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Increase of Serum CXCL16 Level Correlates Well to Microembolic Signals in Acute Stroke Patients with Carotid Artery Stenosis

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ABSTRACT

Background: The majority of strokes are combined with the instability of atherosclerotic plaques. Microembolic signals (MES) have been considered as evidence of plaque destabilization. We found that increased CXCL16 correlated to atherosclerotic ischemic stroke. Thus, we explored whether CXCL16 correlates to MES.
Methods: The study recruited 104 controls and 118 patients with acute ischemic stroke that has an ipsilateral carotid artery stenosis of >50%. The ipsilateral middle cerebral artery of patients was insonated for 60 min using Doppler device within 72 h of their clinical presentation.
Results: We found that CXCL16 was significantly increased in the stroke patients. Furthermore, there was a significant difference in CXCL16 between the MES-positive and MES-negative patients. Using CXCL16 to distinguish the controls and stroke patients, the area under the ROC curve (AUC) was 0.722; the cut-off value was 2.015 ng/ml. The sensitivity and specificity were 70.5% and 67.9%, respectively. Furthermore, if we used CXCL16 to distinguish the MES-positive and MES-negative patients, the AUC was 0.736; the cut-off value was 2.115 ng/ml. The sensitivity and specificity were 88.5% and 56.5%, respectively.
Conclusions: Higher levels CXCL16 may be a biomarker for predicting stroke incidence and might contribute to plaque destabilization.

1. Introduction

Stroke is a personal, familial and social disaster. In China, ischemic stroke accounts for >80% of all strokes [1]. The majority of strokes (85–90%) result from cerebral ischemia. In most cases, extra - and intracranial vessel atherosclerotic changes are considered to be responsible for cerebral ischemia. Carotid artery atherosclerosis is a major risk factor for stroke and subsequent cognitive impairment [2]. A sudden failure of cerebral circulation is usually combined with the instability of atherosclerotic plaques. Plaque destabilization is evidenced clinically by the preoperative occurrence of ischemic symptoms and microembolic signals (MES). MES have been considered a readily measurable marker of increased stroke risk [3,4] and can be detected by transcranial Doppler (TCD) [5].

Accumulating evidence indicates that inflammation is not only a component of atheromatous plaques, which promotes the initiation and evolution of atheroma [6], but, importantly, it also plays a crucial role in the destabilization of vulnerable plaques with consequent thrombosis and distal thromboembolism [7,8], thus converting chronic atherosclerosis into an thrombo-embolic disorder [9]. Recently, a protein called CXCL16 was discovered. It is a member of inflammatory chemokine superfamily with putative roles in directing leukocyte migration and functioning as a scavenger receptor for oxidized low-density lipoprotein (ox-LDL) and chemotactic properties [10,11]. A high expression of CXCL16/SR-PSOX mRNA and protein in the plaques of coronary and carotid atherosclerosis was observed, and CXCL16 has been proposed to act as a pathogenic mediator in atherosclerosis [10–13].

However, the relationship between the circulating levels of soluble CXCL16 and atherosclerotic disorders remains controversial in the clinic. Both decreased and increased CXCL16 levels have been reported in patients with atherosclerotic disorders [14,15]. Our previous research found that an elevation of serum CXCL16 level correlated well with atherosclerotic ischemic stroke [16]. However, there are no reports about the relationship between the levels of CXCL16 and microembolic signals

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(MES) in symptomatic carotid artery stenosis. The role of CXCL16 in the destabilization of atheromatous plaques has never been measured in vivo before. The goal of the present study was to investigate the serum levels of CXCL16 in patients with acute ischemic stroke from atherosclerosis of carotid artery to explore whether the circulating levels of CXCL16 correlates to MES and to further evaluate its potential value as a diagnostic biomarker for the destabilization of atheromatous plaques.

2. Materials and Methods

2.1. Subjects

A total number of 222 subjects were enrolled in the present study, including 104 controls and 118 consecutive patients with acute ischemic stroke (≤3 days) clinically localized to the carotid territory and with an ipsilateral carotid artery stenosis of >50% by the North American Symptomatic Carotid Endarterectomy Trial criteria on carotid duplex ultrasonography [17]. The work was carried out in accordance with The code of ethics of the world medical association for experiments involving humans. This study was cleared by our institution ethics review board for human studies, and the patients signed an informed consent form. The patients were all admitted to the stroke unit in the Affiliated Hospital of Qingdao University. There were 82 men and 36 women between the ages of 40–87 y in the patient group. The control subjects were selected from the Healthcare Clinic and were matched for sex and age (39–85 y).

A detailed history inquiry, neurological examination, risk factor assessment and imaging studies, such as CT or/and MRI of the brain, transcranial Doppler (TCD), carotid duplex ultrasonography and echocardiography were performed in all of the participants. A CT angiography of the brain arteries was also performed to confirm the degree of carotid artery stenosis in the patients with acute ischemic stroke after hospitalization. Digital subtraction angiography (DSA) was performed among those with unconfirmed display.

