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## ORIGINAL ARTICLE

# SIRT1 and miR-34a as Potential Plasma Biomarkers in the Acute Phase of Ischemic Stroke

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## ABSTRACT

Stroke is the major cause of disability and mortality worldwide. Identification of molecular biomarkers in the early hours after stroke is important in terms of both diagnostic and therapeutic applications. miR-34a, a highly expressed miRNA, is involved in many pathological mechanisms in the central nervous system. This miRNA targets sirtuin 1 (SIRT1) gene. Here, the efficacy of miR-34a/SIRT1 axis as a potential biomarker in the acute phase of ischemic stroke has been evaluated. 100 patients (in the first 12 hours after ischemic stroke) and 100 healthy subjects were examined. miR-34a expression level was assessed using real-time polymerase chain reaction and SIRT1 level was measured using Enzyme-linked immunosorbent assay. Stroke etiology and infarct size were investigated in the patients. The National Institutes of Health Stroke Scale (NIHSS) was also evaluated. Compared to the healthy controls, ischemic stroke patients showed increased miR-34a expression ( $P < 0.0001$ ) and decreased SIRT1 levels ( $P < 0.0001$ ). The levels of miR-34a and SIRT1 showed significant differences among various subtypes of stroke etiology and infarct size. The baseline NIHSS values were correlated negatively with SIRT1 ( $r = -0.89$ ) and positively with miR-34a ( $r = 0.81$ ). Our results suggested that dysregulation in miR-34a/SIRT1 may be a potential biomarker in occurrence and severity of ischemic stroke.

**Keywords:** Ischemic stroke, Sirtuin 1, Biomarker, miR-34a



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## Introduction

Stroke is a medical emergency that results from acute focal damage to the central nervous system (CNS) due to cerebrovascular accident(1). An estimated 15 million new stroke cases are reported worldwide every year, and stroke is the second major cause of mortality in people over 60 years of age (1). The outcome of stroke causes significant family and social burden, which has become an important challenge in the health system (2). Stroke can lead to a wide range of complications, mainly resulting in physical, cognitive and emotional impairments, significantly impacting recovery and quality of life (1).

FAST (face, arms, speech, time) is an acronym to help identify the signs and symptoms of stroke early, with emphasis on facial paralysis and drooping, arm or leg weakness, speech difficulties and time to prompt initiation of treatment following these symptoms (3). Long-term complications can include post-stroke fatigue, urinary incontinence, respiratory infections, malnutrition and social isolation, which significantly impact patients and their caregivers (1). Given that stroke is a complicated neurological disorder, genetic and environment are involved in its development. Genetic factors have principal role in the occurrence of stroke and the resulting injuries (4). More than 85% of stroke incidences are categorized as ischemic stroke (5). miRNAs (microRNAs) are non-coding RNA molecules of about 21 nucleotides in length with conserved sequences that can suppress gene expression via specific binding to 3' untranslated regions (3'-UTR) of target mRNAs (6). These short RNAs have crucial roles in various pathophysiological mechanisms (7).

Alterations in miRNA expression levels can lead to dysregulation in expression and function of proteins involved in the development of various cerebrovascular diseases including cerebrovascular accident, cerebral aneurism and cerebral thrombosis (8). Emerging evidence proposes the promising roles of miRNAs in physiological processes associated with ischemic stroke, such as atherosclerosis and hypertension (9). miR-34a, a highly expressed miRNA, is involved in many pathological and physiological mechanisms in the CNS such as neuronal morphology, synaptic plasticity, blood brain barrier (BBB) regulation, neuronal loss and neuroinflammation (10, 11). miR-34a is involved in apoptosis in vascular endothelial cells (12). Overexpression of miR-34a in the brain of

the rat model of middle cerebral artery occlusion has been reported, and its knockout reduces neurodegeneration following ischemic stroke (13, 14). Upregulation of miR-34a in the cerebrovascular cells caused oxidative stress, apoptosis and BBB disruption (13). Inhibition of miR-34a significantly suppressed apoptosis in the CNS, diminished cerebral edema and cerebral infarct volume, and improved locomotion following ischemic stroke(15).

It also enhanced the survival of neural stem cells in the brain after ischemic stroke (15). These reports propose that miR-34a may be a potential biomarker in ischemic stroke and the new target for treatment of stroke. It has been reported that miR-34a inhibits SIRT1 expression via targeting a binding site in 3'UTR of SIRT1 mRNA (11, 16). SIRT1 is a nicotinic adenine dinucleotide (NAD<sup>+</sup>)-dependent deacetylase and plays a role in the regulation of gene expression and cellular metabolism through the modification of transcription factors (17). SIRT1 is widely expressed in various tissues. In the CNS, SIRT1 is expressed in neurons, neural stem cells, astrocytes, microglia and cerebral endothelial cells(18). SIRT1 is crucially involved in the modulation of neural development, cognitive functions, and neuronal metabolism. SIRT1 levels are decreased in neurodegenerative diseases including Alzheimer's and Parkinson's diseases (19). Increased expression of SIRT1 shows obvious neuroprotective effects in ischemic stroke and other neurological diseases (20, 21).

