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Correlation of serum homocysteine levels with nerve injury and atherosclerosis in patients with stroke

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ABSTRACT

Objective: To study the correlation of serum homocysteine levels with nerve injury and atherosclerosis in patients with stroke. **Methods:** Patients who were diagnosed with ischemic stroke in our hospital between January 2014 and December 2016 were selected and then divided into moderate-severe stenosis group (C group), mild stenosis group (B group) and no stenosis group (A group) according to carotid artery ultrasonography; healthy volunteers who received physical examination during the same period were chosen as control group. The serum levels of homocysteine, nerve injury indexes and atherosclerosis indexes were detected. **Results:** Serum Hcy, S100B, NSE, UCH-L1, GFAP, FGF23, CD36, ox-LDL, MMP8 and MMP9 levels of C group, B group and A group were significantly higher than those of control group, and the severer the carotid stenosis, the higher the serum S100B, NSE, UCH-L1, GFAP, FGF23, CD36, ox-LDL, MMP8 and MMP9 levels; serum S100B, NSE, UCH-L1, GFAP, FGF23, CD36, ox-LDL, MMP8 and MMP9 levels in stoke patients with high Hcy were significantly higher than those of patients with normal Hcy. **Conclusions:** Serum homocysteine levels increase in patients with stroke and are closely related to the nerve injury and atherosclerosis.

1. Introduction

Stroke is a cerebrovascular disease with high disability and fatality rate, and the incidence is increasing year by year. Carotid atherosclerosis is the important pathologic basis of stroke, and also the independent risk factor of stroke, and stroke will occur when the atheromatous plaque stability reduces, and the plaques fall off and enter into the intracranial blood vessels with blood circulation[1,2]. Recent study on cardiovascular and cerebrovascular diseases shows that hyperhomocysteinemia is closely related to the occurrence of a variety of cardiovascular and cerebrovascular diseases, and homocysteine can participate in a variety of pathological links of atherosclerosis through inflammation and oxidative stress. Clinical research shows that homocysteine levels increase significantly in patients with stroke[3], but there is still no clear report about the relationship of hyperhomocysteinemia with nerve damage and atherosclerosis in patients with stroke. In the following study, the correlation of serum homocysteine levels with nerve injury and atherosclerosis in patients with stroke was analyzed.

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2. General information and clinical research methods

2.1 General information of subjects

A total of 560 patients who were diagnosed with ischemic stroke in Yulin Third Hospital between January 2014 and December 2016 were selected, all patients were in accordance with the diagnostic criteria for the disease, and the patients with hemorrhagic stroke and malignant tumor, and the patients who recently took vitamin B and other drugs that might affect homocysteine metabolism were ruled out. According to the results of carotid artery ultrasonography, they were divided into moderate-severe stenosis group (C group), mild stenosis group (B group) and no stenosis group (A group). C group (n=189) were with carotid artery stenosis rate ＞30%, including 118 men and 71 women that were 47-62 years old; B group (n=246) included 129 men and 117 women that were 44-63 years old; A

Table 1.
Serum nerve injury indexes S100B, NSE, UCH-L1 and GFAP levels.

Groups	*n*	S100B	NSE	UCH-L1	GFAP
Control group	80	0.62±0.09	10.38±1.58	44.38±7.29	0.69±0.09
A group	125	1.25±0.19*	36.49±5.48*	74.29±9.15*	0.93±0.11*
B group	246	1.84±0.25*a	49.24±7.02*a	125.23±15.32*a	1.18±0.16*a
C group	189	2.55±0.37*ab	75.49±9.34*ab	178.44±20.35*ab	1.42±0.19*ab

*: compared with control group, $P<0.05$; a: compared with A group, $P<0.05$; b: compared with B group, $P<0.05$.

Table 2.
Serum S100B, NSE, UCH-L1 and GFAP levels in stoke patients with different Hcy levels.

Stroke patients	*n*	S100B	NSE	UCH-L1	GFAP
High Hcy	367	2.49±0.33	85.51±9.94	183.51±22.35	1.68±0.22
Normal Hcy	193	1.32±0.19	29.42±4.58	70.23±9.35	0.79±0.09
T		8.394	15.327	13.428	11.039
P		<0.05	<0.05	<0.05	<0.05

group (n=125) included 67 men and 58 women that were 45-65 years old. A total of 80 healthy volunteers who received physical examination during the same period were chosen as control group, including 45 men and 35 women that were 42-65 years old. There was no significant difference in the general data among the four groups (P>0.05).

2.2 Carotid artery ultrasonography

Color Doppler diasonograph (Philips, iE33 type) was used for carotid artery ultrasonography, probe frequency was set to 5-10 MHz, the carotid artery running from bottom to top was referred to scan the bilateral common carotid arteries, the carotid bifurcation and extracranial segment of internal carotid artery, carotid intima-media thickness (IMT) was measured, IMT>1.2 mm indicated carotid atherosclerosis, and the results of ultrasonography were referred to judge the degree of stenosis, including A, B and C.