The exclusion criteria for the ischemic stroke patients and controls included previous stroke, any history of valvular heart disease, atrial fibrillation, peripheral vascular disease, the use of anticoagulants therapy in the past two weeks, liver or renal insufficiency, systemic inflammation, autoimmune diseases and cancer. Patients with potential cardiac sources of emboli were also excluded. The controls had no neurological abnormality, and their brain CT or MRI showed no silent brain infarction. The study was approved by the Institute Ethical Committee, and informed consent to participate in this study was given by all of the participants.

2.2. Microembolic Signal Detection

The ipsilateral (to the side of the symptomatic carotid artery) middle cerebral artery of the patients was insonated continuously for 60 min using a pulsed Doppler device (DWL Doppler box/DWL multidrop ×4, 2 MHz probe) within 72 h of their clinical presentation. The middle cerebral arteries were identified within depths of insonation between 50 and 60 mm from the temporal window. A sample volume of 8 mm in length and a low gain provided a setting optimal for discriminating the emboli from the background setting. Microembolic signals were identified as high-intensity signals that were unidirectional within the velocity spectrum, lasted <300 ms, had an intensity >7 dB above the background velocity spectrum and were associated with a characteristic "chirping" sound [18,19].

2.3. Serology Sample Collection and Storage

Venous blood samples were drawn from the antecubital vein after an overnight fast. For the ischemic stroke patients, the blood samples were obtained within 24 h of admission. Isolated by centrifugation at 3000 ×g for 10 min, the serum or plasma samples were aliquoted and stored at −70 °C until analysis. All of the samples were thawed only once.

2.4. Laboratory Measurements

The serum-soluble CXCL16 concentration was measured with the human CXCL16 Quantikine ELISA Kit (R&D Systems) according to the manufacturer's instructions. The absorbance was measured by a Benchmark Microplate reader (Bio-Rad). Total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglycerides (TG) were assessed by routine methods using a fully automatic biochemical analyzer (Hitachi 7600–020).

2.5. Statistical analysis

The statistical analyses were performed using SPSS 17.0 for Windows software. Normally distributed variables are presented as the mean ± SD. The t (t') test was used to test for differences between the 2 groups, and 1-way analysis of variance (ANOVA) was used to test for differences among several groups. For the enumeration data, the chi-square test was used to compare the means. A $p < 0.05$ was considered statistically significant. A multinomial logistic regression analysis was used to balance the statistically significant factors, such as hypertension, diabetes, smoking, drinking, etc. The ability to evaluate the accuracy and specificity of CXCL16 as biomarkers was done by using the receiver-operating characteristic curve (ROC curve).

3. Results

3.1. Statistical analysis of the patients' characteristics

The clinical characteristics of the patients and the controls are shown in Table 1. There were no significant differences in age, gender, active smoking and alcohol abuse between the patients and the controls ($p>0.05$). Additionally, no differences were observed in the co-existence between coronary artery disease (CAD) and diabetes between the two groups ($p>0.05$). However, significant differences were detected in the history of hypertension between the patients and the controls ($p<0.05$). The laboratory findings are described in Table 1. Serum levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were significantly increased in the ischemic stroke patients compared to the controls ($p<0.001$, $p = 0.035$, $p = 0.008$, $p = 0.032$).

3.2. Laboratory Results

Using a multinomial logistic regression analysis to adjust for the different dangerous factors (hypertension, diabetes mellitus, smoking, drinking, HDL, TC, TG and LDL), Serum CXCL16 concentrations were

Table 1
Basic data of the ischemic stroke patients and the controls.

Variables	Controls ($n = 104$)	Ischemic stroke ($n = 118$)	*P* value
Age (years)	65.40 ± 11.23	68.60 ± 10.89	NS
Gender			
Male (%)	60 (58.7)	82(69.5)	NS
Active smoker (%)	40 (38.5)	35 (29.7)	NS
Alcohol abuse (%)	23 (22.1)	34 (28.8)	NS
Hypertension (%)	59 (56.7)	85 (72.0)*	0.044*
Diabetes (%)	27 (26.0)	39 (33.1)	NS
CAD (%)	27 (26.0)	35 (29.7)	NS
TC (mmol/l)	4.51 ± 1.01	4.89 ± 1.15*	0.035
TG (mmol/l)	1.43 ± 0.37	1.79 ± 0.57△	<0.001
LDL (mmol/l)	2.51 ± 0.71	2.83 ± 0.89△	0.008
HDL (mmol/l)	1.31 ± 0.31	1.23 ± 0.24	0.032

ischemic stroke vs. controls, △$p<0.01$, *$p<0.05$.