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Research Article

Role of microRNA-9-5p as a diagnostic biomarker of acute stroke

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Abstract

Stroke is the second leading cause of death worldwide. MicroRNA-9-5p has been reported to be upregulated in ischemic stroke; however, no previous study has explored its expression in hemorrhagic stroke. The objective of this study was to investigate microRNA-9-5p as a biomarker to differentiate between ischemic and hemorrhagic stroke. This was a case–control study. It was found that microRNA-9-5p was upregulated in both ischemic stroke (IS) and hemorrhagic stroke (HS) compared to controls ($p < 0.0001$). However, no significant difference was observed between the IS and HS groups ($p > 0.9999$). Moreover, microRNA-9-5p could distinguish IS and HS from controls with AUC = 0.9225 and AUC = 0.9245, respectively. However, it could not differentiate IS from HS (AUC = 0.592). There was also no significant correlation between microRNA-9-5p expression fold and either IL-6 or CRP. In conclusion, microRNA-9-5p was upregulated in both IS and HS and may serve as a biomarker to distinguish stroke patients from controls, but it cannot differentiate between stroke types.

Keywords

biomarkers, hemorrhagic stroke, inflammation, ischemic stroke, microRNA-9-5p

Introduction

Stroke is known as a neurological impairment caused by an acute focal injury of the central nervous system (CNS) due to a vascular cause, classified mainly into ischaemic stroke (IS), intracerebral hemorrhage (ICH), and subarachnoid hemorrhage (SAH) (American Heart Association 2019). Stroke is the second greatest cause of mortality and causes long-term disability in approximately 5 million people (Al-Obaidi et al. 2023). Approximately 20% of stroke cases are hemorrhagic stroke (HS), while the majority are ischemic stroke (IS) (Alrabghi et al. 2018). Stroke has many risk factors some are modifiable, such as hypertension and diabetes mellitus, while others are nonmodifiable, such as age and sex (Alrabghi et al. 2018; Maida et al. 2024). Diagnosis of stroke depends on neuroimaging techniques such as computed tomography (CT) scans and magnetic resonance imaging (MRI) to differentiate ischemic stroke from hemorrhagic stroke and then initiate appropriate therapy (Warner et al. 2019). However, these diagnostic techniques have some limitations. For example, MRI has greater sensitivity than other techniques for acute ischemic stroke (AIS) identification, but it is not widely available in all hospitals and requires longer imaging times; it is mostly used as follow-up imaging in patients with cerebrovascular

accidents (Patil et al. 2022). In addition, MRI cannot be utilized in most elderly patients because of pacemakers or implants (Alotaibi et al. 2020). The treatment of AIS depends on the time of symptom onset (it should be given within 4.5 hours of symptoms), and there is currently no specific blood marker for it (Wang et al. 2023). Stroke is one of the major healthcare issues worldwide due to its high mortality and morbidity and the significant socioeconomic impact on patients, families, and society (Johnson et al. 2019). Furthermore, treatment of AIS cannot depend solely on thrombolytic therapy and mechanical thrombectomy, as these treatment methods require certain patient criteria and have a high risk of hemorrhagic complications (Kadir and Bayraktutan 2020). Thus identification of blood-based biomarkers has great potential to improve stroke diagnosis, prognosis, and treatment monitoring. They offer a minimally invasive method for early stroke detection and outcome prediction (Babić et al. 2025). It has been revealed that a number of central nervous system (CNS) disorders, including stroke, multiple sclerosis (MS), and Alzheimer's disease are accompanied by changes in specific circulating microRNAs (miRNAs). Furthermore, miRNAs have been found to be involved in the pathogenesis, diagnosis, and treatment of CNS disorders (Zhang et al. 2022). This has led to the suggestion that these circulating miRNAs could be employed as clinical biomarkers (Bejleri et al. 2021). MicroRNAs (miRNAs) are single-stranded noncoding RNAs (about 19 to 23 base pairs). MiRNAs regulate gene expression by targeting messenger RNA (mRNA), leading to either mRNA degradation or translational suppression (Searles 2024). MicroRNAs are involved in the modulation of many cellular processes, for example, cell metabolism, proliferation, and death (Todoran et al. 2023). It has been demonstrated that miRNAs play critical roles in the main mechanisms of stroke pathology, such as energy failure, inflammation, and cell death. Therefore, miRNAs may serve as reliable blood-based markers for risk prediction, diagnosis, and prognosis of ischemic stroke (Kadir et al. 2022). MiRNAs are released as circulating molecules into body fluids such as cerebrospinal fluid (CSF), blood, and urine (Hussein and Magdy 2021). A number of investigations have identified specific circulating miRNAs for stroke patterns that are associated with symptom severity, infarct volume, and prediction of functional outcomes. Circulating levels of miR-125a-5p, miR-125b-5p, and miR-143-3p have been found to be upregulated in patients with AIS compared to those with transient ischemic attack (TIA) or normal subjects, supporting the applicability of these miRNAs for early stroke diagnosis in the emergency setting (Tiedt et al. 2017). In a recent study, researchers investigated the diagnostic value of miR-9-5p for asymptomatic carotid artery stenosis (CAS) and its predictive value for future cerebrovascular events within 5 years (Liu et al. 2021). microRNA-9-5p has been investigated for its role in the pathological mechanism of IS (Chi et al. 2019). In the present study, we aimed to investigate levels of microRNA-9-5p in ischemic stroke and hemorrhagic stroke and to explore whether it can be used as a diagnostic biomarker to discriminate between IS and HS in acute stroke, as well as to investigate its correlation with inflammatory markers.