SIRT1 enhances mitochondrial biogenesis and function, which are critical for cellular energy metabolism (22). Improving mitochondrial metabolism can protect neurons from oxidative damage, a common consequence of ischemic stroke (23). SIRT1 increases the expression of antioxidant enzymes and protect neurons against oxidative stress following stroke(24). SIRT1 can acetylate and inhibit transcription factors involved in inflammation, such as nuclear factor kappa B (NF- $\kappa$ B) (25). Therefore, SIRT1 reduces the expression of pro-inflammatory cytokines including interleukin 6 (IL-6) and IL-1 $\beta$  and ameliorates neuroinflammation following ischemic stroke (26). SIRT1 also regulates the activation of microglia and modulates the microglia-induced inflammatory damages after stroke (27, 28). The present study aimed to evaluate the miR-34a and SIRT1 levels in acute phase of ischemic stroke and to assess the efficacy of miR-34a/SIRT1 axis as a promising biomarker in the

incidence and the severity of ischemic stroke. miR-34a and SIRT1 are involved in oxidative stress, neuroinflammation, and neurodegeneration, which are critical components of stroke pathobiology (13, 18). Analysis of miR-34a and SIRT1 in relation to stroke outcomes may shed light on the pathophysiological processes that occur during and after ischemic stroke and lead to the development of novel therapeutic strategies to modulate these mechanisms to improve post-stroke outcomes.

## Methods

### Ethical considerations

The processes of the current study have been conducted with regard to the principles stated in the Declaration of Helsinki and approved by Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (ethical code IR.AJUMS.HGOLESTAN.REC.1402.111). All patients or their families agreed to participate in this research. In addition, the control subjects also signed a consent form to participate in the research.

### Individuals and groups

A total of 100 acute ischemic stroke patients ( $\leq 12$  hours between the onset of cerebral infarction and admission) who were admitted to Golestan Hospital in the western region of Iran from April 2023 to September 2023 participated. The sample size was calculated considering the statistical power of 95% (29). The patients included in the study had the following conditions: (1) the patient's first stroke complication and (2) less than 12 hours had passed since their stroke. Malignancy, serious hepatic and kidney disorders, intracranial bleeding, previous neurodegenerative disorder and acute infarction for more than 12 hours were exclusion criteria in the study.

One hundred samples from unrelated healthy subjects were obtained from the biochemical examination laboratory of Golestan Hospital. In the control subjects, samples of individuals with cardiac disease, transient ischemic attack, hemorrhagic stroke, dementia, Parkinson's disease, malignancy and infection were excluded. TOAST (Trial of ORG 10172 in Acute Stroke Treatment) is the most commonly used ischemic stroke categorization system regarding etiology. In accordance with the TOAST categorization, the causes of ischemia were categorized in large artery atherosclerosis, small vessel occlusion, cardiac

embolism and stroke with other/undetermined causes (30). Based on T2-weighted MRI, the infarct sizes were classified as large infarct (more than 1 brain lobe, diameter more than 5.0 cm), medium infarct (one lobe, 3.1-5.0 cm), small infarct (1.6-3.0 cm), and lacunar infarct ( $<1.5$  cm) (31). The lacunar infarct subgroup was not included in statistical comparisons due to the limited sample size ( $n=3$ ). Other clinical indicators including blood pressure, body mass index (BMI), low-density lipoprotein (LDL) and smoking status were investigated in all subjects. Information about stroke patients at Golestan Hospital was recorded in the STROK (Stroke Registry of Khuzestan) registration system.

### Blood sampling

Peripheral blood samples from control and patient subjects, were collected in ethylenediaminetetraacetic acid (EDTA) coated tubes. The blood samples centrifuged with 5000 rpm for 8 minutes. The plasma was separated in the sterile tube and kept at  $-80^{\circ}\text{C}$ . In all patients, blood sampling was performed before any therapeutic intervention.

### NIHSS scores

The National Institutes of Health Stroke Scale (NIHSS) is a general and numerical examination method used to measure stroke-related neurological deficits. Scores range from 0 to 42, that higher ratings representing greater severity (32). During the examination, NIHSS evaluation was measured by a resident and reviewed and confirmed by an expert neurologist.

### Quantitative Reverse Transcription Polymerase Chain Reaction (q-RT PCR)

RNA was isolated with the miRNeasy Serum/Plasma Kit (Qiagen, Germany) in accordance with the manufacturer's instruction. The quantity and quality of RNA were measured with Nanodrop equipment (ThermoFisher, USA). The 260/280 and 260/230 ratios were checked to confirm acceptable RNA purity.

