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ORIGINAL RESEARCH

The expression of microRNA 146a in patients with ischemic stroke: an observational study

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Aim: We conducted the present prospective study to assess the level of microRNA (miRNA) 146a in patients with ischemic stroke and its correlation with patients' characteristics.

Methods: We conducted an observational study that included adult patients (≥18 years old) who presented within 24 hrs after the onset of the symptoms of acute ischemic stroke. In addition, age- and sex-matched healthy volunteers were included as control group. The primary outcome in the present study was the difference in miRNA 146a expression between patients with ischemic stroke and control group participants. The expression of miRNA 146a was measured using quantitativereal-time PCR. Quantitativereal-time PCR amplification and analysis were performed using Rotor-Gene Q thermal cycler.

Results: The present study included 44 patients with ischemic stroke and 22 matched controls. Regarding the primary outcome of the present study, the median expression of miRNA 146a in patients with ischemic stroke was −1.98 fold (IQR −27.1–3.9) compared to 1.75 fold (IQR −2.25–5.27) in control group (P<0.001). However, the subgroup analysis showed that the expression of miRNA 146a was significantly downregulated in comatosed patients only (P<0.001). The expression of miRNA 146a correlated negatively with Glasgow Coma Scale score in comatose patients (r=−0.352, P=0.022).

Conclusion: In conclusion, the expression of miRNA 146a is significantly downregulated in ischemic stroke patients. Further studies are needed to assess its diagnostic utility and therapeutic potentials.

Keywords: ischemic stroke, coma, cerebral infarction, miRNA 146a

Introduction

Stroke is one of the major public health burdens worldwide and a leading contributor to the global mortality and morbidities. According to recent epidemiological figures, stroke affects 16.9 million people worldwide every year and accounts for 6.5 million deaths globally in 2016 alone; which ranked it as the second most common cause of global mortality.[1,2] The condition is defined as the acute development of vascular insult (whether ischemic or hemorrhagic) that leads to the sudden onset of acute, symptomatic, neurological deficit lasting for >24 hrs or accompanying by death.[3] Ischemic strokes account for the vast majority of (≈85%) of cerebrovascular accident and characterized by sustained occlusion of the cerebral blood vessel, with the subsequent reduction in regional blood flow and central area of irreversibly damaged tissue within few hours.[4] Patients with ischemic strokes can present with a wide range of clinical features, according to the size and location of the vascular insult, including aphasia, hemiparesis, and cranial nerve dysfunction.[5] In addition to the increased risk of mortality, ischemic stroke is a leading cause of short- and long-term disabilities in affected individuals; patients with ischemic

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strokes are at increased risk of cerebral edema, hemorrhage within the infarct area, venous thrombosis, seizures, depression, and functional disabilities.[6,7] Moreover, improper management of ischemic strokes can increase health care expenditure and represents an economic burden.[8] Therefore, prompt diagnosis and treatment of ischemic strokes are critical for improving patients' outcomes, the current mainstay management options for ischemic strokes include thrombolytic and/or endovascular treatment.[9]

On the other hand, the diagnosis of acute ischemic stroke is challenging due to the wide range of possible differential diagnosis, in which thrombolysis is contraindicated in some of them, and the time-sensitive nature of the condition.[10] Owing to the major technological advances in neuroimaging, the diagnostic accuracy and prognosis of stroke have improved dramatically in the past few decades. However, imaging studies are limited by low sensitive to infarction in the first 2 hrs and unavailability in limited-resource areas.[11] Thus, innovative diagnostic tools and biomarkers have been proposed including acute phase reactants, IL-6, and microRNAs (miRNA).[12]

miRNAs are a group of non-protein encoding, small, RNAs that act as inhibitors of translation or degradation of messenger RNAs (mRNAs).[13] Over the past decade, a growing body of evidence showed that many circulating miRNAs are major regulators of different cellular processes and pathological conditions such as neurodegenerative diseases and tumorigenesis.[14,15] Recently, it was reported that miRNAs are key regulators of many pathological processes in ischemic stroke such as excitotoxicity, oxidative injury to neurons, and post-ischemic inflammation.[16] Particularly, miRNA 146a was shown to be significantly involved in downregulation of pro-inflammatory cytokines involved in neurological disorders.[17]

Nevertheless, the role of miRNA 146a in the diagnosis and prognosis of ischemic stroke is still unclear. Therefore, we conducted the present prospective study to assess the level of miRNA 146a in patients with ischemic stroke and its correlation with patients' characteristics.

