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Relationship between serum inflammatory response cytokines and the change of plaque properties in patients with H−type hypertension complicated by carotid atherosclerosis

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ABSTRACT

Objective: To study the relationship between serum inflammatory response cytokines and the change of plaque properties in patients with H-type hypertension complicated by carotid atherosclerosis. **Methods:** Patients who were diagnosed with H-type hypertension complicated by carotid atherosclerosis in Yulin Hospital of Traditional Chinese Medicine between May 2014 and December 2016 were selected as group A, patients with H-type hypertension complicated by carotid atherosclerosis and normal Hcy levels were selected as group B, patients with carotid atherosclerosis and normal blood pressure levels and Hcy levels were selected as group C, and healthy volunteers during the same period were selected as control group. Serum levels of inflammatory response cytokines and plaque property-related cytokines were detected. **Results:** Serum Hcy, sICAM-1, MCP-1, YKL-40, IL-6, PTX3, FGF23, MMP9 and Caspase-3 levels of group A, group B and group C were significantly higher than those of group D, serum Hcy, sICAM-1, MCP-1, YKL-40, IL-6, PTX3, FGF23, MMP9 and Caspase-3 levels of group A and group B were significantly higher than those of group C, and serum Hcy, sICAM-1, MCP-1, YKL-40, IL-6, PTX3, FGF23, MMP9 and Caspase-3 levels of group A were significantly higher than those of group B; serum Hcy, sICAM-1, MCP-1, YKL-40, IL-6, PTX3, FGF23, MMP9 and Caspase-3 levels of group A with vulnerable plaques were significantly higher than those of patients with stable plaques. Serum Hcy, sICAM-1, MCP-1, YKL-40 and IL-6 levels of group A were positively correlated with PTX3, FGF23, MMP9 and Caspase-3 levels. **Conclusion:** Serum inflammatory response cytokine secretion increases in patients with H-type hypertension complicated by carotid atherosclerosis, and it can result in the change in plaque properties and the decrease in stability.

1. Introduction

Hypertension is a common disease in the cardiovascular system and also a risk factor for many cardiovascular events. Some hypertensive patients are combined with hyperhomocysteinemia, it is also known as H-type hypertension, and abnormally elevated homocysteine levels will further increase the risk of cardiovascular and cerebrovascular events[1,2]. Atherosclerosis is a common pathological basis for cerebral infarction, myocardial infarction and other cardiovascular events, and is also regarded as an independent risk factor for cardiovascular events[3]. Both hypertension and hyperhomocysteinemia promote the atherosclerosis, but the molecular mechanism is not clear. Carotid artery position is relatively superficial, ultrasound examination is simple, and the evaluation of carotid atherosclerosis condition provides the reference for judging the degree of atherosclerosis of coronary artery, intracranial artery and so on. In the following studies, the relationship between serum inflammatory response cytokines and the change of plaque properties in patients with H-type hypertension complicated by carotid atherosclerosis was analyzed.

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2. Subject information and research methods

2.1 Subject information

Patients who were diagnosed with carotid atherosclerosis by ultrasound in Yulin Hospital of Traditional Chinese Medicine between May 2014 and December 2016 were selected, patients with H-type hypertension complicated by carotid atherosclerosis were selected as group A, patients with H-type hypertension complicated by carotid atherosclerosis and normal Hcy levels were selected as group B, and patients with carotid atherosclerosis and normal blood pressure levels and Hcy levels were selected as group C. Healthy volunteers during the same period were selected as control group. All subjects signed informed consent and serum samples were kept. Group A (n=39) included 22 men and 17 women that were 39-55 years old; Group B (n=53) included 33 men and 20 women that were 42-54 years old; group C (n=64) included 38 men and 26 women that were 41-53 years old; group D (n=60) included 36 men and 24 women, that were 40-55 years old. There was no significant difference in the general data among the four groups.