The integrity of purified RNA was confirmed by 1.5% agarose gel electrophoresis. miR-34a was amplified using specific stem-loop method. 500 ng of isolated RNA was entered in RT-PCR using cDNA Master Mix (KarmaniaParsGene, Iran) containing 5 unite reverse transcriptase enzyme, adequate buffer system and deoxynucleotide triphosphates. The RT-

PCR was performed in the conditions of 25°C for 10 minutes, followed by 47°C for 60 minutes and 95°C for 5 minutes. The miRNA level was evaluated by RealQ Plus 2x Master Mix Green (Amplicon, Denmark) on Applied Biosystems 7900 PCR equipment (Life Technologies) at conditions of 95°C for 5 minutes and 40 cycles of 95°C for 15 seconds and 60°C for 15

seconds 72°C for 25 seconds. U6 small RNA was also considered as the internal control to determine the relative levels of miR-34a. The sequences of primers are presented in Table 1.

The cycle thresholds (CTs) were determined in the qRT-PCR and the relative amount of miR-34a expression was evaluated using  $2^{-\Delta\Delta CT}$  analysis.

**Table 1. List of primer sequences used for Real-Time PCR analysis**

Gene	Primer	Sequence
miR-34a-5p	loop	5'- GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCACTGGATACGACACAA CC-3'
miR-34a-5p	Forward	5'-AACACGCTGGCAGTGTCTTA-3'
miR-34a-5p	Reverse	5'- GTCGTATCCAGTGCAGGGT-3'
U6	Forward	5'- GTGCTCGCTTCGGCAGCACA-3'
U6	Reverse	5'-TGGAACGCTTCACGAATTTGCGTG-3'

#### Enzyme-linked immunosorbent assay (ELISA)

The concentration of SIRT1 was determined with human SIRT1 ELISA kit (SunLongBiotech, China). The plasma samples were centrifuged at 3000 rpm for 20 minutes and added to the wells along with the dilution buffer.

After 30 minutes incubation at 37°C and washing, 5 µl of conjugated secondary antibody was added to every sample. Samples incubated 30 minutes at 37°C then the wells were washed and chromogen solutions were added. After reaction termination and washing, the final optical density (OD) of each sample was assessed with a micro-plate reader (Biotek, USA) at the wavelength of 450 nm.

#### Statistical analysis

The normality of the data was analyzed by the Shapiro-Wilk test. The ischemic stroke and healthy groups were compared by the nonparametric Mann-Whitney U-test. Finally, receiver operating characteristic (ROC) analysis was conducted to evaluate the diagnostic potential of the miR-34a and SIRT1. Kruskal-Wallis test was performed for comparison between multiple groups. Spearman correlation analysis was applied to correlate between the values. SPSS 16.0 and GraphPad Prism 6 tools were

used for data analysis. The significance level in comparisons was considered less than 0.05.

#### Result

The clinical characteristics of the two groups consisting 100 acute ischemic stroke patients and 100 control subjects are presented in Table 2.

#### Plasma miR-34a expression level and SIRT1 concentration in acute ischemic stroke

The expression levels of miR-34a, as observed in Figure 1A, showed that those significantly increased in the ischemic stroke patients when compared to those in the control individuals (3.19 fold change,  $P < 0.0001$ ). Moreover, the evaluation of SIRT1 concentration in the plasma of ischemic stroke patients significantly showed decreased SIRT1 levels in comparison with it in the healthy control individuals ( $P < 0.0001$ ) (Figure 1B). ROC analysis was applied to assess miR-34a and SIRT1 as potential plasma biomarkers for acute ischemic stroke. The area under curve (AUC) measurements for miR-34a and SIRT1 were calculated 0.96 and 0.91 respectively verifying the efficacy of miR-34a/SIRT1 axis in the diagnosis of ischemic stroke (Figure 1C and D).

### Effect of infarction classification and etiological subtypes on miR-34a/STRT1 axis

Plasma miR-34a expression levels in acute ischemic stroke patients with different etiological subtypes were compared, and there was a significant difference between etiological subtypes ( $P < 0.001$ ). As shown in Figure 2A, the relative miR-34a expression was significantly higher in large artery atherosclerosis subtype compared to cardiac embolism subtype ( $P < 0.01$ ). The levels of miR-34a in all etiological subtypes of stroke were remarkably higher compared to control individuals ( $P < 0.001$ ). Furthermore, miR-34a expression levels were significantly different among the different subtypes of cerebral infarct sizes ( $P < 0.0001$ ). As shown in Figure 2B, the expression of this miRNA was greater in large infarct group in comparison with small infarct group ( $P < 0.05$ ). The expression levels of miR-34a in all infarct size groups were higher compared to control group ( $P < 0.001$ ). SIRT1 plasma concentration in acute ischemic stroke patients with different etiological subtypes was compared (Figure 2C). The