Materials and methods

We followed the recommendations of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement during the preparation of the present study.[18] The present study runs in concordance with the Declaration of Helsinki principles and the guidelines of the International Committee of Medical Journal. The study's protocol gained the approval of the local ethics and research committee of Al-Zahraa university hospital.

Study design and patients selection

We conducted an observation, prospective, study at Internal Medicine Department and intensive care unit of Al-Zhraa University Hospital through the period from August 2018 to February 2019. Adult patients (≥18 years old) who presented to the Emergency Department within 24 hrs after the onset of the symptoms of acute ischemic stroke were included. Only patients with thrombotic stroke due to the affection of anterior circulation were included. The eligible patients were then grouped according to the presence of coma. Patients with intracerebral hemorrhage, hemorrhagic infarction, or extensive infarction who received anticoagulants or thrombolytic therapy, and patients with other neurological disorders, were excluded. Also, we excluded only patients with extensive infarction who received anticoagulants and/or thrombolysis.[19] In addition, age- and sex-matched healthy volunteers were included as control group. A non-probability convenient sampling method was utilized to enroll eligible participants. Written informed consents were obtained from eligible participants or their first-degree relatives prior to study enrollment.

Data collection and mirna 146a assessment

We collected the following data from eligible participants: demographic characteristics, complete medical history, full medical examination, Glasgow Coma Scale (GCS),[20] lipid profile, complete blood count (CBC), serum creatinine level, and miRNA 146a level. Blood specimens (5 mL) were collected immediately after admission. The CBC profile was done using Sysmex KX21N (Kobe, Japan); while the biochemical analysis using Cobas C311 (Mannheim, Germany) was done. The expression of miRNA 146a was measured using quantitative real-time PCR.

Quantitative PCR for serum gene expression of miR-146a

Total RNA was extracted from serum samples using miRNeasy Mini kit (Qiagen, Hilden, Germany) according to instructions of manufacturer. The isolated miRNA was reverse transcribed into cDNA using miScript II Reverse Transcription Kit (Qiagen). Quantitativereal-time PCR amplification and analysis were performed using Rotor-Gene Q thermal cycler using 20 µL reaction mixture consisting of 10 µL SYBR Green PCR Master Mix (Qiagen), 1 µL forward primer (nM), 1 µL reverse primer (nM), 3 µL cDNA and 5 µL RNase free water performing the following thermal cycling conditions: 95°C for 10 mins, followed by 40 cycles at 95°C

for 15 s and 60°C for 1 min. All values of miRNA 146a were normalized to the snoRD68 gene (housekeeping gene). The primer sequences for miRNA 146a was as follow: sense, 5′-CAG-CTG-CAT-TGG-ATTTAC-CA-3′ and anti-sense, 5′-GCC-TGA-GAC-TCT-GCC-TTC-TG-3′. Relative expression of the miRNA 146a was calculated using by $2^{-\Delta\Delta CT}$ method.

Study's outcomes

The primary outcome in the present study was the difference in miRNA 146a expression between patients with ischemic stroke and control group. The secondary outcomes included: 1) the difference in miRNA 146a expression between patients with and without coma and 2) the correlation between miRNA 146a and clinical characteristics of the patients.

Statistical analysis

Data entry, processing, and statistical analysis were carried out using Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 22 for Microsoft Windows. Quantitative data were described in terms of mean±SD, while qualitative data were expressed as frequencies (number of cases) and relative frequencies (percentages). Comparisons of quantitative variables between stroke patients and control were done using unpaired Student *t*-test for parametric data or Mann–Whitney Rank Sum test for non-parametric data. While the comparisons between the three study's groups were done using ANOVA for parametric data and Kruskal–Wallis H test for non-parametric data. Chi-square test was performed for categorical variables. A $P<0.05$ was considered statistically significant.