Method

This study was conducted on 50 patients suffering from stroke at Al-Yarmook Teaching Hospital in Baghdad, Iraq, and 25 subjects as a controls (controls did not have stroke or a history of stroke). Some control subjects had risk factors for stroke such as, hypertension, diabetes or other predisposing factors. The study was performed during the period from October 2024 to March 2025. Blood samples were collected from patients within 24 hours after the onset of symptoms to determine the expression fold of microRNA-9-5p and the concentration of interleukin 6 (IL-6), C-reactive protein (CRP). All patients were diagnosed by neurologists, who determined whether the patient had ischemic stroke or hemorrhagic stroke. About 4–5 ml of venous blood was collected from each patient within 24 hours of stroke symptom onset and placed in gel tubes. after approximately 30 minutes and no longer than 1 hour, the blood samples were centrifuged at 4,100 rpm for 8 minutes to obtain serum. The serum was then stored at –20 °C until analysis. About 250 µl of isolated serum from each subject was placed in PCR tubes containing 750 µl of Trizol for microRNA-9-5p extraction, which was performed on the day of analysis. All participants provided written informed consents to participate in the study.

Inclusion criteria

All patients aged ≥18 years with acute stroke who presented within 24 hours.

Exclusion criteria

Patients with autoimmune diseases, infections, tumours, head trauma, intracranial tumors, or neurodegenerative diseases.

Design of the study

The design of the currrent study was a case–control study. Participants were divided into three groups: the ischemic stroke (IS) group (n = 25), the hemorrhagic stroke (HS) group (n = 25) and the control group (n = 25)

Reagents and kits

Transzol Up Plus RNA Kit (ER501-01) was used to extract total RNA from all samples. EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix Kit (AE311-02) was used for total RNA reverse transcription to complementary DNA (cDNA). TransStart® Top Green

qPCR SuperMix (AQ131-01) was used to assess the expression levels and fold changes of the microRNA-9-5p and U6 genes. The miR U6 was utilized as an endogenous control. MiRNA-specific primers were obtained from Alpha DNA Ltd. (Canada). Fold expression of the mature miRNA was calculated by the relative cycle threshold ($2^{-\Delta\Delta Ct}$) method originally published by Livak and Schmittgen (2001). Human IL-6 (Interleukin 6) ELISA Kit (E-EL-H6156; Elabscience. China) and Human CRP (C-reactive protein) ELISA Kit (Elabscience, China) were used to determine concentration of IL-6 and C-reactive protein, respectively (Hashim et al. 2021). Chemicals used in the study included chloroform and 100% ethanol during total RNA extraction.

Quantitative real time PCR (qRT–PCR) was carried out using the QIAGEN Rotor-Gene Q Real-Time PCR System (Germany).

An ELISA reader (Human Reader HS, Germany) was used for measuring absorbance and quantifying biomarkers levels.

Pipettes and microplates were used for handling and transferring samples during the ELISA procedure.