levels of SIRT1 protein were significantly lower in large artery atherosclerosis subtype compared to small vessel occlusion ( $P < 0.05$ ) and cardiac embolism ( $P < 0.001$ ). The SIRT1 levels in all etiological subtypes of stroke were significantly lower compared to control individuals ( $P < 0.0001$  for large artery atherosclerosis subtype compared to control and  $P < 0.05$  for small vessel occlusion and cardiac embolism subtypes compared to control). Moreover, SIRT1 concentration levels were different among the patients with various cerebral infarct sizes ( $P < 0.0001$ ) (Figure 2D).

The SIRT1 level was lower in large infarct group compared to small infarct group ( $P < 0.001$ ). Furthermore, SIRT1 concentrations showed lower levels in patients with moderate infarct subtypes compared to small infarct subtypes ( $P < 0.01$ ). The SIRT1 levels in all groups of cerebral infarct sizes were significantly lower in comparison with control group ( $P < 0.0001$  for large infarct size and moderate infarct size subtypes compared to control and  $P < 0.05$  for small infarct subtype compared to control).

**Table 2. Clinical Characteristics of Stroke Patients and Control Individuals**

	Stroke patient(n=100)	Control(n=100)	<i>p</i>
Age	63.44±10.32	63.56±9.02	0.93
Female/male	49/51	52/48	0.67
Hypertension	28	15	0.02*
Smoker	35	19	0.01*
BMI	23.22±5.02	23.85±4.66	0.36
LDL(mmoL/L)	2.69±0.58	2.29±0.53	<0.001***
<b>Cause of stroke</b>			
Large artery atherosclerosis	68	-	-
Small vessel occlusion	13	-	-
Cardiac embolism	19	-	-
<b>Infarct size</b>			
Large infarct	28	-	-
Medium infarct	50	-	-
Small infarct	19	-	-
Lacunar infarct	3	-	-
*The difference between groups was less than 0.05; *** The difference between groups was less than 0.001. LDL, Low-density lipoprotein; BMI, Body mass index.			

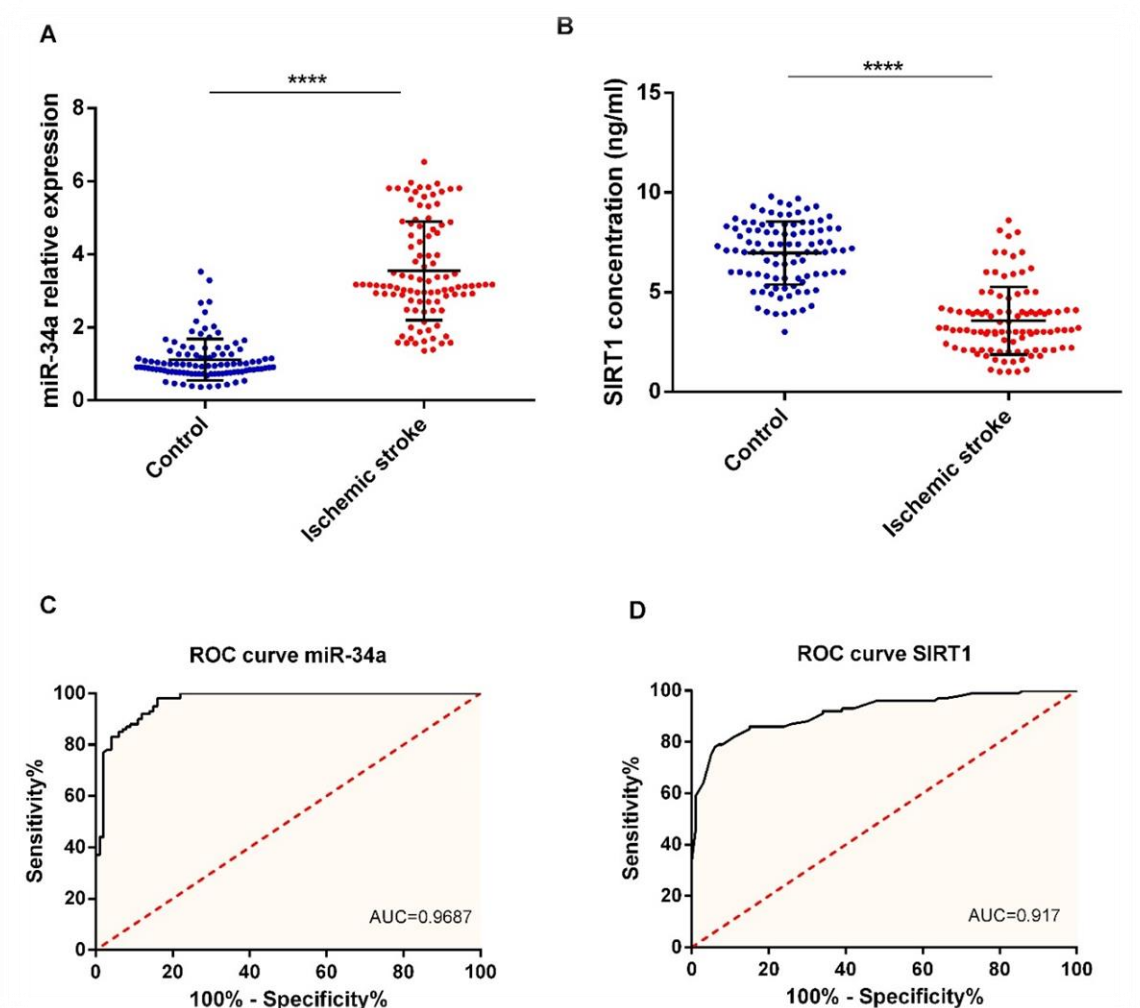
### Disregulation in miR-34a/SIRT1 axis correlated with disabilities in acute phase of ischemic stroke

