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ORIGINAL ARTICLE

Peripheral Blood miR-451 May Serve as a Biomarker of Ischemic Stroke

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SUMMARY

Background: **The current study aims to investigate the correlation between the expression level of miR-451 in peripheral blood and the risk of ischemic stroke, and its feasibility as a biomarker of ischemic stroke.**
Methods: **Three hundred and two cases of ischemic stroke diagnosed in Qianfoshan Hospital Affiliated to Shandong University from April 2017 to Dec 2017 and 302 cases matched for age and gender were selected from routine health examination subjects. Real-time quantitative PCR was used to detect the expression of microRNA in peripheral blood. Receiver operating curve (ROC) was used to analyze whether miR-451 could be used as a basis for judging ischemic stroke.**
Results: **The expression level of miR-451 in peripheral blood of patients with ischemic stroke was higher than that of the control group ($p < 0.05$). ROC analysis demonstrated that peripheral blood miR-451 could screen ischemic stroke patients from healthy controls, with the AUC of 0.912. In addition, the expression level of miR-451 was negatively correlated with the number of platelets and platelet hematocrit ($p < 0.05$).**
Conclusions: **There is a significant correlation between the expression level of miR-451 in peripheral blood and the occurrence of ischemic stroke. miR-451 is expected to be a biomarker of ischemic stroke.**
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KEY WORDS

miR-451, peripheral blood, ischemic stroke, biomarker

INTRODUCTION

Stroke is one of the most common cardiovascular and cerebrovascular diseases [1]. Stroke has surpassed heart disease and become the first cause of death and disability among adults in China [2]. Ischemic stroke is a complex disease caused by both environmental and genetic factors, accounting for 60% to 70% of the total number of stroke patients [3].

In recent years, with the development and application of epigenetics, the role of non-coding small RNA (microRNA, miRNA) in the occurrence and development of diseases has attracted widespread attention in academia [4]. MicroRNAs are small non-coding RNAs with a length of about 20 - 25 nucleotides [5,6]. They can identify 3'untranslated regions (3'UTR) of target genes by

base complementary pairing, which can guide silencing complexes to degrade target genes or block the translation of target genes [7]. It has been found that human microRNAs can regulate the expression of about 1/3 of human genes, so they can participate in regulating various physiological and pathological states [8]. Current studies have clearly shown that microRNAs play an important role in many pathological processes related to the occurrence and development of ischemic stroke, such as atherosclerosis, cerebral edema, and cerebral ischemia-reperfusion[4,9,10].

Because of the important biological role of microRNAs, this study aims to explore whether circulating microRNAs can be used as a biomarker of ischemic stroke by detecting the expression level of microRNAs in the blood circulation of patients with ischemic stroke. Previously, we used a microarray to detect the expression of microRNAs in peripheral blood of 24 patients with ischemic stroke and 8 normal controls matched for age and gender, and screened out a series of differentially expressed microRNAs (unpublished data), including miR-451, which was the highest overexpressed miRNA in the peripheral blood of patients with ischemic stroke. We intend to further detect the expression of miR-451 in a large sample of case-control population, to clarify the relationship between miR-451 and the occurrence of ischemic stroke and the feasibility of miR-451 as a biomarker of ischemic stroke.

MATERIALS AND METHODS

General information

A total of 302 new cases of ischemic stroke diagnosed in Qianfoshan Hospital Affiliated to Shandong University from April 2017 to December 2017 and 302 people who had routine health examinations in Qianfoshan Hospital Affiliated to Shandong University were selected as case group and control group. There was no significant difference in gender composition and age distribution between the two groups ($p > 0.05$). The information of population biological samples and questionnaires was complete. The diagnostic criteria of ischemic stroke, according to WHO International Classification of Diseases (9th edition), are having more than one neurological symptoms or signs which lasts at least 24 hours, and positive changes are found by head CT (or MRI) and other auxiliary examinations. Patients with history of stroke, coronary heart disease, peripheral artery occlusion, and cancer were excluded. All selected controls were checked by medical history, physical examination, and general biochemical indicators, excluding people with previous history of stroke, cardiac surgery, cancer, and neurological diseases. Questionnaires were used to investigate the general situation of cases and controls. The contents of the questionnaires included general situation, past medical history, family history, exposure to environmental factors and so on. Blood samples were collected within 24 hours after admission. The study was approved by the Ethics Committee of Qianfoshan Hospital Affiliated to Shandong University before its launch. All the subjects signed an informed consent.