Information of participants

All participant information was collected using a questionnaire that included demographic data and medical history.

Statistical analysis

All statistical analyses were performed using GraphPad Prism version 10.5. Normality was assessed using the Shapiro–Wilk test. Depending on data distribution, either one-way ANOVA or Kruskal–Wallis tests were used for groups comparisons, with appropriate post hoc analyses. The Mann–Whitney test was used as appropriate. Chi-square or Fisher's exact tests were applied to categorical data. ROC curve analysis was used to assess diagnostic performance. A p-value < 0.05 was considered statistically significant.

Result

The patients and the control groups were presented by their age, body mass index (BMI), sex, smoking status, hypertension, diabetes mellitus, and previous history of stroke, as shown in Table 1. With respect to the distribution of age, the mean age for the control group was 54.04 ± 9.93 years, the mean age for the ischemic stroke group was 66.92 ± 12.11 years, and the mean age for the hemorrhagic stroke group was 52.20 ± 11.53 years. There was a significant difference between the age distribution in the study groups (p < 0.0001). IS patients were significantly older than control and HS patients (p = 0.0002 and p < 0.0001, respectively), and no significant differences were observed between controls and HS (p = 0.5641). With respect to BMI, there was no significant difference between the groups (p > 0.05). Regarding sex distribution, there were statistically significant differences in sex proportions across groups (p = 0.0056). The control group exhibited a balanced male-to-female ratio (13 males: 12 females), but both the IS and HS groups showed a marked male predominance – 92% and 76%, respectively. In addition, it was found that there was a significant difference in the prevalence of smokers among the study groups (p = 0.004), in which HS patients had the highest proportion compared to control and IS. Furthermore, it was found that there was a significant difference in the prevalence of smokers among the study groups (p = 0.004), in which HS patients had the highest proportion compared to control and IS. Regarding hypertension, there were highly significant differences in hypertension prevalence among the groups (p < 0.0001), in which IS patients (100%) and HS patients (92%) had the highest prevalence of hypertension compared to the control group, which had the lowest prevalence (56%). In addition, there were significant differences in diabetes prevalence across groups (p = 0.0155), with the highest prevalence in IS (60%) and HS (40%) patients. It was also noted that there were significant differences with regard to previous history of stroke across groups (p < 0.001), with the highest percentage in IS (48%). The median time of taking the blood after symptom onset was 12 hours (range: 1–24 hours), and 11.5 hours (range: 1–24 hours) in IS and HS, respectively.

Table 1. Demographic distribution data and the clinical characteristics of the patients and the control groups.

Characteristics	Control (n = 25)	IS (n = 25)	HS (n = 25)	P-value
Age (years)	54.04 a ± 9.93	66.92 b ± 12.11	52.20 a ± 11.53	*0.001
BMI (kg/m^2)	29.30a (25.99–35.57)	27.68a (24.74–32.05)	30.07a (25.54–33.51)	**>0.05
Sex Male (%)	52% (13/12)	92% (23/2)	76% (19/6)	0.0056
Smoking %Yes/no	12% (3/22)	44% (11/14)	56% (14/11)	0.004
HTN %Yes/no	56% (14/11)	100% (25/0)	92% (23/2)	<0.001
DM %Yes/no	20% (5/20)	60% (15/10)	40% (10/15)	0.0155
Previous stroke%Yes/no	0% (0/25)	48% (12/13)	32% (8/17)	<0.001
Time of taking the blood after symptom onset median (range)	–	11.5 hour (1–24)	12 hour (1–24)	–

*The data were expressed as mean ± standard deviation; **data were expressed as median (interquartile range); mean or median values followed by the same letter are not significantly different, while mean or median values followed by different letters are significantly different; IS: ischemic stroke; HS: hemorrhagic stroke; BMI: body mass index; n: number; %: percentage; DM: diabetes mellitus; HTN:hypertension.

MicroRNA-9-5p expression fold and serum levels of interleukin 6 and C-reactive protein in the patients and the control groups

In the present study it was found that microRNA-9-5p was significantly upregulated in both the ischemic stroke (IS) and hemorrhagic stroke (HS) groups compared to controls ($p < 0.0001$). However, there was no significant difference between the IS and HS groups ($p > 0.9999$) (Table 2).