Results

The present study included 44 patients with ischemic stroke and 22 matched controls. The mean ages of the included patients and control group were 63.7±6.4 and 64.4±6.2, respectively ($P=0.05$). Patients with ischemic stroke had significantly higher cholesterol level ($P<0.001$), serum urea level ($P<0.001$), and serum creatinine level ($P<0.001$). On the other hand, patients group had significantly lower serum hemoglobin and hematocrit values ($P<0.05$). Table 1 shows the baseline demographic and laboratory characteristics of the included participants.

Regarding the primary outcome of the present study, the median expression of miRNA 146a in patients with ischemic stroke was −1.98 (−27.1–3.9), compared to 1.75 (−2.25–5.27) in control group ($P<0.001$). However, the subgroup analysis showed that the expression of miRNA 146a was significantly downregulated in comatose patients compared to control group ($P<0.001$), while there was no statistically significant difference in the levels of miRNA 146a between non-comatose patients and healthy individuals ($P=0.53$; Figure 1). The expression of miRNA 146a correlated negatively with GCS in comatose patients ($r=-0.352$, $P=0.022$; Figure 2). Similarly, the expression of miRNA 146a correlated positively with hemoglobin and hematocrit values, and negatively with platelet count ($P<0.05$; Table 2).

Table 1 The baseline demographic and laboratory characteristics of the included participants

	Patients (N=44)					Control (N=22)					*P*-value
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum	
Age, years	63.69	6.42	62.50	54.00	81.00	64.38	6.23	65.00	56.00	81.00	0.501
Cholesterol, mg/dL	208.71	71.33	202.00	104.00	320.00	156.31	15.10	157.00	132.00	179.00	0.001
Triglyceride, mg/dL	129.43	35.20	140.00	66.00	183.00	127.46	22.74	133.00	54.00	145.00	0.250
Hemoglobin, g/dL	12.68	1.21	12.90	11.00	16.00	13.32	0.69	13.60	11.90	14.10	0.001
Hematocrit, %	35.01	12.71	39.50	5.10	45.00	39.42	2.72	39.90	32.10	43.00	0.551
Platelets, x10^9/L	255.64	100.49	220.00	133.00	431.00	297.51	49.61	301.00	196.00	372.00	0.021
WBCs, 10X3 cell/mm^3	6.86	2.51	5.70	4.00	12.00	6.70	1.70	6.50	4.50	10.00	0.733
Urea, mg/dL	52.57	51.24	32.00	22.00	221.00	26.46	4.89	27.00	18.00	33.00	<0.001
Serum creatinine, mg/dL	1.42	0.61	1.16	0.90	2.80	0.86	0.12	0.90	0.70	1.00	<0.001

Abbreviation: WBCs, white blood cells.

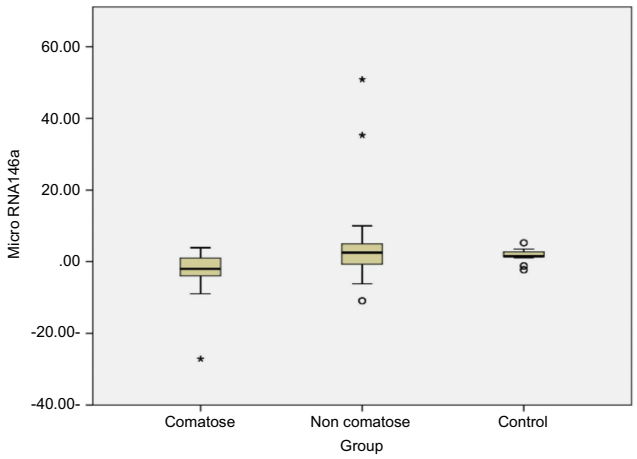

Figure 1 The distribution of miRNA 146a levels across studied groups.
Note: The * symbols represent outliers.
Abbreviation: miRNA, microRNA.

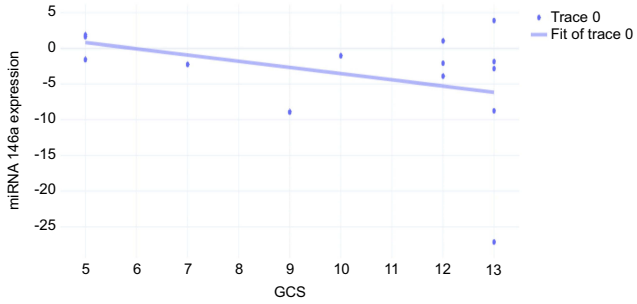

Figure 2 The correlation between miRNA 146a expression and GCS.
Abbreviations: GCS, Glasgow Coma Scale; miRNA, microRNA.