Blood sample collection

From all subjects, 5 mL of venous blood were collected the next morning following an overnight 12-hour fast. The whole blood was stored in a freezer at -80^{o}C for reserve.

Real-time quantitative PCR

Total RNA was extracted from 200 μL whole blood by mirVana PARIS microRNA extraction kit (AM1556, Life Technologies Inc.). The TaqMan microRNA reverse transcription kit (Life Technologies Inc) was used to retrieve the microRNA. The components of the reverse transcription system were 100 mmol/L dNTPs (0.05 μL), reverse transcriptase (0.33 μL), 10 x reverse transcription buffer (0.5 μL), reverse transcriptase inhibitor (0.063 μL), 5 x reverse transcriptase primer (1 μL), RNA (1.67 μL), RNA-free water (1.387 μL), and the reaction system was 5 μL. The reverse transcription products were diluted 5 times by RNAase-free water and amplified by real-time quantitative PCR using TaqMan miRNA detection kit (Life Technologies, USA). The components of the PCR system were TaqMan universal PCR solution (5 μL), 20 x TaqMan microRNA probe (0.5 μL), reverse transcription products (4.5 μL), and the reaction system was 10 μL. The relative expression of microRNAs was calculated by -ΔCt method. $\Delta Ct = Ct_{miR-451} - Ct_{U6}$, in which U6 was an internal reference gene.

Statistical analysis

SPSS 11.0 software package was used for statistical analysis. The chi-square test was used to compare the counting data. Multivariate logistic regression was used to analyze the expression level of microRNAs and ischemic stroke. The area under the curve (AUC) of miR-451 as a molecular marker of ischemic stroke was analyzed by ROC curve. Spearman's rank correlation analysis was used to analyze the correlation between miR-451 and clinical indicators. All statistical analyses were bilateral tests, with $p < 0.05$ as the statistical significance.

RESULTS

Basic situation

The general information of the subjects was shown in Table 1. There were no significant differences in age, gender, smoking, drinking, total cholesterol, triglycerides, LDL cholesterol, platelet count, average platelet distribution width, and platelet hematocrit between the case group and the control group ($p > 0.05$). There were 172 cases (57.0%) with hypertension and 54 cases (17.9%) with diabetes mellitus in the case group, which

Table 1. Comparison of basic situation between two groups.

Variables	Case group (302)	Control group (302)	p-value
Age (years)	57.50 ± 10.40	55.87 ± 10.42	0.054
Gender (male/female)	179/123	179/123	1.000
Smoking (%)			0.279
No	235 (77.8)	254 (84.1)	
Yes	67 (22.2)	52 (17.2)	
Drinking history			0.054
No	277 (91.7)	250 (82.8)	
Yes	25 (8.3)	52 (17.2)	
History of hypertension			< 0.001
No	130 (43.0)	241 (79.8)	
Yes	172 (57.0)	61 (20.2)	
History of diabetes			0.009
Yes	248 (82.1)	285 (94.4)	
No	54 (17.9)	17 (5.6)	
FG (mmol/L)	5.55 (4.98, 6.89)	5.28 (4.93, 5.86)	< 0.001
TC (mmol/L)	5.02 (4.22, 5.88)	5.05 (4.32, 5.62)	0.467
TG (mmol/L)	1.33 (0.96, 1.84)	1.37 (0.94, 2.28)	0.163
HDL-c (mmol/L)	1.05 ± 0.27	1.26 ± 0.31	< 0.001
LDL-c (mmol/L)	2.89 (2.44, 3.73)	3.00 (2.58, 3.46)	0.540
PLT (10^9/L)	219 (175.75, 266)	228 (197, 262)	0.071
PDW (%)	16.00 (12.8, 16.6)	16.00 (15.70, 16.40)	0.504
MPV	9.20 (7.80, 10.20)	8.40 (7.55, 9.30)	< 0.001
PCT	0.19 (0.16, 0.24)	0.19 (0.16, 0.22)	0.306

