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Plasma miR-124-3p and miR-16 concentrations as prognostic markers in acute stroke

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ABSTRACT

Objectives: This study aimed to investigate plasma concentrations of miR-124-3p and miR-16 as prognostic markers in emergency department patients with acute stroke.

Design and methods: Plasma concentrations of miR-124-3p and miR-16 of 84 stroke patients (presenting to the emergency department within 24 h from onset of symptoms) were determined by RT-qPCR. The primary outcome measure was 3-month mortality and the secondary outcome measure was post-stroke modified Rankin Score (mRS).

Results: Twelve patients (14.3%) died within 3 months of hospital admission and forty-one (48.8%) patients as achieved a 3-month mRS > 2. Median plasma miR-124-3p concentrations were elevated in patients who died compared to patients who survived ($p = 0.0052$), and its levels were found to be higher in patients with a 3-month mRS > 2 compared with patients with mRS ≤ 2 ($p = 0.0312$). Higher plasma miR-16 concentrations were observed in patients who survived than in patients who died ($p = 0.0394$), while its concentrations were lower in patients achieving mRS > 2 than in patients with mRS ≤ 2 ($p = 0.0124$). For a subgroup of cases presenting to the emergency department within 6 h from time of symptom onset (n = 36), plasma miR-124-3p concentrations predicted 3-month mortality with an area under the ROC curve of 0.87 (95%CI: 0.72–0.96).

Conclusions: Plasma miR-124-3p and miR-16 are molecular markers which could be useful for the early prediction of mortality and mRS.

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1. Introduction

Stroke is a leading cause of death and disability in the world [1]. The diagnosis is made primarily through clinical examination and neuroimaging [2]. Early prediction and risk-stratification in patients with acute stroke are important for stroke management and for improving patient outcomes and quality of life [3].

microRNAs (miRNAs) are small non-coding RNAs of approximately 22 nucleotides (nt) in length. They are involved in the gene regulation of different physiological and pathological functions such as development, differentiation, apoptosis and metabolism [4]. High expressions of miR-124-3p in the brain have been reported [5]. There is an association between high plasma levels of miR-124-3p and severity of stroke [6]. Thus, this brain-enriched miRNA, miR-124-3p might be a useful marker for prognostic stratification in stroke. After stroke onset, neurons and non-neuronal cells undergo apoptosis [7], which is controlled in part by miR-16 [8]. Thus, there are changes in miR-16 in response to stroke progression and these changes may be useful prognostic markers. In this study, brain-specific miRNA, miR-124-3p and apoptotic-related miRNA, miR-16 were determined in the plasma of stroke patients presenting within 24 h of symptom onset in order to assess whether these could predict 3-month mortality using the modified Rankin Score (mRS) as a marker of functional outcome.

2. Materials and methods

2.1. Subjects and data collections

Approval was obtained from Institutional Review Board of the Chinese University of Hong Kong (reference no.: CRE-2011.015) to conduct this prospective study investigating circulating miRNAs as predictors of mortality and functional outcome.

Eligible patients aged 18 years and above, presenting to the Emergency Department of the Prince of Wales Hospital, Hong Kong, with stroke-like symptoms within 24 h of onset, were recruited. In all

Abbreviations: miRNA, microRNA; CT, computed tomography; MRI, magnetic resonance imaging; DWI, diffusion weighted imaging; NIHSS, National Institutes of Health Stroke Scale; GCS, Glasgow Coma Score.

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cases, informed, written consent was obtained either from patients or relatives.

2.2. Definitions and diagnostic imaging

Stroke was defined as focal or global neurological deficit lasting for more than 24 h in a different neuroanatomical location from that of any previous stroke, or worsening of an existing deficit that lasted for more than 1 week, or accompanied by a new lesion on neuroimaging [9].

Patients underwent standard clinical investigations, including CT without contrast enhancement within 24 h of symptom onset, and magnetic resonance imaging (MRI) with diffusion weighted imaging (DWI) scans as clinically indicated. CT scans and MRI scans were performed on a 64 slice multidetector CT (Lightspeed VCT, GE Healthcare) and a 3T system (Achieva; Philips Healthcare) respectively. Details of the diagnostic imaging were described previously [6].

2.3. Preparation of plasma, RNA extraction, reserve transcription (RT), standard and quantitative real-time polymerase chain reaction (qPCR) for miRNAs

A 10 ml venous blood sample was taken by standard venipuncture and collected into EDTA-tubes, and centrifuged at 1500*g* for 20 min at 4 °C. Plasma preparation and RNA extraction have been previously described [6].

For miRNA analysis, RT reaction was performed by miRNA-specific stem-loop primers using TaqMan miRNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's protocols. RT-qPCR was performed using the TaqMan miRNA RT-qPCR Assay (Applied Biosystems) in the Applied Biosystems 7500 System (Applied Biosystems) as previously described [6]. PCR reactions were performed in duplicate. Concentrations of miR-124-3p and miR-16 were expressed as copies per ml plasma.