2.2 Carotid atherosclerosis evaluation methods

Four groups of subjects received carotid artery ultrasonography by IE33 type color Doppler diasonograph of Philips company, the frequency of the probe was 5-12 MHz, the common carotid artery, internal carotid artery and external carotid artery were scanned from bottom to top, intima-media thickness 2cm from proximal end of carotid artery bifurcation as well as the intima-media thickness of internal carotid artery and external carotid artery 1cm from distal end of common carotid artery enlargement, and the intima-media thickness > 1.2 mm was the standard of plaque formation. The flat plaques and hard plaques were judged as stable plaques, and the mixed plaques and soft plaques were judged as vulnerable plaques.

2.3 Serum index detection methods

5 mL of fasting venous blood was collected from four groups of subjects before carotid artery ultrasonography, let stand at room temperature for 30 min and then centrifuged in the centrifuge for 10 min at 3 000 r/min to separate upper serum, Hcy content was determined by radioimmunoprecipitation kits, and enzyme-linked immunosorbent kit was used to determine sICAM-1, MCP-1, YKL-40, IL-6, PTX3, FGF23, MMP9 and Caspase-3 levels.

2.4 Statistical methods

SPSS 18.0 software was used for variance analysis of measurement data among four groups and for t test of measurement data between two groups, and P<0.05 indicated statistical significance in differences.

3. Results

3.1 Serum inflammatory response cytokine levels in four groups of subjects

Analysis of serum inflammatory response cytokines Hcy (μmol/L), sICAM-1 (ng/mL), MCP-1 (pg/mL), YKL-40 (pg/mL) and IL-6 (ng/mL) levels in four groups of subjects was as follows: serum Hcy, sICAM-1, MCP-1, YKL-40 and IL-6 levels of group A, group B and group C were significantly higher than those of group D, serum Hcy, sICAM-1, MCP-1, YKL-40 and IL-6 levels of group A and group B were significantly higher than those of group C, and serum Hcy, sICAM-1, MCP-1, YKL-40 and IL-6 levels of group A were significantly higher than those of group B. Differences in pair-wise comparison of serum Hcy, sICAM-1, MCP-1, YKL-40 and IL-6 levels were statistically significant among four groups of subjects (P<0.05).

3.2 Serum inflammatory response cytokine levels in group A with different carotid plaque properties

Analysis of serum inflammatory response cytokines Hcy, sICAM-1, MCP-1, YKL-40 and IL-6 levels in group A with different carotid plaque properties was as follows: serum Hcy, sICAM-1, MCP-1, YKL-40 and IL-6 levels of group A with vulnerable plaques were significantly higher than those of patients with stable plaques. Differences in serum Hcy, sICAM-1, MCP-1, YKL-40 and IL-6 levels were statistically significant between group A with different carotid plaque properties (P<0.05).

Table 1.
Serum inflammatory response cytokine levels in four groups of subjects.

Groups	n	Hcy	sICAM-1	MCP-1	YKL-40	IL-6
Group A	39	14.59±2.23^{*bc}	3.95±0.54^{*bc}	35.29±4.52^{*bc}	59.62±7.72^{*bc}	298.42±33.25^{*bc}
Group B	53	8.38±1.05^{*c}	3.11±0.47^{*c}	26.51±3.98^{*c}	40.33±5.68^{*c}	227.59±28.39^{*c}
Group C	64	7.52±0.89*	2.57±0.34*	22.14±3.53*	32.15±4.52*	176.59±20.25*
Group D	60	6.03±0.79	2.03±0.29	17.63±2.04	26.76±3.35	124.28±16.84

*: compared with group D, P<0.05; ^{c}: compared with group C, P<0.05; ^{b}: compared with group B, P<0.05.

Table 2.
Serum inflammatory response cytokine levels in group A with different carotid plaque properties.

Groups	n	Hcy	sICAM-1	MCP-1	YKL-40	IL-6
Vulnerable plaque	22	18.52±2.64	4.66±0.67	42.03±5.45	72.31±9.35	363.74±42.39
Stable plaque	17	10.12±1.45	3.20±0.46	28.23±3.58	46.58±6.69	238.60±32.52
T		9.282	7.478	8.319	8.847	7.624
P		<0.05	<0.05	<0.05	<0.05	<0.05

Table 3.
Serum plaque property-related cytokine levels in four groups of subjects.

