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Research paper

Clinical predictor and circulating microRNA profile expression in patients with early onset post-stroke depression

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ABSTRACT

Objective: We aim to explore the clinical factors and blood biomarker for predicting the early-onset post-stroke depression (PSD).
Methods: 251 acute ischemic stroke patients were divided into PSD group and non-PSD group by Hamilton depression scale in 2 weeks after stroke. The clinical data, the severity, etiology and location of stroke were recorded. The analysis of inflammatory mediator, glycose and lipid metabolism was performed on the day of admission. The association between clinical factors and early onset PSD was studied by logistic regression analysis. In addition, the differentially expressed miRNAs in plasma between the two groups were screened by gene chip and the bio-information was further investigated by GO and KEEG analysis.
Results: Among 251 patients, 45 (17.93%) were diagnosed as early onset PSD. NIHSS score (>3) and carotid stenosis were independent relative factors with early-onset PSD (OR 3.479 and 2.617, $p=0.000$ and 0.009, respectively). Moreover, lower LDL trended toward association with early onset PSD in minor stroke subgroup ($p=0.084$). MiRNA profile demonstrated 25 differential expressed circulating miRNAs with FC ≥ 2 and $P \leq 0.05$ between the two groups. The target genes of these miRNAs were enriched in pathways of cancer and MAPK signaling.
Limitations: The sample of the study was small. The results should be further confirmed in large cohort patients.
Conclusions: Early onset PSD was more likely in patients with severe neurological deficits and carotid artery stenosis, also note the possible association between lower LDL and depression in minor stroke. Blood miRNAs may be served as a potential biomarker for PSD diagnosis.

1. Introduction

Post-stroke depression (PSD) is one of the most common complications after cerebral infarction. PSD may occur in the acute stage of stroke within 2 weeks following ischemia, namely early-onset PSD, and last several months or years. The prevalence of PSD vary from 25% to 70% according to different study subject, evaluation criteria and time (Ayerbe et al., 2013; Fang et al., 2011; Huff et al., 2001; Mei et al., 2013; Shi et al., 2010; Toso et al., 2004; Yuan et al., 2012). Studies demonstrated that PSD was associated with the worse neurological functional recovery, the higher recurrence rate and mortality rate of stroke (Ayerbe et al., 2013; House et al., 2001; Yuan et al., 2012). Hence, it is important to make timely a diagnosis and treatment for PSD.

However, the pathogenesis of PSD remained unclear. At present, several hypotheses were responsible for PSD, such as neuroanatomy, neurobiochemistry and neurogenetics. The frontal subcortical circuit (FSC) and the limbic-cortical-striatal-pallidal-thalamic circuit (LCSPTC) were thought to be associated with the occurrence of post-stroke depression (Santos et al., 2009; Terroni et al., 2011; Zhang et al., 2012). After stroke, the human body could quickly respond to immune response, release a large number of inflammatory factors, and then affect the hypothalamic pituitary adrenal (HPA) axis function. In the cell, the expression level of 5-hydroxytryptamine (5-HT), dopamine (DA) and norepinephrine decreased (Pascoe et al., 2011; Su et al., 2012), and the depression occurred. In addition, it had been reported that the 5-HT transporter encoding *SLC6A4* gene (Kohen et al., 2008; Pascoe et al., 2011) and brain-derived neurotrophic factor (BDNF) *Val66Met* gene polymorphism (Kim et al., 2007, 2013) were related with

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PSD. However, due to the wide variety of theories, the pathogenesis of post stroke depression remain unclear. We think that PSD may be a result of joint action of multiple mechanisms. The combination of clinical factor and blood biomarker may be the potential approach for predicting early-onset PSD.

In recent years, although there were a lot of related literature reports, but the PSD related relevant factors were not consistent, including gender, age, income level, education level, past stroke history, the location of the lesion, and so on. This might be due to the differences in the study plan or the regional policy. In addition, some biological markers had been reported their association with depression, such as dopamine, 5-HT and inflammatory cytokines ect. However, clinical predictors in the PSD patients were still lacking. In this study, we aimed to find clinical factors and potential biomarkers for predicting early-onset PSD, and to further explorer the pathogenesis of PSD. MicroRNAs are a group of 18–25nts noncoding short RNAs. It could regulate multiple downstream targets and partipate multiple functional pathways. Moreover, microRNAs were stable in plasma and detectable. Hence, microRNAs were the potential blood biomarkers in many diseases including PSD. In this study, we performed the microRNA profile analysis to find the differential expressed microRNAs. The further bioinformation analysis was beneficial for understand the pathogenesis of early-onset PSD and provide potential blood biomarkers. Meanwhile, complex clinical factors were also discussed their association with early-onset PSD in this paper.