The amplification plot of microRNA-9-5p and miRNA U6 and the melt curve of microRNA-9-5p and miRNA U6 genes, are shown in Fig. 1A–D. The expression fold of microRNA-9-5p among the control and patient groups is presented in Fig. 1E.

Regarding inflammatory markers, highly significant differences were revealed in IL-6 levels ($p < 0.0001$). The Ischemic stroke (31.45 (30.07–33.46)) and HS (40.60 (30.62–45.02)) groups exhibited significantly higher IL-6 levels compared to the control group (17.13 (14.87–17.93)) ($p < 0.0001$). However, no significant difference was observed between IS and HS ($p = 0.2327$), as illustrated in Table 2.

It was also demonstrated that there were highly significant differences in CRP levels ($p < 0.0001$). According to Dunn's post hoc tests, HS patients exhibited the highest CRP concentrations (10.24 (9.34–10.70)), significantly exceeding both controls (3.69 (3.06–4.22)) ($p < 0.0001$) and IS patients (7.88 (7.24–8.43)) ($p = 0.0011$). IS patients also showed significant elevation compared to controls ($p < 0.0001$), as shown in Table 2.

Receiver operating characteristic (ROC) curve of microRNA-9-5p

MicroRNA-9-5p could diagnose IS and HS from controls with AUC = 0.9225 and AUC = 0.9245, respectively, and the best cut-off value of >2.242, with 96% sensitivity and 84% specificity (Fig. 2A and Fig. 2B, respectively). However, it could not differentiate between IS and HS; AUC was 0.592, with 84% sensitivity and 48% specificity; cut-off value was 2.442 ($P = 0.252$), as illustrated in Table 3 and Fig. 2C.

Correlation between microRNA-9-5p and IL-6 and CRP

This study found that there was no significant correlation between microRNA-9-5p expression fold and either IL-6 and CRP ($p > 0.05$), as shown in Table 4.

Association between microRNA-9-5p and clinical parameters among stroke patients

It was found that there were significant associations between microRNA-9-5p and previous history of stroke ($p < 0.05$). However, there were no significant associations between microRNA-9-5p and hypertension, diabetes, or smoking ($p > 0.05$), as shown in Table 5.

Table 2. Expression fold of microRNA-9-5p and serum levels of interleukin 6 and C-reactive protein among the patients and the control groups.

Biomarkers	Control (n = 25)	IS patients (n = 25)	HS patients (n = 25)	P-value
Fold (2^-ΔΔCt) of microRNA9-5p	2.09 a (1.18–2.61)	4.01 b (2.37–7.82)	4.32 b (2.86–8.59)	p <0.0001
IL 6 (pg/ml)	17.13 a (14.87–17.93)	31.45 b (30.07–33.46)	40.60 b (30.62–45.02)	p <0.0001
CRP (ng/ml)	3.69 a (3.06–4.22)	7.88 b (7.24–8.43)	10.24 c (9.34–10.70)	p <0.0001

Data presented as median (interquartile range). Median values followed by different letters are significantly different according to Dunn's post hoc test; median values followed by the same letter are not significantly different; Fold (2^-ΔΔCt): fold expression of microRNA9-5p; IL-6: interleukin 6; CRP: C-reactive protein.

Table 3. Receiver operating characteristic curve data of microRNA-9-5p.

Biomarker	Group	AUC	Explanation	P-value	Best cut off	Sensitivity	Specificity
MicroRNA-9-5p	IS vs. control	0.9225	excellent	<0.0001	>2.242	96%	84%
	HS vs. control	0.9245	excellent	<0.0001	>2.242	96%	84%
	IS vs. HS	0.592	no discrimination	0.252	2.442	84%	48%

AUC: area under the curve; IS: ischemic stroke; HS: hemorrhagic stroke.

Table 4. Spearman correlation among the studied biomarkers.

Variable 1	Variable 2	R value	P-value	Correlation type
Fold (2^-ΔΔCt) of miRNA-9-5p	IL-6 (pg/ml)	–0.346	0.0905	Weak negative
Fold (2^-ΔΔCt) of miRNA-9-5p	CRP (ng/ml)	–0.098	0.6415	No/very weak

Correlation is significant at the 0.05 level; R: Pearson correlation coefficient; Fold (2^-ΔΔCt): fold expression of microRNA-9-5p; IL-6: interleukin 6; CRP: C-reactive protein.