Groups	*n*	PTX3	FGF23	MMP9	Caspase-3
Group A	39	3.48±0.51^{*bc}	6.79±0.89^{*bc}	289.43±33.52^{*bc}	47.48±6.72^{*bc}
Group B	53	2.77±0.37^{*c}	5.12±0.57^{*c}	186.53±23.16^{*c}	35.65±5.48^{*c}
Group C	64	2.21±0.29*	3.83±0.52*	135.46±16.74*	26.74±3.86*
Group D	60	1.67±0.20	2.44±0.37	89.45±9.35	15.64±1.86

*: compared with group D, $P<0.05$; c: compared with group C, $P<0.05$; ^{b}: compared with group B, $P<0.05$.

3.3 Serum plaque property–related cytokine levels in four groups of subjects

Analysis of serum plaque property-related cytokines PTX3 (ng/mL), FGF23 (μg/mL), MMP9 and Caspase-3 (pg/mL) levels in four groups of subjects was as follows: serum PTX3, FGF23, MMP9 and Caspase-3 levels of group A, group B and group C were significantly higher than those of group D, serum PTX3, FGF23, MMP9 and Caspase-3 levels of group A and group B were significantly higher than those of group C, and serum PTX3, FGF23, MMP9 and Caspase-3 levels of group A were significantly higher than those of group B. Differences in pair-wise comparison of serum PTX3, FGF23, MMP9 and Caspase-3 levels were statistically significant among four groups of subjects ($P<0.05$).

3.4 Serum plaque property–related cytokine levels in group A with different carotid plaque properties

Analysis of serum plaque property-related cytokines PTX3, FGF23, MMP9 and Caspase-3 levels in group A with different carotid plaque properties was as follows: serum PTX3, FGF23, MMP9 and Caspase-3 levels of group A with vulnerable plaques were significantly higher than those of patients with stable plaques. Differences in serum PTX3, FGF23, MMP9 and Caspase-3 levels were statistically significant between group A with different carotid plaque properties ($P<0.05$).

3.5 Correlation between serum inflammatory resposne cytokines and plaque property–related cytokines in group A

Pearson correlation analysis showed that serum inflammatory resposne cytokines Hcy, sICAM-1, MCP-1, YKL-40 and IL-6 levels of group A were positively correlated with plaque property-related cytokines PTX3, FGF23, MMP9 and Caspase-3 levels.

4. Discussion

Hypertension, carotid atherosclerosis and hyperhomocysteinemia are all considered to be independent risk factors of cardiovascular and cerebrovascular events, and the three diseases are closely related and have many common pathological basis[4]. Inflammation is an important pathological change involved in multiple links of cardiovascular and cerebrovascular diseases, and Hcy, sICAM-1, MCP-1, YKL-40, IL-6 and other cytokines are confirmed to be closely related to the inflammatory reaction in the course of cardiovascular and cerebrovascular diseases[5,6]. In the course of hypertension, there is microinflammatory response in the body and it is involved in the vascular remodeling process; in the course of carotid atherosclerosis, inflammatory response is involved in various steps such as the formation of atheromatous plaques and the changes in the plaque properties; in the process of hyperhomocysteinemia, the abnormally elevated homocysteine content significantly promotes the activation of inflammatory response[7,8]. In order to define the changes of inflammatory response in the course of H-type hypertension complicated by carotid atherosclerosis, serum levels of inflammation cytokines were analyzed in the study, and the results showed that serum Hcy, sICAM-1, MCP-1, YKL-40 and IL-6 levels significantly elevated in A-C group of patients with carotid atherosclerosis, serum Hcy, sICAM-1, MCP-1, YKL-40 and IL-6 levels in patients with hypertension complicated by carotid atherosclerosis were significantly higher than those in patients with carotid atherosclerosis alone, and serum Hcy, sICAM-1, MCP-1, YKL-40 and IL-6 levels in patients with H-type hypertension complicated by carotid atherosclerosis were significantly higher than those in patients with hypertension complicated by carotid atherosclerosis and normal Hcy level. This means that the inflammatory response is involved in the process of carotid atherosclerosis, hypertension complicated by carotid atherosclerosis can aggravate the inflammatory response and the increase in serum homocysteine levels will further aggravate the inflammatory response.