2.3 Serum index detection methods

5-8 mL of cubital venous blood was collected from A-C group of patients on admission, 5-8 mL of cubital venous blood was collected from control group of volunteers during physical exams, the blood was let stand for coagulation and then centrifuged for 10 min at 3 000 r/min, serum was taken, and Elisa kit was used to determine S100B protein, neuron-specific enolase (NSE), ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), glial fibrillary acidic protein (GFAP), fibroblast growth factor 23 (FGF23), CD36, oxidized low density lipoprotein (ox-LDL), matrix metalloproteinase (MMP8) and MMP9 levels.

2.4 Statistical methods

SPSS 20.0 software was used to input and analyze data, measurement data comparison among four groups was by variance analysis and $P<0.05$ was the standard of statistical significance in differences between groups.

3. Results

3.1 Serum Hcy levels

Serum Hcy levels of the four groups were (39.42±5.42) μmol/L, (28.31±3.58) μmol/L, (21.24±2.98) μmol/L and (7.64±0.93) μmol/L respectively, and specific analysis was as follows: serum Hcy levels of C group, B group and A group were significantly higher than that of control group; serum Hcy levels of C group and B group were significantly higher than that of A group; serum Hcy level of C group was significantly higher than that of B group. Differences in pair-wise comparison of serum Hcy levels were statistically significant among four groups of subjects (P<0.05).

3.2 Serum nerve injury index levels

Analysis of serum nerve injury indexes S100B (μg/L), NSE (μg/L), UCH-L1 (ng/L) and GFAP (μg/L) levels among four groups of subjects was as follows: serum S100B, NSE, UCH-L1 and GFAP levels of C group, B group and A group were significantly higher than those of control group; serum S100B, NSE, UCH-L1 and GFAP levels of C group and B group were significantly higher than those of A group; serum S100B, NSE, UCH-L1 and GFAP levels of C group was significantly higher than those of B group. Differences in pair-wise comparison of serum S100B, NSE, UCH-L1 and GFAP levels were statistically significant among four groups of subjects (P<0.05).

Analysis of the correlation of serum Hcy levels with nerve injury indexes S100B, NSE, UCH-L1 and GFAP levels in patients with stroke was as follows: serum S100B, NSE, UCH-L1 and GFAP levels in stoke patients with high Hcy were significantly higher than those of patients with normal Hcy. Differences in serum S100B, NSE, UCH-L1 and GFAP levels were statistically significant between patients with high Hcy and normal Hcy (P<0.05).

Table 3.
Serum atherosclerosis index levels in four groups of subjects

Groups	*n*	FGF23	CD36	Ox-LDL	MMP8	MMP9
Control group	80	2.03±0.34	2.77±0.35	103.24±13.84	36.48±5.41	79.41±9.33
A group	125	3.77±0.52*	3.84±0.51*	153.32±17.67*	55.29±7.43*	95.23±11.26*
B group	246	4.94±0.72*a	4.53±0.76*a	203.42±26.93*a	70.32±9.35*a	126.73±15.62*a
C group	189	7.24±1.03*ab	5.96±0.89*ab	264.94±33.25*ab	98.34±11.25*ab	162.35±20.34*ab

*: compared with control group, $P<0.05$; a: compared with A group, $P<0.05$; b: compared with B group, $P<0.05$.

Table 4.
Serum atherosclerosis index levels in stoke patients with different Hcy levels.

Stroke patients	*n*	FGF23	CD36	Ox-LDL	MMP8	MMP9
High Hcy	367	7.03±0.93	6.14±0.83	272.13±35.62	103.41±14.85	179.32±22.35
Normal Hcy	193	3.94±0.56	3.55±0.59	142.39±14.59	52.32±7.03	89.34±10.39
T		8.938	8.328	9.382	9.928	9.018
P		<0.05	<0.05	<0.05	<0.05	<0.05

3.3 Serum atherosclerosis index levels

Analysis of serum atherosclerosis indexes FGF23 (mg/L), CD36 (μg/L), ox-LDL (mg/L), MMP8 (μg/L) and MMP9 (μg/L) levels among four groups of subjects was as follows: serum FGF23, CD36, ox-LDL, MMP8 and MMP9 levels of C group, B group and A group were significantly higher than those of control group; serum FGF23, CD36, ox-LDL, MMP8 and MMP9 levels of C group and B group were significantly higher than those of A group; serum FGF23, CD36, ox-LDL, MMP8 and MMP9 levels of C group was significantly higher than those of B group. Differences in pair-wise comparison of serum FGF23, CD36, ox-LDL, MMP8 and MMP9 levels were statistically significant among four groups of subjects ($P<0.05$).