To determine disability after ischemic stroke, motor and cognitive indices was performed with NIHSS scoring. The correlation of plasma miR-34a expression in the first 12 hours of ischemic stroke with baseline NIHSS outcomes was analyzed with Spearman correlation coefficient and data revealed the positive correlation between miR-34a expression and baseline NIHSS outcomes ( $P < 0.0001$ ,  $r=0.81$ , Figure 3A). Moreover, there was a negative correlation between SIRT1 plasma levels in the first 12 hour after

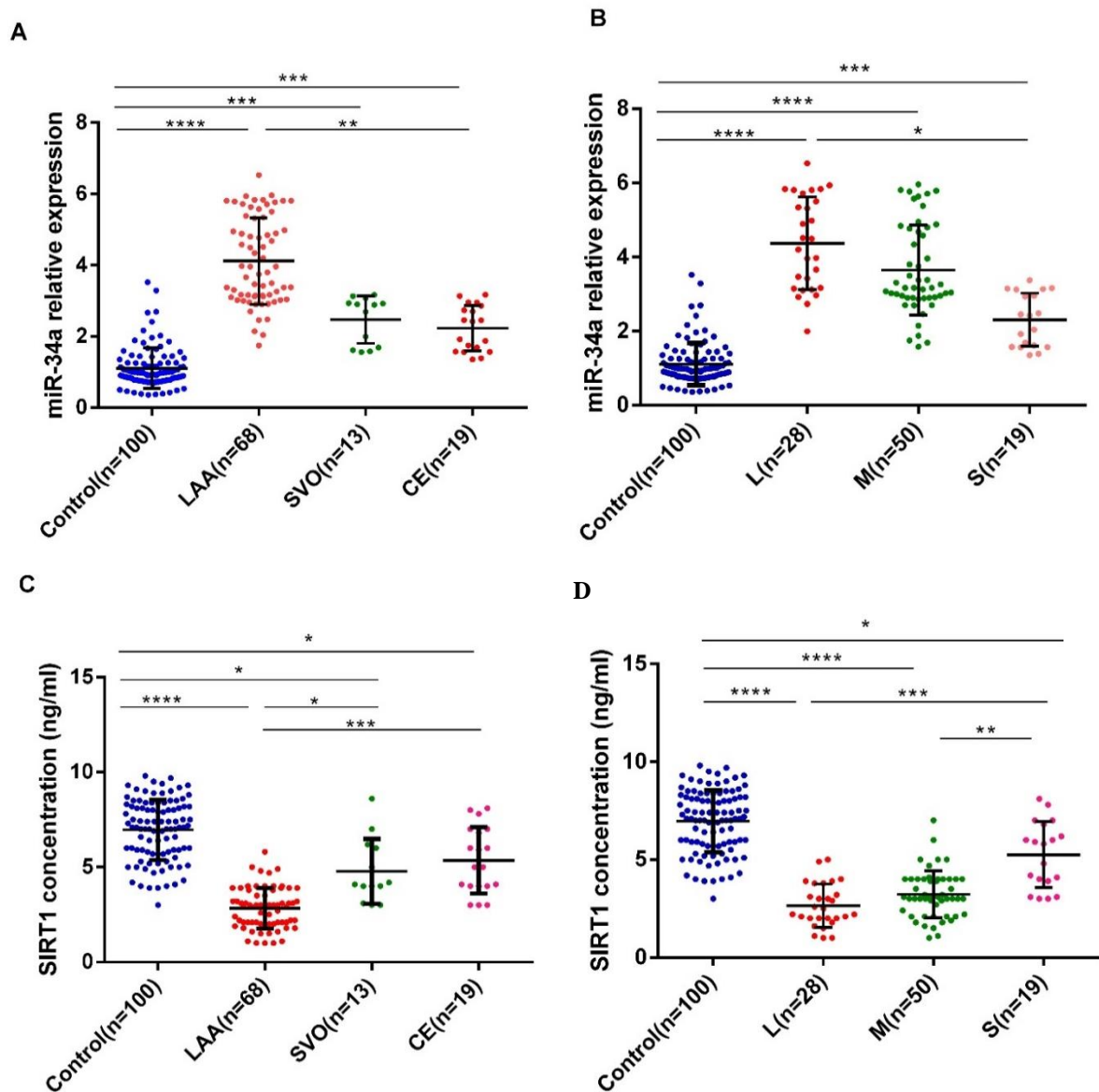
ischemic stroke and baseline NIHSS outcomes ( $P < 0.0001$ ,  $r=-0.89$ , Figure 3B).

