The relationship between serum CXCL16 level and carotid vulnerable plaque in patients with ischemic stroke

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Abstract. – OBJECTIVE: We investigated the relationship between the serum macrophage chemokine ligand 16 (CXCL16) levels and the vulnerable carotid plaque in patients with ischemic stroke.

PATIENTS AND METHODS: We successively selected 118 cases of patients with an initial diagnosis of acute ischemic stroke (time of onset < 72 h), recorded risk factors, including gender, age, family history, smoking, body mass index, blood glucose levels, blood lipid levels, average systolic pressure and diastolic blood pressure (DBP) and homocysteine levels. ELISA was used to detect the levels of serum CXCL16. GE-3000 color Doppler ultrasound diagnostic instrument was applied for the detection of the cervical artery (including a bilateral common carotid artery, internal carotid artery and external carotid artery) intima-media thickness (IMT), plaque number, size, nature (stable and vulnerable) and luminal stenosis rate. Delica EMS-9EBx2P type transcranial Doppler ultrasound (TCD) was used to detect micro-arterial micro-embolic signals (MES). Stroke, according to etiologic type, was divided into large artery atherosclerosis (LAA), small artery occlusion (SAO) and others.

RESULTS: Serum CXCL16 levels were not significantly correlated with sex, age, family history, smoking, BMI, blood glucose levels, blood lipid levels, mean systolic blood pressure, diastolic blood pressure, and homocysteine levels. Serum CXCL16 levels increased with an increase of IMT, plaque area and lumen stenosis rate. Serum CXCL16 levels of vulnerable plaques were significantly higher than those of stable plaques; differences were statistically significant (p<0.05).

It has nothing to do with the number of atherosclerotic plaques. The levels of serum CXCL16 in MES positive group were significantly higher than that in MES negative group; differences were statistically significant (p<0.05). The serum CXCL16 levels of LAA patients were significantly higher than that of SAA and other types of patients; differences were statistically significant (p<0.05).

CONCLUSIONS: The levels of serum CXCL16 are not related to high-risk factors for acute stroke and closely related to characteristics of atherosclerotic plaque, micro-embolic signals and stroke subtypes.

Key Words: Ischemic stroke, Serum CXCL16, Vulnerable plaque, High-risk factors, Micro-embolic signal.

Introduction

Ischemic stroke accounts for 60-80% of all cerebral vascular diseases with high disability and mortality, and has become a major threat to human health. Pathology and clinical studies confirm that the formation of atherosclerotic plaques is an important mechanism of stroke, and that the rupture and loss of vulnerable plaques are the main cause of acute stroke. Also, various circulating emboli that block the cerebral artery is also a cause of stroke. Dyslipidemia, endothelial dysfunction, and persistent chronic inflammation are closely related to the formation of atherosclerotic plaques. CXCL16 functions as a trend factor, scavenger receptor, and adhesion molecule and can effectively gather CXCL16+T lymphocyte, and mediate inflammatory reactions. Lv et al finds that the serum CXCL16 levels were elevated and closely related to the formation of coronary heart disease and carotid atherosclerotic plaque. The trend can earlier reflect the formation of a “vulnerable plaque”, and has a higher sensitivity and specificity in early stroke. Based on this, our study further analyzed the relationship between CXCL16 levels and high-risk factors such as plaques, micro-arterial micro embolic signals (MES) and the stroke subtypes formed in acute stroke in order to provide reference to the establishment of
the accuracy and sensitivity of the CXCL16 levels in the early diagnosis of acute stroke.

**Patients and Methods**

**Patients**

We selected 118 cases of patients with an initial diagnosis of acute stroke from January 2015 to January 2016 at our hospital. Inclusion criteria: 1. Time of onset <72 h; 2. The patient did not receive any treatment (thrombolysis, anticoagulation, antiplatelet, nutrition, etc.); 3. Perfect clinical data. Exclusion criteria: 1. Combined diagnosis cerebral hemorrhage, brain tumor, cerebral vascular malformation and brain injury; 2. Stroke Central Asian type cannot be clear; 3. Other serious underlying diseases such as heart, liver, lung, kidney and other organs dysfunction, autoimmune diseases, infection, pregnancy, lactation women, awareness barriers, etc. This study was approved by the Ethics Committee of our hospital. We obtained written informed consent from the patients and their families, including 68 males and 50 female. Patients were aged from 46 to 76 years old with an average age of 56.4 ± 11.2 years old. 10 cases had a family history (8.5%), 29 cases smoke (24.6%), body mass index (BMI) was between 21.3-23.6 kg/m² and averaged at (20.6±2.2) kg/m². Fasting blood glucose was between 5.5-8.6 mmol/L and averaged at (6.7±2.3) mmol/L. Total cholesterol was between 6.1-6.8 mmol/L and averaged at (6.7±0.7) mmol/L. Low-density lipoprotein was 4.2-5.0 mmol/L and averaged at (4.8±0.5) mmol/L. Mean systolic blood pressure was between 146.7-167.8 mmHg and averaged at (152.3±6.7) mmHg. Mean diastolic blood pressure was between 82.6-91.3 mmHg and averaged at (85.6±4.7) mmHg. Homocysteine was between 6.7-22.5 μmol/L and average (16.8±5.7) μmol/L.