2.4. Statistical analysis

Descriptive statistics and data comparison tests were determined by chi-squared, Fisher's exact, Mann–Whitney, and Kruskal–Wallis tests as appropriate, and correlations were determined by Spearman Rank test. Receiver operator characteristic (ROC) curve analysis and forward stepwise multiple logistic regression analysis were also performed. All the tests were carried out using MedCalc12.3 software version 12.3 (MedCalc Software bvba).

2.5. Outcome

The primary outcome was the 3-month all-cause mortality. The secondary outcome was 3-month post-stroke modified Rankin Score (mRS) [10].

3. Results

3.1. Baseline characteristics

Table 1 shows the characteristics of the 84 patients with stroke who were enrolled in the study (median age 72 years; 51.2% male). Twelve patients died with 3 months of hospital admission and a 3-month post-stroke modified Rankin Score (mRS) > 2 was observed in 41 patients (48.8%).

3.2. Plasma miR-124-3p and miR-16 in stroke prognosis

Plasma miRNAs concentrations in stroke patients with of 3-month mRS 0–2, 2–5 and 6 are shown in Fig. 1. Median plasma concentration of miR-124-3p in patients who scored 3-month mRS 0–2, 2–5 and 6 are 1.9, 2.5 and 5.3×10^5 copies/ml respectively. Median plasma concentration of miR-16 in patients with mRS 0–2, 2–5 and 6 are 1.8, 1.4, and 1.1×10^9 copies/ml respectively. In the present study, it is found that median plasma concentrations of miR-124-3p were 2.5 fold higher in patients who died than patients who survived (5.3×10^5 copies/ml vs 2.1×10^5 copies/ml; $p = 0.0052$), whereas median plasma miR-16 concentrations were 1.5 fold higher in patients who survived compared to those who died (1.6×10^9 copies/ml vs 1.1×10^9 copies/ml; $p = 0.0394$) (Table 2).

Table 1
Characteristics of 84 patients presenting to hospital with stroke.

Factor	Value	
Age — years	72	[18] 43–92
Male sex, no. of patient (%)	43	(51.2%)
Stroke risk factors, no. of patients (%)		
Hypertension	62	(73.4%)
Diabetes mellitus	29	(34.5%)
Ischemic heart disease	9	(10.7%)
Atrial fibrillation	15	(17.9%)
Hyperlipidemia	17	(20.2%)
Active smoking	13	(15.5%)
Ex-smoking	17	(20.2%)
Previous stroke	26	(31.0%)
Pulse rate (per min)	79	[23] 47–119
Blood pressure (mm Hg)		
Systolic	168	[46] 102–283
Diastolic	84	[30] 50–144
Blood glucose (mmol/L)	7.3	[3.6] 4.4–18
Time from symptom onset to blood sample (h)	7.8	[10.9] 1.0–24
Lesion volume on CT (cm^3)	21	[73] 1.5–205
Lesion volume on MRI (cm^3)	1.5	[2.7] 0.1–59
ROSIER Score		
—2–0	5	(6.0%)
1–5	79	(94.0%)
NIHSS		
0–1	6	(7.1%)
2–8	47	(56.0%)
9–40	27	(32.1%)
GCS		
3–8	6	(7.1%)
9–12	1	(1.2%)
13–15	77	(91.7%)
Stroke types, no. of patients (%)		
Hemorrhagic stroke	15	(17.9%)
Ischemic stroke	69	(82.1%)
TOAST, no. of patients (%)		
Cardioembolic ischemic stroke	18	(21.4%)
Large artery ischemic stroke	7	(8.3%)
Small artery ischemic stroke	26	(31.0%)
Undetermined ischemic stroke	18	(21.4%)
Hemorrhage	15	(17.9%)

All continuous data are expressed as medians [interquartile range] range.
Categorical variables are given as values (percentages).
TOAST, Trial of Org 10172 in Acute Stroke Treatment [24].

The ROC analysis of plasma miR-124-3p of 3-month mortality in patients presenting within 6 h of symptom onset is shown in Fig. 2. The area under the curve (AUC) was 0.87 (95%CI: 0.72–0.96). The sensitivity and specificity of plasma miR-124-3p at $>3.5 \times 10^5$ copies/ml plasma were 88.9% and 77.8% respectively. The ROC analysis of prediction of plasma miRNA concentrations in 3-month mortality in stroke patients presenting at different time of symptoms onset is shown in Table 4. The AUC for plasma miR-124-3p and miR-16 were 0.75 (95%CI: 0.65–0.84) and 0.69 (95%CI: 0.58–0.79) respectively. The optimal cut off plasma miR-124-3p at $>3.5 \times 10^5$ copies/ml plasma generates a sensitivity and specificity of 75% and 73.2%, respectively. Use of a plasma miR-16 cutoff of $>1.3 \times 10^9$ copies/ml, yields a sensitivity of 75% and specificity of 68.6%. At these cutoffs, the odds ratio for miR-124-3p was 8.21 (95%CI: 2.01–33.57) and for miR-16 was 4.78 (95%CI: 1.19–19.23). For patients presenting more than 6 h but within 24 h of symptom onset, the AUC

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