Discussion

Over the last few years, a growing body of evidence showed that miRNAs play key regulatory roles in many physiological processes that can limit the onset and progression of ischemic strokes. However, there is no consensus regarding their diagnostic and therapeutic values.[16] In the present study, we found that the expression of miRNA 146a was significantly downregulated in comatose patients with severe ischemic stroke, compared to healthy volunteers and ischemic patients without coma. The expression of miRNA 146a was negatively correlated with GCS as well.

Recently, the concept of NeurimmiR has emerged in line with the dramatic increase in our understanding of the impact of the biological functions of several miRNAs on the pathogenic processes of neurological disorders. Based on the findings of Iyer et al,[17] the expression of miRNA 146a exhibited anti-inflammatory effect during the astrocyte-mediated inflammation by suppressing pro-inflammatory cytokines, as IL-1β and IL-6. Moreover, the miRNA 146a was found to downregulate cyclooxygenase-2 (COX-2) mRNA in the glial cell.[21] Thus, we hypothesized that the expression of miRNA 146a is significantly reduced in the setting of ischemic stroke which can be used as a promising biomarker. The present study showed that the expression of miRNA 146a was correlated with the severity of the ischemic stroke and it was significantly downregulated in patients with coma, compared to healthy controls. In agreement with our findings, Li et al[22] reported that the level of miRNA 146a was significantly lower in ischemic stroke patients in the acute phase, compared to patients in the subacute phase and healthy controls. Such findings were further supported by genetic studies which demonstrated a significant association between miRNA 146a polymorphisms and the risk of ischemic stroke[23,24]; however, this association is still debatable.[25]

Despite the exact function of miRNA 146a downregulation during the acute phase of ischemic stroke is unclear, the previous work by Zhou et al[26] demonstrated that the miRNA 146a downregulation has a protective effect on neural cells and it targeted pro-apoptotic genes to reduce apoptosis. Notably, a more recent report found that miRNA 146a/b significantly downregulated tumor necrosis factor receptor-associated factor 6 and IL-1 receptor-associated kinase 1 during the recovery phase of ischemic stroke, which in return favor the proliferation and migration of endothelial progenitor cells.[27] miRNA 146a was found to enhance stroke-induced oligodendrogenesis as well.[28] These findings, along with the finding of Iyer et al[17] about the inhibitors of miRNA 146a on pro-inflammatory cytokines and COX-2 in

Table 2 The correlation between miRNA 146a and the clinical/laboratory parameters of the included patients

Patients (N=44)	microRNA146a	
	Correlation coefficient	*P*-value
Age	0.072	0.651
Cholesterol	−0.015	0.923
Triglyceride	0.189	0.230
Fasting blood glucose	−0.004	0.978
Post-prandial glucose	0.086	0.588
Hemoglobin	0.656	<0.001
Hematocrit	0.392	0.010
Platelets	−0.317	0.041
WBCs	0.252	0.107
Urea	−0.249	0.112
Serum creatinine	0.053	0.739
Glasgow Coma Scale	−0.352	0.022

Abbreviation: WBCs, white blood cells.

various brain cells, highlight the potential role of miRNA 146a as promising therapeutic target during the acute and recovery phases of ischemic stroke.[29] Therefore, future research is still needed to evaluate the potential therapeutic implications of miRNA 146a in ischemic stroke.

We acknowledge that the present study has a number of limitations. The study included a small number of patients and the power analysis was not planned prior to study enrollment. Moreover, the study was a single-center experience. Such factors may affect the generalizability of our findings. Moreover, patient-centered and long-term outcomes were not utilized in the present study.

Conclusion

The expression of miRNA 146a is significantly downregulated in patients with severe ischemic stroke. The expression of miRNA 146a showed a declining trend in patients with more severe status. Nevertheless, further studies are needed to assess its diagnostic utility and therapeutic potentials.

Disclosure

The authors report no conflicts of interest in this work.

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