**Research Method and Observation Index**

The ELISA method was used to detect the levels of serum CXCL16 within 24 h after admission to the hospital. 5 ml of peripheral venous blood was obtained and coagulated for 20 min. The blood was centrifuged at 3500 g for 15 min, and the upper serum was taken and kept at -80°C for detection. Kits were purchased from the Sigma-Aldrich Company (St. Louis, MO, USA). The standard enzyme instrument was purchased from Bio-Rad Company (Hercules, CA, USA). Instructions were followed in strict accordance with manufacturer’s guidelines.

We utilized the GE-3000 color Doppler ultrasound diagnostic instrument (GE Company, Fairfield, CT, USA) IE33 probe; the frequency was between 7-10 MHz. While the patient was in a supine position, the front of the neck was fully exposed and an ultrasonic probe was passed along the carotid artery from the proximal to the distal longitudinal scan including the bilateral common carotid artery, internal carotid artery and external carotid artery. We measured the carotid artery intima-media thickness (IMT), plaque number, size, nature stable and vulnerable and luminal stenosis. The criteria for the stability of plaque was the eccentric thickening of the arterial wall, and the strong echo with a more uniform low echo or irregular shape. Vulnerable plaque (unstable plaque) was a heterogeneous, low echo or mixed echo in the lumen.

We applied EMS-9EBx2P type delikai transcranial Doppler (TCD) for the detection of microembolic signals (MES). While the patients was in a supine position, we used a 2 MHz probe to detect bilateral middle cerebral artery blood flow signal, fixed the Spencer head frame with a depth of 50 to 60 mm, the distance ≥ 6 mm, sample volume 6-12 mm, relative intensity thresholds for MES 4dB in order to try and reduce gain value and to gain clear frequency spectrum. We recorded and analyzed the results by TCD software V1.3.3. The MES criteria for time is <300 ms, signal intensity compared with background blood flow signal ≥3 dB, one-way appears in the blood flow spectrum and the audio signal is “crackling” or “birds” sound that appeared in a cardiac cycle arbitrarily; also, the two detection depths have a time delay. The ultrasonic examination was judged by two experienced physicians.

Acute ischemic stroke patients were divided, according to the therapeutic effect of cerebral apoplexy, into large artery type (LAA), small artery occlusion type (SAA) and others. LAA ultrasound showed that the carotid stenosis was more than half of the lumen, CTA, MRA or DSA examination confirmed that the occlusion or stenosis of relevant intracranial or extracranial artery trunk and branches of the artery were more than 50%. SAA in MRI showed lacunar infarction and maximum diameter of stroke range was less than 15 mm.

**Statistical Analysis**

We used the SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) for data analysis; measurement data was expressed by mean ± standard de-
viation. Comparison between the two groups was tested by the independent sample $t$-test. Comparison among groups was analyzed by the single factor analysis of variance (ANOVA); the pairwise comparison was tested with the LSD-$t$ method. Count data was expressed as a ratio, and comparison was tested by $\chi^2$-test. $p<0.05$ indicated that the difference was statistically significant.

### Results

#### Analysis of the Relationship Between Serum CXCL16 Level and High-risk Factors for Stroke

Serum CXCL16 levels did not correlate with sex, age, family history, smoking, BMI, blood glucose, blood lipid, mean systolic blood pressure, diastolic blood pressure, and homocysteine levels (Table I).

#### Analysis of the Relationship Between Serum CXCL16 Level and Plaque Characteristics

Carotid artery IMT was between 8-16 mm and averaged at (12.5±3.3) mm. Atherosclerotic plaque 1-5 and averaged at (2.5±1.4). Plaque area was between 2.3-9.6 mm$^2$ and averaged at (5.6±2.4) mm$^2$. A stable plaque was present in 49 cases and vulnerable plaque was present in 69 cases. Lumen stenosis rate was between 46-98% and averaged at (75.6±24.3%). Serum CXCL16 levels increased with an increase of IMT, plaque area and lumen stenosis rate. Serum CXCL16 levels of vulnerable plaques were significantly higher than those of stable plaques; differences were statistically significant ($p<0.05$). CXCL16 levels are not related to the number of atherosclerotic plaques (Table II).

#### Analysis of the Relationship Between Serum CXCL16 level and MES

Total MES was detected in 72 cases (61%), serum CXCL16 levels in MES positive group were significantly higher than those in the MES negative group; differences were statistically significant [(2.23±0.46) compared to (1.24±0.36) ng/ml, $t=5.764$, $p=0.020$].

#### Analysis of the Relationship Between Serum CXCL16 Level and stroke Subtype

LAA was detected in 57 cases (48.3%), SAA in 54 cases (45.8%), and the other subtype in 7 cases. The serum CXCL16 levels of LAA patients were significantly higher than those of SAA and other types of patients. The differences were statistically significant [(2.18±0.35) compared to (1.24±0.36) ng/ml, F=5.438, $p=0.027$].