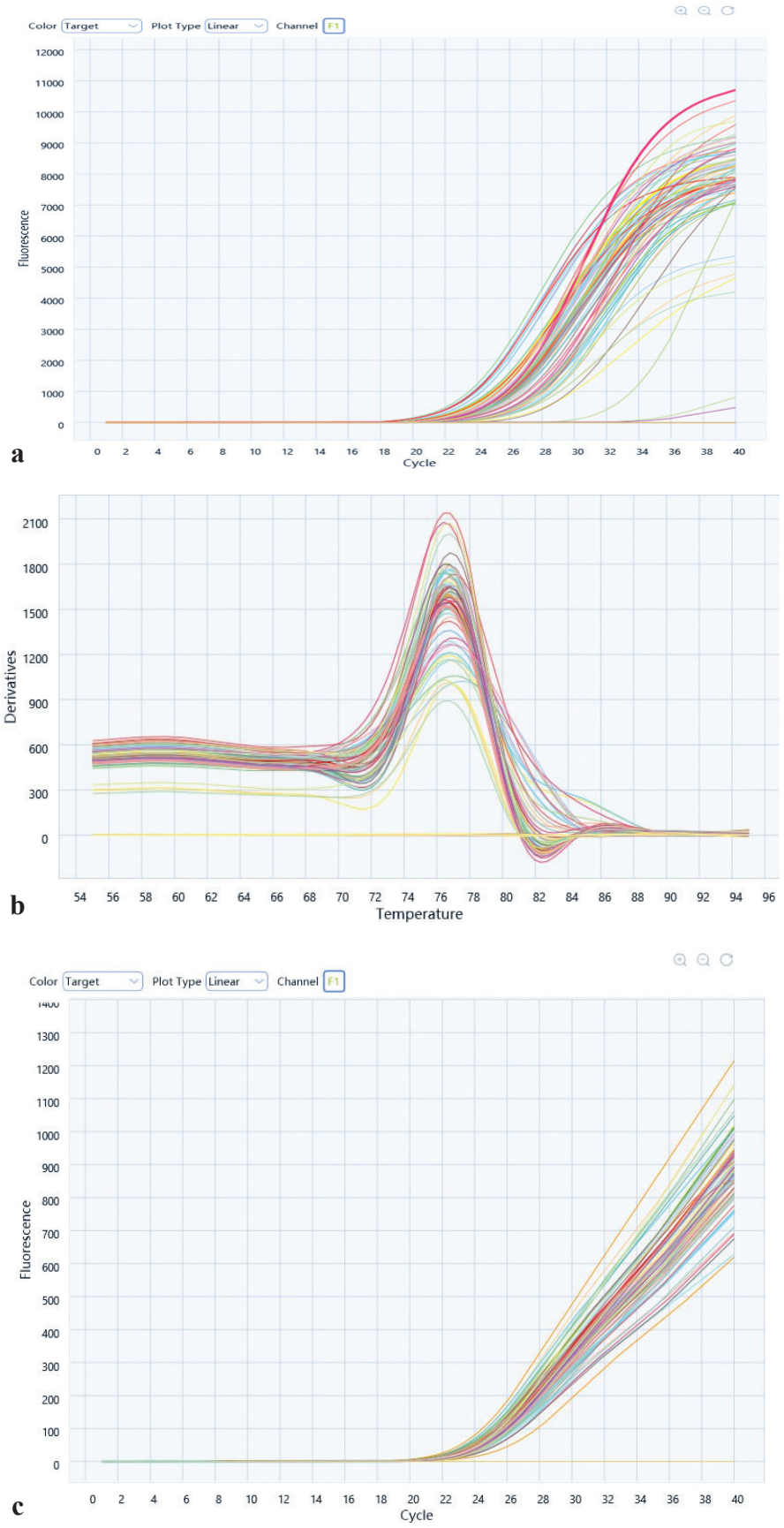

Figure 1. a. The amplification curve of microRNA-9-5p gene by RT-PCR. The picture was taken directly from the device. **b**. Melting curve of microRNA-9-5p gene. **c.** The amplification curve of miRNA U6 gene by RT-PCR. The picture was taken from qRT-PCR. **d.** Melt curve of miRNA U6 gene amplicons describing the peak following analysis by RT-qPCR. **e.** Expression fold of microRNA-9-5p among the control and patient groups. ns: not significant ($P > 0.05$). **** Extremely highly significant ($P \leq 0.0001$).