FG - fasting blood, TC - total cholesterol, TG - total triglycerides, HDL-c - high density lipoprotein cholesterol, LDL-c - low density lipoprotein cholesterol, PLT - platelet count, PDW - average platelet distribution width, MPV - mean platelet volume, PCT - platelet crit.

were significantly higher than those in the control group ($p < 0.05$). The fasting blood glucose and platelet volume in the case group were 5.55 (4.98, 6.89) mmol/L and 9.20 (7.80, 10.20) respectively, which were significantly higher than those in the control group ($p < 0.05$), while the HDL cholesterol concentration in the case group was 1.05 ± 0.27 mmol/L, which was significantly lower than that in the control group 1.26 ± 0.31 mmol/L, with statistical significance ($p < 0.01$).

Expression of miR-451 in patient and control groups

The relative expression levels of miR-451 in the case group and the control group were 5.04 ± 6.62 and 1 ± 1.39, respectively (Figure 1). The expression levels of miR-451 in the case group were significantly higher than those in the control group ($p < 0.01$).

Feasibility of miR-451 as molecular biomarkers of ischemic stroke

In this study, ROC curve was used to analyze the feasibility of miR-451 as a molecular biomarker of ischemic stroke. ROC analysis demonstrated that peripheral blood miR-451 could screen ischemic stroke patients from healthy controls, with an AUC of 0.912 (95% CI: 0.819 - 1.000), while sensitivity and 1-specificity were 89.8% and 96.8%, respectively.

Analysis of the correlation between miR-451 and the clinical indicators of the subjects

This study continued to analyze the correlation between miR-451 and variables in ischemic stroke population, as shown in Figure 3. MiR-451 was negatively correlated with platelet count ($r = -0.234$, $p < 0.01$) and thrombocytocrit ($r = -0.234$, $p < 0.01$), but not with other clinical indicators.

DISCUSSION

Early diagnosis and treatment are of great significance in reducing the mortality rate and improving the prognosis of stroke [11]. At present, the diagnosis of ische-

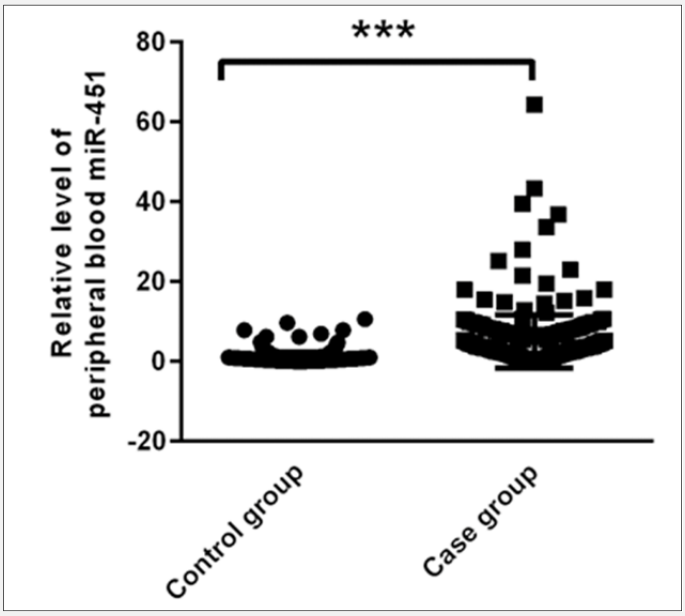

Figure 1. Real time PCR analysis demonstrated that the expression levels of miR-451 in the case group were significantly higher than those in the control group.