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<table>
<thead>
<tr>
<th>Table I. Analysis of the relationship between serum CXCL16 level and high risk factors of stroke.</th>
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<tbody>
<tr>
<td><strong>High-risk factor</strong></td>
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<tr>
<td>Male</td>
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<tr>
<td>Female</td>
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<tr>
<td>&lt;56.4 years old</td>
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<td>≥56.4 years old</td>
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<tr>
<td>No family history</td>
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<tr>
<td>Have a family history</td>
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<tr>
<td>No smoking</td>
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<tr>
<td>BMI&lt;20.6 kg/m$^2$</td>
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<tr>
<td>Fasting blood glucose&lt;6.7 mmol/L</td>
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<td>≥6.7 mmol/L</td>
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<td>Total cholesterol&lt;6.5 mmol/L</td>
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<td>≥6.5 mmol/L</td>
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<tr>
<td>Low density lipoprotein&lt;4.8 mmol/L</td>
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<td>≥4.8 mmol/L</td>
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<tr>
<td>Mean systolic blood pressure&lt;152.3 mmHg</td>
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<td>≥152.3 mmHg</td>
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<td>Mean diastolic blood pressure&lt;85.6 mmHg</td>
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<td>≥85.6 mmHg</td>
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<td>Homocysteine&lt;16.8 μmol/L</td>
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<td>≥16.8 μmol/L</td>
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Discussion

The body’s inflammatory response plays an important role in plaque formation, progression, rupture and embolism, which is an independent risk factor for an atherosclerotic stroke. CXCL16 is closely related to the occurrence of atherosclerosis; it is present in both bound and secreted form, expressed on the surface of macrophages, functions to phagocytosis oxidized LDL, participates in the formation of foam cells, is deposited in the endometrium, and becomes the core of the lipid plaque. CXCL16 binding on the surface of the T lymphocyte CXCR6 drives T cell migration to atherosclerotic parts. While there, it secretes a variety of chemokines, growth factors, interleukin, tumor necrosis factor to activate endothelial cells, and promote the formation and development of plaques. T cells, through the activation of matrix metalloproteinases, prompt plaque rupture and exacerbate the vulnerability of plaque. As a blood vessel derived factor, it results in intimal neovascularization, plaque rupture and bleeding. Wang et al confirm that the expression of CXCL16 in atherosclerotic plaques abnormally increases, which increases the level of INF-γ, and plays a positive role in atherosclerosis. The elevation of CXCL16 level can promote the transformation of stable plaque to vulnerable plaque. Through the present work, we concluded that there was no significant correlation between serum CXCL16 levels and gender, age, family history, smoking, BMI, blood glucose, blood lipid, mean systolic blood pressure, diastolic blood pressure and homocysteine levels. However, gender, age, family history, smoking, BMI, blood glucose, blood lipid, the average systolic pressure and diastolic blood pressure, homocysteine levels are traditional risk factors which were confirmed to be closely related to the occurrence of stroke. This suggests that the increase of CXCL16 levels in the occurrence of stroke is mainly mediated by the inflammatory response, and promotes the formation and rupture of the plaque.

Serum CXCL16 levels increased with an increase of IMT, plaque area and lumen stenosis rate. The serum CXCL16 levels of vulnerable plaques were significantly higher than those of stable type, but not related to the number of atherosclerotic plaques. The serum CXCL16 levels of LAA patients were significantly higher than those of SAA and other types of patients. Scholars confirm that vulnerable plaques are closely related to the occurrence of LAA and SAA. NF-κB pathway study shows that CXCL16 and its receptors in vascular smooth muscle further increase the ability of cells to proliferate and lead to cell adhesion of smooth muscle. However, Aslanian et al observe that the ability of CXCL16 gene knockout mice (CXCL16-/-) macrophages to combine oxidize LDL is significantly reduced which leads to the progression of atherosclerosis in CXCL16-/- and LDLR-/- double deficient mice to be accelerated, which suggests that CXCL16 may also have anti-atherosclerotic effect. Carotid artery high-resolution magnetic resonance imaging in the detection of the neck plaque and determination of the vulnerable nature displays a better diagnostic value. The level of serum CXCL16 in MES positive group was significantly higher than that in MES negative group; differences were statistically significant (p<0.05). The serum CXCL16 levels of LAA patients were significantly higher than those of SAA and other types of patients; the differences were statistically significant (p<0.0). Therefore, MES suggests plaque instability and TCD is the only way to detect MES.
Conclusions

The serum CXCL16 levels are not related to high-risk factors for acute stroke and are closely related to characteristics of atherosclerotic plaques, micro-emboli and stroke subtypes. CXCL16 mediated inflammatory response can promote the formation of vulnerable atherosclerotic plaques, which are important for the early diagnosis of ischemic stroke by detection of the levels of serum CXCL16.

Conflict of interest

The authors declare no conflicts of interest.

References


