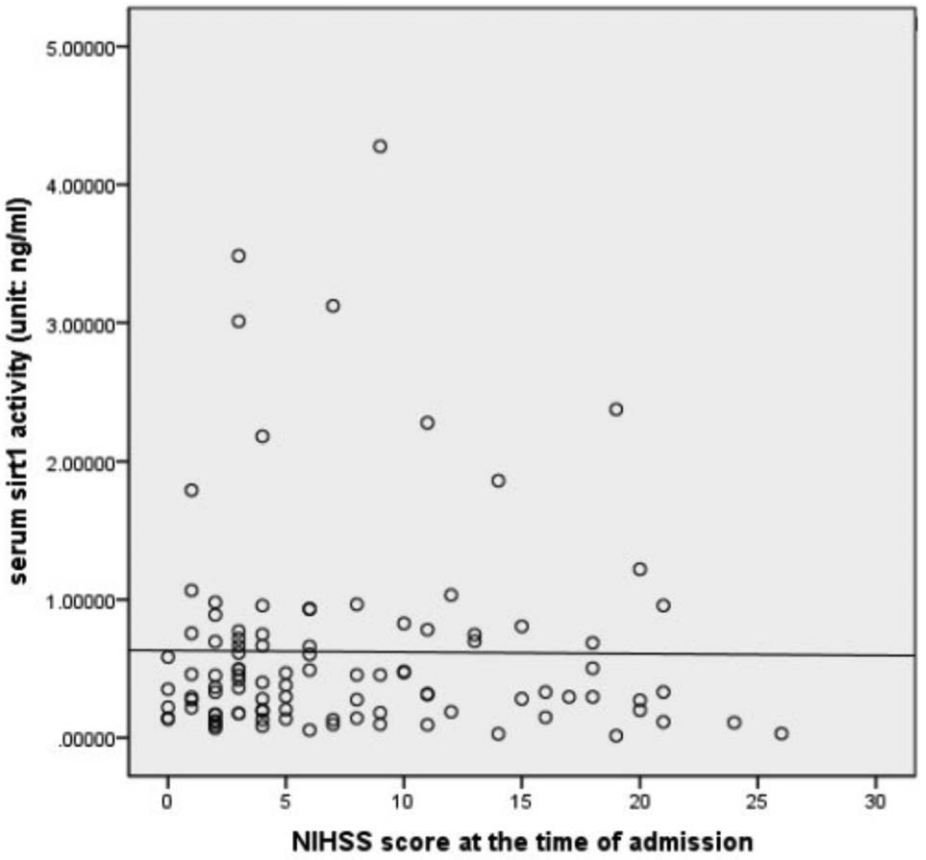

Figure 1. Linear correlation between serum SIRT1 concentration and NIHSS score. Serum SIRT1 concentration not significantly correlated with NIHSS score ($r = -0.01$, $P = .920$). NIHSS = National Institutes of Health Stroke Scale, SIRT1 = Sirtuin1.

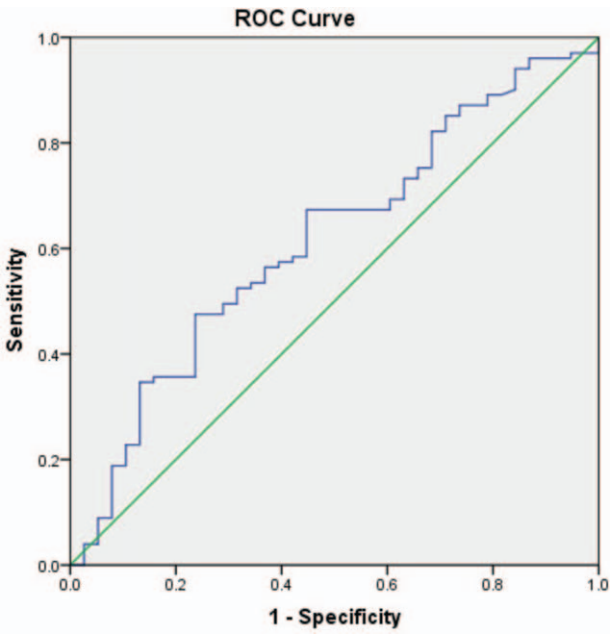

Figure 2. ROC curve of serum SIRT1 activities for diagnosing ischemic stroke patients. ROC = receiver-operating characteristic, SIRT1 = Sirtuin1.