2. Materials and methods

2.1. Study subjects

From May 2013 to September 2014, we recruited acute cerebral infarction patients in the Department of neurology, Ruijin hospital, Shanghai Jiaotong University School of Medicine, China. Inclusion criteria: (1) The diagnosis of ischemic stroke was in accordance with the World Health Organisation diagnostic criteria for cerebral infarction, and was confirmed by CT or MRI; (2) Age >18 years old; (3) Course of disease <1 week. Exclusion criteria: (1) Patients with psychosis. (2) Patients with serious illness or with disturbance of consciousness could not be matched for examination. (3) Patients with aphasia or severe cognitive dysfunction could not be matched for examination. (4) Patients with other serious systemic diseases such as infection, heart and lung functional failure or liver and kidney dysfunction. (5) Patients with past depression or taking antidepressants. The study was approved by the ethics committee of Ruijin hospital. All the patients were given informed consent and recruited into the ongoing prospective study.

2.2. Clinical evaluations

On the admission day, all the clinical data were recorded including age, gender, educational level, vascular risk factors (hypertension, diabetes, heart disease, and hyperlipidemia, smoking), and previous history of stroke. All patients were evaluated by National Institute of Health Stroke Seale (NHISS). The routine laboratory analysis were performed including white blood cell count, blood glucose, glycosylated hemoglobin and blood lipid indexes (total cholesterol, triglyceride, high density lipoprotein cholesterol HDL-C, low density lipoprotein cholesterol LDL-C, apolipoprotein A, apolipoprotein B). The evaluations of intra- and extra- cranial artery were conducted within one week using cervical Doppler echography, CTA or MRA. ECG and cardiac echography were processed to evaluate the thrombus from the heart. The location and the size of the cerebral infarction were confirmed by cranial CT or MRI. Definite TOAST type and OCSP type were determined by two neurologist according patient's medical history and auxiliary examination at the time of discharge. The treatment of all patients was based on the guidelines for the prevention of stroke in patients with stroke and transient ischemic attack in China.

Diagnostic criteria of vascular risk factors: Hypertension: Systolic pressure >140 mmHg and/or diastolic pressure >90 mmHg; Diabetes: Fasting blood glucose >7.1 mmol/L and 2 h postprandial blood glucose >11.1 mmol/L, or 2 random blood glucose >11.1 mmol/L; Hyperlipidemia:Triglyceride >1.7 mmol/L, and/or total cholesterol >5.7 mmol/L, and/or low density lipoprotein cholesterol >4.3 mmol/L. TOAST type (1) Large artery atherosclerosis(LAA): Extracranial and intracranial arterial stenosis rate $>50\%$, infarct diameter ≥ 1.50 cm; (2) Cardiac embolism(CE): Caused by a variety of heart diseases which can produce cardiogenic emboli; (3) Small artery occlusion(SAO): Intracranial small artery stenosis or occlusion, infarct diameter <1.50 cm; (4) Stroke of other determined etiology(SOD): Caused by other rare diseases; (5) Stroke of undetermined etiology(SUD): Multiple causes, not clear cause or no relevant inspection. OCSP type (1) Total anterior circulation cerebral infarction (TACI): Cause 3 following symptoms: higher dysfunction, homonymous hemianopsia, motor and sensory defects ($\geq 2/3$ of face, arm, leg); (2) Partial anterior circulation cerebral infarction (PACI): Cause 2 out of 3 features of the symptoms of total anterior circulation cerebral infarction, or higher dysfunction alone, or partial motor or sensory defect; (3) Posterior circulation cerebral infarction(POCI): Vertebrobasilar ischemic stroke, including the brainstem lacunar infarction; (4) Lacunar cerebral infarction(LACI): The clinical manifestations is lacunar syndrome, infarct diameter <1.50 cm.

2.3. Assessment of depression

The diagnosis of post-stroke depression was in accordance with the DSM-IV criteria for depression. The Hamilton depression-17 scale (HAMD-17 scale) was used to evaluate the depressive symptoms in patients with acute ischemic stroke by bridle-wise neurologists two weeks following ischemia. Score ≥ 7 points indicated the presence of depression. According to whether depression, patients were divided into PSD group and non-PSD group. And we choose 3 patients from two groups separately, which were matched by gender, age, vascular risk factor, stroke location, infarction size, NHISS score, to the further study of microRNA profile.

2.4. Blood sample collection and RNA extraction

The 4 ml venous blood of patients was collected into the EDTA containing Tube (Becton, Dickinson and Company, USA) on the day of admission. The blood samples were fractioned by the centrifuge (Beckman, USA) with 3000 rpm, 5 min. The upper plasma were collected into EP tube(Xingyao, China), and putted into $-80\,^{\circ}C$ freezer storage(Thermo, USA). The mirVanaTM RNA Isolation Kit (Applied Biosystem p/n AM1556, USA) were used to extract the total RNA from the plasma. All the procedure was according to the manufacture protocol. In brief, the plasma was added 10 volumes of lysis/binding buffer and 1/10 volume of miRNA homogenate, and mixed well. A volume of acid–phenol:chloroform that was equal to the lysate volume was added into the addition of the miRNA Homogenate Additive. After centrifugation, we removed the aqueous (upper) phase and transfer it to a fresh tube. After adding 1.25 volumes 100% ethanol, we passed the lysate/ethanol mixture through a Filter Cartridge, and washed the filter with 700 μl miRNA Wash Solution 1 and 500 μl Wash Solution 2/3. The filter was eluted with 100 ml of nucleasefree water. Subsequently, we collected the eluate (which contains the RNA) and store it at