### SIRT1 concentrations negatively correlated with miR-34a expression levels in ischemic stroke patients

We performed Spearman correlation between miR-34a and SIRT1 levels in the plasma of stroke patients (Figure 4). Data revealed that SIRT1 was negatively correlated with miR-34a ( $P < 0.0001$ ,  $r=-0.91$ ) highlighting the possible negative regulation of SIRT1 by this miRNA.

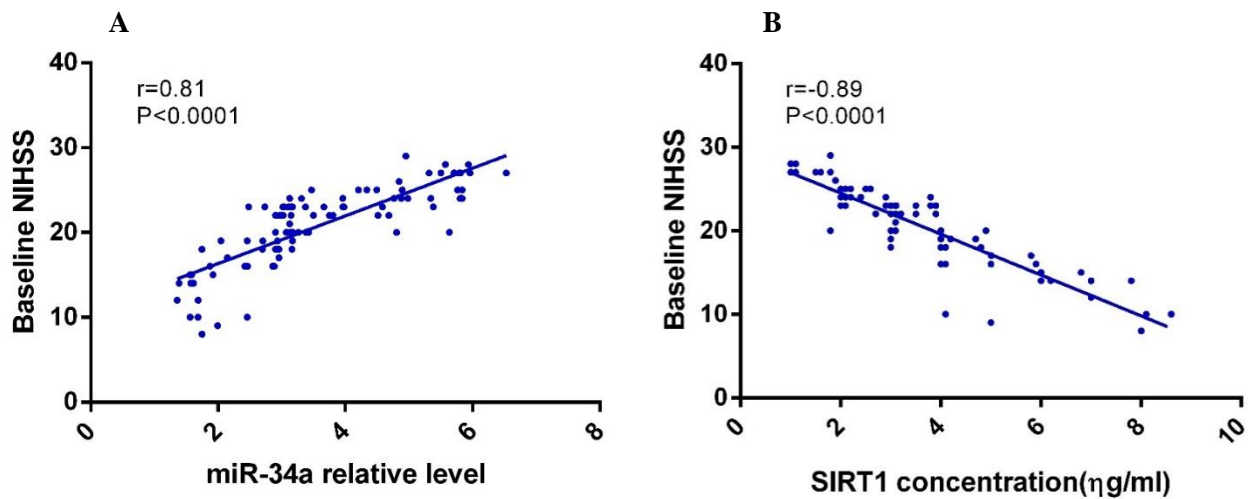


**Figure 1.** The differences in miR-34a and SIRT1 levels between ischemic stroke patients and controls. Data showed that in the acute phase of ischemic stroke, the expression of miR-34a in plasma increased (A). Moreover; the level of SIRT1 in patients has decreased significantly (B). ROC curve analysis performed to verify the efficacy of miR-34a and SIRT1 axis in the diagnosis of ischemic stroke in the acute phase. AUC value for miR-34a was 0.96 (C). AUC for SIRT1 was 0.91(D). \*\*\*\*  $P < 0.0001$