** **- p < 0.01 vs. control group.**

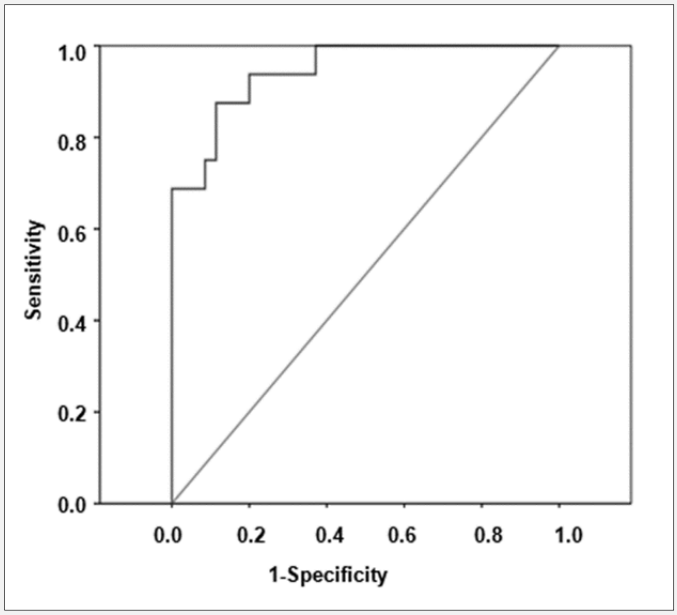

Figure 2. ROC analysis was carried out to evaluate the feasibility of miR-451 as a molecular biomarker of ischemic stroke.