Analysis of the correlation of serum Hcy levels with atherosclerosis indexes FGF23, CD36, ox-LDL, MMP8 and MMP9 levels in patients with stroke was as follows: serum FGF23, CD36, ox-LDL, MMP8 and MMP9 levels in stoke patients with high Hcy were significantly higher than those of patients with normal Hcy. Differences in serum FGF23, CD36, ox-LDL, MMP8 and MMP9 levels were statistically significant between patients with high Hcy and normal Hcy ($P<0.05$).

4. Discussion

Ischemic stroke is closely related to carotid atherosclerosis, carotid atheromatous plaque stability will reduce after the properties change, and then plaques will rupture, fall off and become emboli, which enter into the intracranial vessels along with the blood circulation, cause vascular obstruction, and lead to cerebral infarction. Complicated pathophysiologic changes are involved in the occurrence and development carotid atheromatous plaque formation and ischemic stroke, and abnormal homocysteine metabolism is thought to be associated with cardiovascular and cerebrovascular disease. Homocysteine is intermediate of methionine metabolism in the body, and abnormal metabolic processes can cause homocysteine accumulation and form hyperhomocysteinemia[4]. Homocysteine participates in the regulation of inflammatory response, oxidative stress and other processes in the body, which can increase the inflammatory mediator and oxygen free radical generation to cause endothelial damage, platelet activation and thrombosis[5,6]. In the study, analysis of serum homocysteine levels in all groups of subjects showed that serum homocysteine levels in stroke patients with degree of carotid atherosclerosis were significantly higher than those in healthy volunteers, and the severer the carotid stenosis, the more obvious the increase of serum homocysteine levels. This indicates that the increase in homocysteine content is closely related to the occurrence of ischemic stroke and the occurrence of carotid atherosclerosis.

In the progression of ischemic stroke, brain damage caused by ischemia hypoxia will cause the rupture of neurons and glial cells in local tissue, and then a variety of molecules in cells are released into the cerebrospinal fluid and then enter in the blood circulation through the blood brain barrier. NSE and UCH-L1 are specific proteins that are expressed in neurons of nervous system and have the function of catalyzing energy metabolism[7,8]; GFAP is a type III intermediate filament protein distributed in astrocytes, which has important value for maintaining the function of the glial cells [9]; S100B is a type of calcium ion-binding protein in neurons and glial cells that is involved in the regulation of calcium ion steady-state[10,11]. In the study, analysis of the contents of these molecules showed that serum S100B, NSE, UCH-L1 and GFAP levels in patients with stroke were significantly higher than those in healthy volunteers, and the severer the carotid stenosis, the more obvious the increase of serum S100B, NSE, UCH-L1 and GFAP levels. Further analysis of the correlation between homocysteine levels and these nerve injury molecule levels showed that serum S100B, NSE, UCH-L1 and GFAP levels in patients with high Hcy were significantly

higher than those of patients with normal Hcy. This suggests that the increased levels of homocysteine in patients with ischemic stroke can aggravate nerve injury.

Abnormally elevated homocysteine in serum of patients with stroke can influence the stability of carotid artery atheromatous plaque by inflammation and oxidative stress, and the decrease of plaque stability is directly related to the occurrence of stroke. FGF23, a new member of fibroblast growth factor family, is a kind of secretory protein that has the function of hormones, and plays an important role in the process of triggering inflammation[12]; CD36 belongs to the scavenger receptor family, is a transmembrane glycoprotein, and can identify the ox-LDL, make the ox-LDL infiltrate in intima and experience endocytosis, and then induce foam cells to form, accumulate in blood vessel walls and become atheromatous plaque[13-15]; MMP8 and MMP9 are the important members of the matrix metalloproteinase family that can work together to participate in the hydrolysis of extracellular matrix, and promote the degradation of plaque fibrous cap and the decrease of plaque stability[16,17]. In the study, analysis of the contents of above atherosclerosis indexes showed that serum FGF23, CD36, ox-LDL, MMP8 and MMP9 levels in patients with stroke were significantly higher than those of healthy volunteers, and the severer the carotid stenosis, the more obvious the increase of serum FGF23, CD36, ox-LDL, MMP8 and MMP9 levels. Further analysis of the correlation between homocysteine levels and these carotid atherosclerosis index levels showed that serum FGF23, CD36, ox-LDL, MMP8 and MMP9 levels in patients with high Hcy were significantly higher than those of patients with normal Hcy. This suggests that the increased levels of homocysteine in patients with ischemic stroke can aggravate carotid atherosclerosis.

To sum up, it is believed that the serum homocysteine levels are unusually high in patients with stroke; the elevated homocysteine levels are closely associated with nerve injury and atherosclerosis.

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