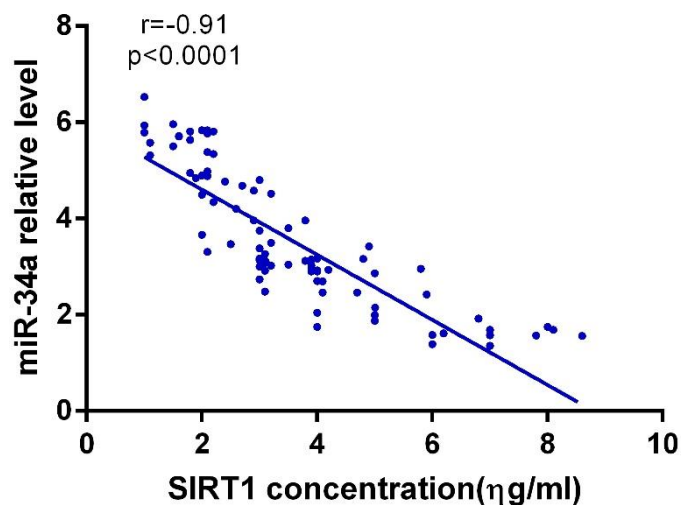


**Figure 2.** Differences in SIRT1 and miR-34a expression levels in different subtypes of infarct size and stroke etiology. miR-34a expression level was significantly higher in all stroke etiological subtypes (large artery atherosclerosis, small vessel occlusion and cardiac embolism) compared to control group. Moreover, the levels of miR-34a expression was significantly higher in large artery atherosclerosis subtype compared to cardiac embolism subtype (A). The relative expression levels of miR-34a in all infarct size subgroups of ischemic stroke were remarkably higher than in the control group, and expression levels of miR-34a were also significantly higher in the large infarct size subgroup of stroke patients compared to the small infarct size (B). SIRT1 concentration was significantly lower in all stroke etiological subtypes compare to control. The SIRT1 level was significantly lower in large artery atherosclerosis group in comparison with small vessel occlusion and cardiac embolism groups (C). SIRT1 levels in all infarct size subgroups of ischemic stroke were remarkably lower than in the control group. The SIRT1 level was significantly lower in large infarct size group in comparison with small infarct size group and there was a significant difference between moderate infarct size and small infarct size groups (D). LAA, large artery atherosclerosis; SVO, small vessel occlusion; CE, cardiac embolism; L, large infarct; M, moderate infarct ; S, small infarct. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; \*\*\*\* $P < 0.0001$





**Figure 3.** Correlation between NIHSS and dysregulation in miR-34a/SIRT1 in the plasma of stroke patients. Data revealed that NIHSS was positively correlated miR-34a expression levels (A) and negatively correlated with SIRT1 levels (B)



**Figure 4.** Correlation between miR-34a and SIRT1 levels in the plasma of stroke patients. Data revealed that SIRT1 was negatively correlated with miR-34a highlighting the possible negative regulation of SIRT1 by miR-34a

## Discussion

In this study, it was observed that expression levels of miR-34a increased in ischemic stroke patients within the first 12 hours after stroke onset, and the increase in miR-34a expression levels corresponded with the functional disability outcomes of stroke.

Moreover, the plasma level of SIRT1 decreased in the first 12 hours following ischemic stroke, and the decrease in plasma SIRT1 was consistent with the functional disability outcomes of stroke. Therefore, the dis-regulation in miR-34a/SIRT1 axis is suggested as an efficient biomarker for early detection and outcomes of ischemic stroke. Since the early diagnosis of stroke



and the time to start treatment in patients are very important as it can be said that “time is brain”, therefore finding efficient biomarkers in the acute phase of ischemic stroke is of great clinical value. In addition, these biomarkers can reveal the pathological mechanisms in stroke and can be considered as therapeutic targets in future studies (33, 34).

Our results in observing the increased expression of miR-34a in stroke patients in the first 12 hours were consistent with the study of Ren et al. on 13 ischemic stroke patients and 15 control individuals. They reported that miR-34a expression levels increased in all patients on days 5, 30 and 90 after ischemic stroke. In addition, they observed a higher serum level of miR-34a in the mice model of experimental ischemic stroke at 6 hours after stroke. The increase in miR-34a was in association with increased opening of the BBB in mice, as verified by a marked enhancement in Evans blue flow to the CNS (13).

Hu et al. showed that miR-34a expression level in cerebrovascular endothelial cells of mice increased one hour after transient middle cerebral artery occlusion, which led to BBB opening. Knockout of miR-34a significantly reduced BBB permeability, reduced tight junction disruption, and improved stroke outcomes in comparison with wild-type animals (14). In a study, Yi W-R showed that increased expression of miR-34a-5p in epithelial cells strongly reduced the mitochondrial respiratory capacity and induced apoptosis. These processes were accompanied by the production of more reactive and selective oxygen species (35).

It has also been seen that the downregulation of miR-34a-5p leads to an increase in mitochondrial biogenesis in vascular endothelial cells, which improved cellular and mitochondrial antioxidant capacity and reduced ROS (36). Upregulation of miR-34a in endothelial cells has resulted in endothelial damage, inflammation and vascular impairment (37, 38). Overexpression of miR-34a triggered endothelial damage by downregulation of SIRT1, and upregulation of p53 (21, 39).