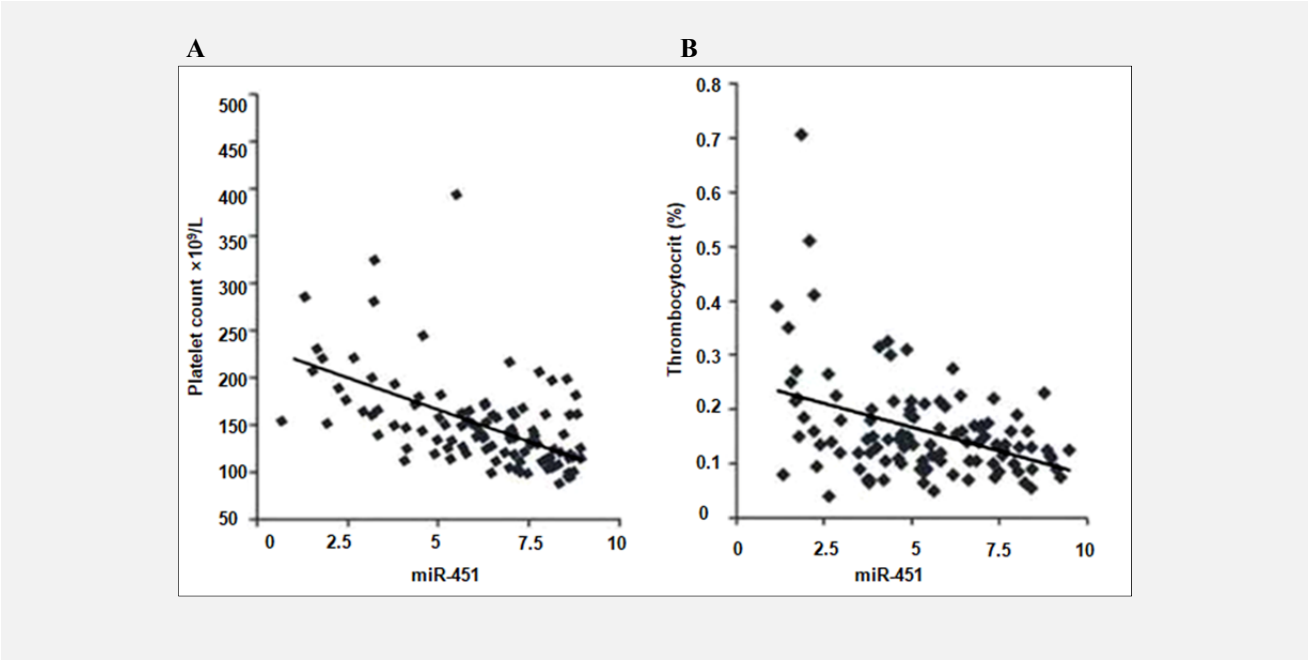

Figure 3. Spearman's correlation assay was performed to explore the correlation between miR-451 and the clinical indicators of the subjects. miR-451 was negatively correlated with platelet count (A) and thrombocytocrit (B).

mic stroke mainly relies on imaging examination and clinical manifestations. There is still a lack of effective and reliable blood biomarkers, which increases the difficulty of early screening for ischemic stroke [12]. Therefore, it is of great clinical value to actively search for effective blood biomarkers [12,13]. In this study, we found that the expression level of miR-451 in peripheral blood of ischemic stroke patients was significantly higher than that of the control group. There was a significant correlation between the increased expression of miR-451 and the increased risk of ischemic stroke. Therefore, we believe that peripheral blood miR-451 may become a biomarker of ischemic stroke and has a certain clinical value.

MicroRNAs have a wide range of biological functions and can be stable in various human body fluids, including serum, plasma, urine, etc., [14,15]. Many studies have proved that microRNAs in human body fluids can be specific in some disease states, so they have potential as biomarkers [5,16,17]. However, because the expression level of microRNAs in plasma or serum is usually low and the source is unknown, many studies have gradually shifted their focus to the study of microRNAs in peripheral blood [8]. In this study, we selected miR-451 for large sample population validation on the basis of previous microarray results. We found that the expression level of miR-451 in peripheral blood of patients with ischemic stroke was significantly higher than that of control group. In evaluating peripheral blood miR-451 as a biomarker of disease, this study found that the area under the curve of ROC was higher, with statistical differences ($p < 0.05$).

The role of miR-451 in cardiovascular diseases and related pathological processes has been documented [18, 19]. These studies have shown that miR-451 may play a role in the pathogenesis of cardiovascular diseases [18, 19]. The results of this study showed that the expression of miR-451 in peripheral blood was negatively correlated with the number of platelets in blood and platelet hematocrit. The main pathological basis of cerebral arterial thrombosis is cerebral atherosclerosis, intimal injury, increased blood viscosity, increased platelet aggregation, resulting in decreased blood fluidity, in which changes in platelet quality and endothelial damage play an important role [20]. In the acute stage of stroke, platelet destruction or consumption increases, platelet count and platelet hematocrit decrease, stimulating bone marrow megakaryocyte proliferation and platelet production [21]. When new platelets increase, platelet average volume and platelet average distribution width increase [22]. The basic information of the population in this study also indicated that the average platelet volume of stroke patients was significantly higher than that of the control group, while the other three indicators showed no significant difference [23]. These platelet parameters are helpful to understand the changes of patients with stroke and have important significance in predicting the evolution of disease [24]. The correlation between miR-451 and platelet parameters indicates that miR-451 may be related to platelet production or plate-

let activation. The mechanism needs further study. However, some limitations still exist in the present study. For instance, only one microRNA was included for verification. Because of the high correlation between the expression levels of microRNAs in peripheral blood, it is generally believed that multiple microRNAs have better sensitivity and specificity as biomarkers of disease. Therefore, the next step is to incorporate multiple microRNAs for analysis.

CONCLUSION

In conclusion, the results of this study showed that the expression level of miR-451 in peripheral blood was significantly correlated with the risk of ischemic stroke, and was expected to be a biomarker of ischemic stroke. The next step is to incorporate multiple microRNAs for detection and analysis and to explore the biological functions of miR-451.

Declaration of Interest:
We declare no conflicts of interest.

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