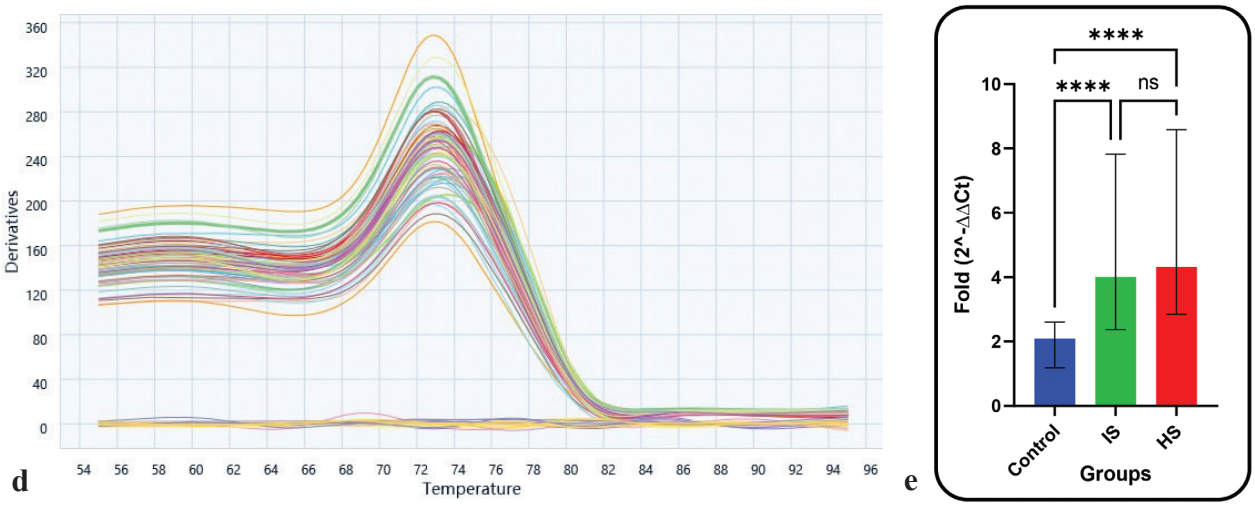

Figure 1. Continued.

Table 5. Association between microRNA-9-5p and clinical parameters among stroke patients.

Biomarker	Risk factor	Group	N	Level	U test	P-value
Fold expression of microRNA-9-5p	HTN	positive	48	4.23(8.15–2.56)	57	0.686
		negative	2	6.13(8.65–3.61)		
	DM	positive	25	4.57(8.97–2.58)	294.5	0.727
		negative	25	4.06(7.83–2.61)		
	Smoking	positive	25	4.15(5.36–2.64)	338	0.621
		negative	25	4.32(9.05–2.57)		
	Previous history of stroke	positive	20	4.75(11.88–4.05)	192.5	0.033*
		negative	30	3.53(6.4–2.39)		

Data were expressed as median (IQR); *$p < 0.05$ was considered as statistically significant; n: number.