By disrupting the mitochondrial membrane potential, miR-34a releases cytochrome c into the cytoplasm and initiates the apoptotic pathway in endothelial cells (21, 39). The data showed that in the first 12 hours after ischemic stroke, SIRT1 protein level decreased in the plasma samples of patients. Furthermore, SIRT1 level had a negative relationship with the stroke severity as determined by NIHSS

measurements and infarct sizes. This result is consistent with Imam et al.' study which reported lower SIRT1 levels in the serum of patients 24 hours after stroke in both ischemic and hemorrhagic groups (40). SIRT1 levels have been shown to be widely reduced in the brain of a rat model of cerebral ischemia-reperfusion injury. Furthermore, overexpression of SIRT1 through adeno-associated viral Sirtuin-1, restored mitochondrial structure and function, which provided neuroprotection against cerebral ischemia-reperfusion injury and inhibited stroke-induced apoptosis and motor damage, representing a potential approach for the treatment of cerebral ischemia (41). Examination of SIRT1 transgenic mice and SIRT1 knockout mice specific to brain endothelial cells showed that SIRT1 expression is crucially involved in BBB integrity (42, 43).

Overexpression of SIRT1 or increased activity of SIRT1, protected CNS against the high permeability of the brain endothelial barrier caused by aging and preserved claudin-5/ZO-1 interactions in the vascular endothelium (43). SIRT1 plays an important role in the clinical pathophysiology of stroke. SIRT1 is involved in oxidative stress reduction and DNA repair pathways (44). Decreased expression and activity of SIRT1 is associated with many age-related diseases (44). Aging processes increase the risk of stroke, which may be related to the reduction of SIRT1 levels and its protective functions (24). Additionally, SIRT1 has neuroprotective effects that can reduce neuronal damage following ischemic stroke. SIRT1 increases the expression of various neuroprotective proteins, including brain derived neurotrophic factor (BDNF) (45). In animal model of stroke, higher SIRT1 activity has been associated with better stroke outcomes (46). In addition, SIRT1 affects endothelial function and vascular health, both of which are critical in the pathophysiology of stroke (46).

It enhances the activation of the enzyme nitric oxide synthase (eNOS), which leads to improved vasodilation and blood pressure (47). Reduced SIRT1 activity can induce endothelial dysfunction and incidence of ischemic stroke (24). Conditions such as obesity, diabetes, and metabolic syndrome are risk factors for stroke (48). SIRT1 is involved in metabolic regulation, including glucose homeostasis and lipid metabolism. Impaired SIRT1 activity in these conditions may contribute to increased risk of stroke accordance and poorer recovery outcomes (24).

miR-34a polymorphisms are associated with increased susceptibility to ischemic stroke, supporting the importance of miR-34a as a biomarker or potential therapeutic target for ischemic stroke (49). SIRT1 upregulation reduced the risk of stroke recurrence (50). After stroke, SIRT1 suppresses matrix metalloproteinase-9 (MMP-9), a member of the zinc-dependent endopeptidase family that is crucially involved in stroke pathogenesis by BBB breakdown, cerebral edema, neuronal cell death, macrovascular spasm, and small vessel thrombosis (51). Increased SIRT1 expression can directly prevent vascular thrombus formation following primary ischemic stroke, as SIRT1 inhibits tissue factor, a major driver of the coagulation cascade (52, 53).

In addition to its direct effects on various intracellular signaling pathways, SIRT1 activation also contributes to the establishment of ischemic tolerance by stabilizing hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ), which can prevent stroke recurrence (54). We observed that increased miR-34a expression levels coincided with decreased concentrations of SIRT1. It has been reported that miR-34a suppresses SIRT1 expression by binding to the 3' UTR of SIRT1 mRNA (11, 16). This pathway is involved in p53 upregulation, cytochrome c release, mitochondrial damage and apoptosis (11, 16, 37). Interestingly, Liu et al. reported decreased mtDNA and SIRT1 and increased miR-34a expression levels in circulating mononuclear cells of patients with cognitive impairment associated with type 2 diabetes. They introduced the miR-34a/SIRT1 axis as a differential biomarker in the diagnosis of type 2 diabetes mellitus with cognitive impairment from type 2 diabetes mellitus without cognitive impairment and healthy control (55).

Conclusively, in the first 12 hours following ischemic stroke, miR-34a expression level increased and SIRT1 concentration decreased in the plasma of patients. There was a positive correlation between plasma miR-34a expression levels and functional disability following ischemic stroke as determined by baseline NIHSS outcomes. Moreover, there was a negative correlation between SIRT1 plasma levels and baseline NIHSS outcomes. Data revealed that SIRT1 was negatively correlated with miR-34a highlighting the possible negative regulation of SIRT1 by this miRNA. Subsequently, miR-34a/SIRT1 axis could be suggested as a potential biomarker in the acute phase of stroke. In addition, the miR-34a/SIRT1 axis may be

a therapeutic target to reduce damage caused by ischemic stroke.

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