4. Discussion

Sirtuins play an important protective role by resisting cellular stress and regulating metabolism in ischemia–reperfusion injury processes. Mammals have several different sirtuins, and SIRT1 can be found in both the cytoplasm and nucleus of cells. It is the most widely studied mammalian sirtuin, is expressed at high levels in the brain, and is required for the extended life-span associated with caloric restriction.[7] SIRT1 regulates cell metabolism by regulating multiple downstream genes in response to cellular stress.[8] For example, in the inflammatory response to ischemia–reperfusion injury, SIRT1 plays an important neuroprotective role by deacetylating several apoptosis-related proteins, such as Smad7, FOXO3, and FOXO4, thus protecting cells against damage-induced apoptosis.[9–11] SIRT1 also deacetylates p53, which destabilizes it and reduces its transcriptional activity, resulting in decreased apoptosis.[12–14] Moreover, previous studies have shown that SIRT1 can mediate the neuroprotective effect of a rate-limiting enzyme of nicotinamide phosphoribosyl transferase (Nampt) in synthetic neuronal cells in the salvage pathway.[15] SIRT1 can also increase mitochondrial oxidative phosphorylation by reducing uncoupling protein 2 transcription.[16,17] Furthermore, SIRT1 eliminates the decrease in Bcl-2 expression and suppresses caspase-3 cleavage in nerve cells damaged by oxygen and glucose deprivation (OGD).[18] In addition, a previous study showed that activation of SIRT1 can prevent hyperglycemia-

Table 2

Clinical characteristics of stroke patients with favorable and unfavorable functional outcome.

Variable (SD/%)	Unfavorable outcome (41)	Favorable outcome (60)	*P*	Variable (SD/%)	Unfavorable outcome (41)	Favorable outcome (60)	*P*
Age (y)	77.73 ± 10.03	71.17 ± 8.22	.001	Hs-CRP (mg/l)	4.40 ± 5.13	3.03 ± 3.15	.001
Sex, male	24 (58.5%)	36 (60.0%)	.883	Leucocytes	8.26 ± 3.03	7.26 ± 2.65	.079
TC (mmol/L)	4.70 ± 1.16	4.33 ± 0.75	.177	Smoking history	21 (51.2%)	17 (28.3%)	.020
Cholesterol (mmol/L)	1.66 ± 1.56	1.50 ± 0.99	.927	Alcohol consumption	17 (41.5%)	16 (26.7%)	.119
LDL (mmol/L)	2.96 ± 1.03	2.76 ± 0.71	.410	Hypertension	29 (70.7%)	39 (65.0%)	.546
HDL (mmol/L)	1.29 ± 0.34	1.23 ± 0.28	.183	Diabetes mellitus	13 (31.7%)	15 (25.0%)	.460
SIRT1	0.58 ± 0.70	0.66 ± 0.79	.224	Hypercholesterolemia	10 (24.4%)	19 (31.7%)	.427
Admission NIHSS	11.76 ± 7.12	4.75 ± 4.11	.000	CHD	11 (26.8%)	7 (11.7%)	.051
HbA1c (%)	7.08 ± 2.28	7.00 ± 2.09	.775	Atrial fibrillation	16 (39.0%)	10 (16.7%)	.012

CHD = coronary heart disease, HbA1c = glycated hemoglobin, HDL = high-density lipoprotein, hs-CRP = high-sensitivity C-reactive protein, LDL = low-density lipoprotein, NIHSS = National Institutes of Health Stroke Scale, SD = standard deviation, TC = total cholesterol.

Table 3

Univariate and multivariate logistic regression analysis for functional outcome.