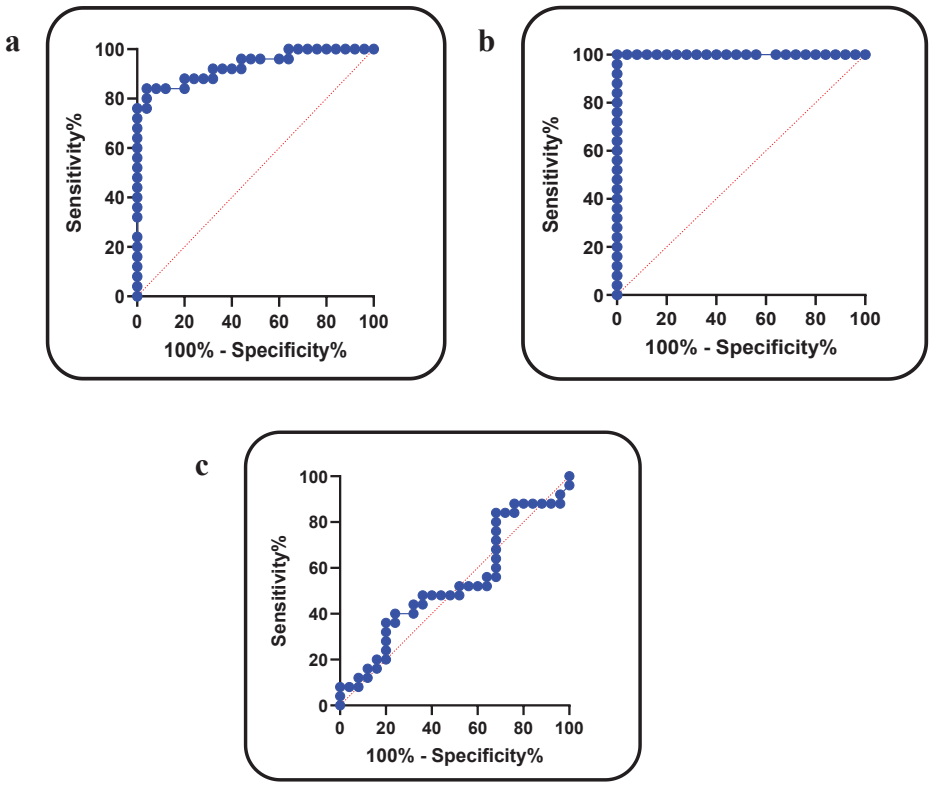

Figure 2. Receiver operating characteristic curve of microRNA-9-5p. **a**. ROC curve of IS versus control; **b**. ROC curve of HS versus control; **c**. ROC curve of IS versus HS.

Discussion

Several studies have been conducted to investigate the role of microRNA-9-5p in the pathophysiology of stroke. In a study performed by Yan Q. et al, using an in vitro oxygen-glucose deprivation/reoxygenation (OGD/R) neuronal cell model, they found that OGD/R-induced injury was accompanied by significant upregulation of several microRNAs, one of which was microRNA-9-5p. OGD/R-induced injury resulted in marked apoptosis as shown by TUNEL analysis. They also revealed that in vitro inhibition of miR-9-5p using miR-9-5p inhibitors decreased cell death, and in vivo inhibition of miR-9-5p (mouse model) resulted in decreased infarct size and caspase-3 activity (Yan et al. 2020).

The present study investigated the role of miR-9-5p in the diagnosis of acute stroke and its correlation with inflammatory biomarkers. Significant upregulation of microRNA-9-5p was found in both ischemic and hemorrhagic stroke. This result is consistent with another study, which found upregulation of both miR-9-5p and miR-128-3p in patients with acute ischemic stroke within 6 hours after symptom onset (Wang et al. 2021). However, other studies found that miR-9 was downregulated in patients with larger strokes (Liu et al. 2015), which does not align with the present findings. Moreover, Ji Q. et al. noted upregulation of miR-9 and miR-124 in acute ischemic stroke within 24 hours after symptom onset (Ji et al. 2016). No previous studies have investigated the expression level of miR-9-5p in hemorrhagic stroke. Additionally, a study conducted in a rat model showed that miR-9a-5p levels were significantly reduced (Wang et al. 2018). However, Sørensen et al. (2017) demonstrated that levels of both miR-9-5p and miR-128-3p were increased in the cerebrospinal fluid of patients if infarct size was greater than 2 cm^3 in volume. Such differences in expression fold may be due to variations in sample types, time of sample collections after stroke, and models used for miRNA detection.