During inflammatory reaction activation process, Hcy, sICAM-1, MCP-1, YKL-40, IL-6 and other cytokines are not only involved in the occurrence of atherosclerosis, but also closely related to the change of atheromatous plaque properties. Hcy is the intermediate product of methionine metabolism cycle, which promotes the activation of various inflammatory cell; sICAM-1 is an important adhesion molecule that promotes inflammation adhesion and infiltration in the plaques and increases inflammation in the plaques[9]; MCP-1 is an important chemokine that can promote monocyte transformation to macrophage, devour the lipid and become foam cells, thus accelerating the atheromatous plaque formation and property change[10]; YKL-40 is secreted by activated macrophages, which can cause vascular endothelial injury, decrease plaque stability and promote local thrombosis[11]; IL-6 is an inflammatory cytokine that can directly mediate the cascade activation of inflammatory reaction[12]. In order to further clarify the correlation between plaque properties and inflammatory reaction in patients with H hypertension complicated by carotid atherosclerosis, serum levels of these inflammation cytokines in H hypertension complicated by carotid atherosclerosis patients with different carotid plaque properties were analyzed in the study, and the results showed that serum Hcy, sICAM-1, MCP-1, YKL-40 and IL-6 levels of patients with vulnerable plaques were significantly higher than those of patients with stable plaques. This means that the activation of inflammatory response and the secretion of the corresponding

cytokines are closely related to the changes in the plaque properties in patients with H-type hypertension complicated by carotid atherosclerosis.

Changes in the carotid plaque properties may increase the risk of plaque loss and thrombosis, resulting in cerebral infarction. The effect of inflammatory response on the properties of plaques is to be achieved through different pathways and multiple molecules. PTX3 is a kind of pentraxin that is derived from the mononuclear macrophage, vascular smooth muscle cells, etc., and directly promotes the reduction in atheromatous plaque stability[13,14]; FGF23 is a new member of the fibroblast growth factor family, which affects the stability of the plaques by promoting the proliferation of fibroblasts[15]; MMP9 is a kind of matrix metalloproteinase, which hydrolyzes the matrix components in plaque fibrous cap, degrades fibrous cap, then cause plaque ruptures and reduce plaque stability[16,17]; Caspase-3 is an executive molecule of apoptosis, which can mediate the apoptosis of endothelial cells and nerve cells, and cause plaque damage and stability change[18]. In the study, analysis of serum levels of plaque property-related cytokines in H-type hypertension complicated by carotid atherosclerosis pateints with different carotid plaque properties showed that serum PTX3, FGF23, MMP9 and Caspase-3 levels in patients with vulnerable plaques were significantly higher than those in patients with stable plaques. This suggests that the increased secretion of PTX3, FGF23, MMP9, Caspas-3 and so on can lead to changes in plaque properties in patients with H-type hypertension complicated by carotid atherosclerosis through a variety of links. Further analysis of the correlation between serum inflammatory cytokines and plaque property-related cytokines showed that serum inflammation response cytokines Hcy, sICAM-1, MCP-1, YKL-40 and IL-6 levels of group A were positively correlated with plaque property-related cytokines PTX3, FGF23, MMP9 and Caspase-3 levels. Thefore, the excessively activated inflammation in patients with H hypertension complicated by carotid atherosclerosis can increase secretion of PTX3, FGF23, MMP9 and Caspase-3 to influence plaque stability and cause property change.

The secretion of serum inflammatory response cytokines increases significantly in patients with H type hypertension complicated by carotid atherosclerosis and it is closely related to the plaque properties. The activation of inflammatory response can change the plaque properties and decrease the plaque stability by increasing the secretion of PTX3, FGF23, MMP9 and Caspase-3.

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