	Univariate		Multivariate	
Variable	**OR (LL-UL)**	***P***	**OR (LL-UL)**	***P***
Age	1.081 (1.032–1.133)	.001	1.073 (1.003–1.148)	.042
Sex, male	1.062 (0.474–2.384)	.883	—	—
TC	1.532 (0.973–2.412)	.066	—	—
Cholesterol	1.105 (0.978–1.529)	.548	—	—
LDL	1.329 (0.818–2.160)	.251	—	—
HDL	1.812 (0.479–6.850)	.381	—	—
Admission NIHSS	1.232 (1.127–1.346)	.000	1.176 (1.054–1.313)	.004
HbA1c %	1.018 (0.808–1.282)	.882	—	—
hs-CRP	1.162 (1.051–1.285)	.003	1.043 (0.923–1.178)	.498
Leukocytes	1.134 (0.981–1.311)	.089	—	—
Smoking history	2.656 (1.157–6.096)	.021	4.820 (1.362–17.053)	.015
Alcohol consumption	1.948 (0.837–4.533)	.122	—	—
Hypertension	1.301 (0.522–3.065)	.547	—	—
Diabetes mellitus	1.393 (0.578–3.358)	.460	—	—
Hypercholesterolemia	0.696 (0.248–1.706)	.468	—	—
CHD	2.776 (0.973–7.918)	.056	—	—
Atrial fibrillation	3.200 (1.270–8.066)	.014	1.449 (0.349–6.020)	.609
Sirt1	0.862 (0.495–1.502)	.600	—	—

CHD = coronary heart disease, HbA1c = glycated hemoglobin, HDL = high-density lipoprotein, hs-CRP = high-sensitivity C-reactive protein, LDL = low-density lipoprotein, LL = lower limit, NIHSS = National Institutes of Health Stroke Scale, OR = odds ratio, TC = total cholesterol, UL = upper limit.

induced vascular cell senescence in mice with diabetes, thus protecting from vascular dysfunction.[19] SIRT1 also suppresses SIRT3 expression via the Adenosine monophosphate activated protein kinase-Peroxisome proliferator-activated receptor coactivator 1 pathway, leading to mitochondrial ROS production, which results in reduced BBB permeability and apoptosis in an OGD model in vitro.[5] In hypoxic exposures of less than 8 to 12 hours in cell culture experiments, SIRT1 demonstrates a biphasic response to hypoxia, indicating a favorable role of SIRT1 in the acute phase of hypoxia and neutral or even deleterious effects in late-stage hypoxia.[20] Resveratrol is reported to be a SIRT1 activator, and preconditioning of rats with low doses of resveratrol before the induction of lethal ischemia may induce neuroprotection, depending on SIRT1 activation.[16] In other studies, resveratrol led to an 8-fold activation of SIRT1.[21]

All these previous results indicate that the activation of SIRT1 by gene overexpression or pharmacological activators exerts neuroprotective effects in neurodegenerative diseases. Additionally, SIRT1 is probably related to the degree of neurological deficit after an ischemic stroke. In the present study, we found significantly increased serum levels of SIRT1 in AIS patients compared with controls, and the values of serum SIRT1 activity for diagnosing ischemic stroke patients was with the AUC at 0.615. However, there was no significant correlation between SIRT1 and NIHSS scores at admission. Additionally, serum SIRT1 concentrations did not predict functional outcome after stroke, with the AUC at 0.572.

In explaining our findings, we must consider some issues. First, we measured SIRT1 in serum instead of measuring it in cerebrospinal fluid. It is uncertain whether peripheral SIRT1 levels reflect central nervous system levels, and this relationship requires further study. Second, SIRT1 measurements were only performed after stroke, which may not accurately reflect prestroke exposure, and we lack a series measurement of circulating SIRT1 levels to accurate evaluate prognosis. Finally, the classical SIRT1 assay using the ELISA method was used in this study; however, it is well known that human serum contains immunoglobulins that affect the results of immunoassays by binding to the reagent antibodies used in the assays. Future studies should consider alternative methods to avoid this problem.

5. Conclusions

Our study investigated a possible association between serum levels of SIRT1 and functional outcomes in stroke patients. We demonstrated that elevated serum SIRT1 level at admission was a novel diagnostic marker of patients with ischemic stroke. However, serum concentration of SIRT1 had no significant predictive value for functional outcome after stroke and did not correlate significantly with NIHSS. More investigation is therefore needed to identify any possible connection between SIRT1 and AIS, especially because our findings are not in agreement with some previous reports.

Author contributions

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