In addition, the current study showed that the inflammatory markers IL-6 and C-reactive protein were significantly higher in ischemic and hemorrhagic stroke compared to controls, as expected since inflammation is part of the pathogenesis of stroke (Simats and Liesz 2022). Previous studies have concentrated on the role of these inflammatory markers in predicting stroke prognosis or severity. For example, IL-6 was upregulated in stroke patients and showed a positive correlation with the National Institutes of Health Stroke Scale (NIHSS) and the modified Rankin Scale (MRS) after 3 months (Aref et al. 2020). Furthermore, Zhu et al. (2024) reported that the median C-reactive protein level in patients with good prognosis was 5 (range 2.28–11) while in patients with poor prognosis it was 8.42 (range 3–17.1). However, in intracerebral hemorrhage (ICH) patients C-reactive protein was not found to predict mortality (Bernstein et al. 2018). The current study focused on the correlation between microRNA-9-5p and IL-6 and C-reactive protein. Interestingly, no significant correlation was found between microRNA-9-5p and these markers. Huang et al. (2025) performed a study in human SH-SY5Y cells, inducing cerebral ischemia/reperfusion injury simulated by OGD/R. They observed that microRNA-9-5p targets NADPH oxidase 4 (NOX4, a member of the NOX family known to generate reactive oxygen species). The mir-9-5p mimic dramatically suppressed NOX4 expression, leading to inhibition of oxidative stress and decreased inflammatory markers such as IL-6. However, in the present study, no correlation was found between microRNA-9-5p and inflammatory markers. This finding is inconsistent with a study that found a positive correlation between microRNA-9-5p and IL-6 levels in ischemic stroke patients (Ji et al. 2016). Other studies found a negative correlation between miR-9 and high-sensitivity CRP (Liu et al. 2015) which also contrasts with the present study as no correlation was observed between microRNA-9-5p and C-reactive protein (CRP).

Regarding diagnostic accuracy, the present study found that the AUC of microRNA-9-5p to differentiate IS from controls and HS from controls was 0.9225 and 0.9245, respectively, with 96% sensitivity and 84% specificity for both. Other studied have recorded lower sensitivity and higher specificity – for instance, one study reported an AUC of 0.9467 for microRNA-9-5p to differentiate IS from controls with 89.75% sensitivity and 82.66% specificity (Wang et al. 2021). Another study found a lower AUC for exosomal miR-9 to differentiate IS from controls (0.8026) without reporting sensitivity and specificity (Ji et al. 2016). However, the area under the cuvre to differentiate IS from HS was 0.592, indicating that it could not discriminate between these stroke types, and no previous studies have reported on this. The present study also found no significant association between microRNA-9-5p and hypertension, diabetes mellitus (DM) or smoking. Kontaraki et al. (2014) found that hypertensive patients had significantly lower miR-9 (9.69 ± 1.56 vs. 41.08 ± 6.06; $P < 0.001$) expression levels compared with healthy controls which contradicts our findings. In addition, Elhag and Al Khodor (2023) found that miR-9-5p was overexpressed in the serum of newly diagnosed individuals with type 2 diabetes (T2D), which does not align with the result of the present study. Conversely, Narayan et al. (2024) reported that miR-9-5p levels were significantly lower in T2DM compared to normal individuals. Regarding smoking, Zhang et al. (2024) conducted a study on patients with non-small-cell lung cancer (NSCLC) and found that miR-9-5p expression in nonmalignat tissue was significantly higher in smokers than in nonsmokers, Furthermore, results of TCGA bioinformatics analysis showed that miR-9-5p levels in NSCLC tumor tissues of smokers were significantly higher than those of nonsmokers. An in vitro study in naïve and desialylated human alveolar epithelial cells (A549 cells) grown for 24 hours in cigarette smoke-conditioned medium found that cigarette smoke decreased miR-9 levels in both cell types by about threefold (Holownia et al. 2019). It was found that patients with a previous history of stroke had significantly higher expression folds of microRNA-9-5p than those without history of stroke. No previous studies have reported on this finding.

Conclusion

In conclusion, microRNA-9-5p was upregulated in both IS and HS and may be used as a biomarker to differentiate stroke patients from control subjects, but it cannot differentiate between types of stroke.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

Clinical trials: This study was based on the local ethical committee recommendations of the College of Pharmacy/Mustansiriyah University. Approval No.71.

The authors declared that experiments on humans or human tissues were performed for the present study.

Informed consent from the humans, donors or donors' representatives: The informed consent have been deposited at department of clinical laboratory science/College of Pharmacy/Mustansiriyah University

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

Use of AI

No use of AI was reported.

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Author contributions

Yassameen Jamal Ali: sample collection, laboratory analysis, writing and statistical analysis; Wassan Abdulkareem Abbas: supervision, editing, reviewing; Suzan Yousif Jasim: supervision, editing, reviewing.

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Data availability

All of the data that support the findings of this study are available in the main text.

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