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Correlation of serum HMGB1 and sTLT−1 contents with oxidative stress response and endothelial injury in patients with ischemic stroke

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ABSTRACT

Objective: To study the correlation of serum HMGB1 and sTLT-1 contents with oxidative stress response and endothelial injury in patients with ischemic stroke. **Methods:** The patients who were diagnosed with ischemic stroke in Zhouzhi County People′s Hospital between February 2015 and March 2017 were selected as the stroke group of the study, and healthy subjects who received physical examination during the same period were selected as the control group of the study. Serum was collected to determine the contents of HMGB1, sTLT-1, oxidative stress reaction molecules and endothelial injury molecules. **Results:** Serum HMGB1, sTLT-1, vWF, vWF-cp, sTM, ET-1, D-D, 8-OHdG, LPO and NOS contents of stroke group were significantly higher than those of control group while T-SOD and GSH-Px contents were significantly lower than those of control group; serum T-SOD and GSH-Px contents of stroke patients with high HMBG-1 content were significantly lower than those of stroke patients with low HMBG-1 content while 8-OHdG, LPO and NOS contents were significantly higher than those of stroke patients with low HMBG-1 content; serum vWF, vWF-cp, sTM, ET-1 and D-D contents of stroke patients with high sTLT-1 content were significantly higher than those of stroke patients with low sTLT-1 content. **Conclusion:** The abnormally elevated HMGB1 and sTLT-1 in serum of patients with ischemic stroke can induce oxidative stress response and aggravate endothelial injury.

1. Introduction

Ischemic stroke is the most common type of stroke in clinic, which is basically pathologically characterized by atherosclerosis, intracranial thromboembolism and cerebral ischemic ischemic injury. In the course of ischemic stroke, oxidative stress and endothelial injury are the important pathological links which are not only involved in the formation of atherosclerosis and the change of the atheromatous plaque nature, but also related to the brain tissue injury after ischemia hypoxia[1,2]. At present, it is still unclear about the specific mechanism of oxidative stress response and endothelial injury in ischemic stroke. High mobility group box 1 (HMGB1) is a nucleoprotein in mononuclear macrophages which is involved in the process of inflammatory response and can affect the generation of oxygen free radicals and regulate oxidative stress response[3]. sTLT-1 is the soluble form of platelet granule membrane-bound protein triggering receptor expressed on myeloid cells-like transcript-1 (TLT-1), which participates in the regulation of thrombosis, platelet activation and endothelial damage process[4]. The correlation of serum HMGB1 and sTLT-1 contents with oxidative stress response and endothelial injury in patients with ischemic stroke was analyzed in the following study.

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2. Clinical information and research methods

2.1 General information of subjects

The patients who were diagnosed with ischemic stroke in Zhouzhi County People′s Hospital between February 2015 and March 2017 were selected as the stroke group of the study, all of them were consistent with the diagnostic criteria for ischemic stroke and with the first onset, and the patients with autoimmune diseases and infectious diseases were excluded. Healthy subjects who received physical examination in Zhouzhi County People′s Hospital during the same period were selected as the control group of the study. There were 58 cases in the stroke group, including 31 male cases

and 27 female cases that were 39-62 years old; there were 46 cases in the control group, including 26 male cases and 20 female cases that were 40-60 years old. There was no significant difference in general data between the two groups (P>0.05).

2.2 Research methods

2.2.1 Clinical sample collecting

3-5 mL of cubital venous blood was collected from the stroke group and control group on admission and during examination respectively and centrifuged to separate serum and then store it at -80 ℃.

2.2.2 Clinical index detecting

Enzyme-linked immunosorbent assay kit was used to detect serum levels of HMBG-1, sTLT-1, vWF, vWF-cp, sTM, ET-1 and D-D in, and the contents of T-SOD, GSH-Px, 8-OHdG, LPO, and NOS were detected by radioimmunoprecipitation kit.

2.3 Statistical methods

SPSS 19.0 software was used to input and analyze data, the median of HMBG-1 and sTLT-1 contents in patients with cerebral stroke were calculated and used to divide them into those with high and low HMBG-1 and sTLT-1 contents respectively, the measurement data between two groups was by t test and P<0.05 indicated statistical significance in differences in test results.

3. Results

3.1 Serum HMBG-1 and sTLT-1 contents

Serum HMBG-1 and sTLT-1 contents of stroke group were (79.41±9.55) pg/mL and (648.3±78.4) pg/mL respectively; serum HMBG-1 and sTLT-1 contents of control group were (23.51±4.49) pg/mL and (371.6±48.1) pg/mL respectively. After t test, serum HMGB1 and sTLT-1 contents of stroke group were significantly higher than those of control group (P<0.05).

3.2 Serum oxidative stress index contents and their correlation with HMBG-1

Analysis of oxidative stress indexes T-SOD (U/mL), GSH-Px (U/mL), 8-OHdG (ng/mL), LPO (mmol/L) and NOS (U/mL) contents between two groups of subjects was as follows: serum T-SOD and GSH-Px contents of stroke group were significantly lower than those of control group while 8-OHdG, LPO and NOS contents were significantly higher than those of control group. Differences in above oxidative stress indexes in serum were statistically significant between two groups of subjects (P<0.05).

Analysis of T-SOD, GSH-Px, 8-OHdG, LPO and NOS contents between cerebral stroke patients with different HMBG-1 contents was as follows: serum T-SOD and GSH-Px contents of stroke patients with high HMBG-1 content were significantly lower than those of stroke patients with low HMBG-1 content while 8-OHdG, LPO and NOS contents were significantly higher than those of stroke patients with low HMBG-1 content. Differences in above oxidative stress indexes in serum were statistically significant between stroke patients with different HMBG-1 contents (P<0.05).

3.3 Serum endothelial injury index contents and their correlation with sTLT-1

Analysis of serum endothelial injury indexes vWF (pg/mL), vWF-cp (pg/mL), sTM (pg/mL), ET-1 (pg/mL) and D-D (μg/mL) contents between two groups of subjects was as follows: serum vWF, vWF-cp, sTM, ET-1 and D-D contents of stroke group were significantly higher than those of control group. Differences in serum vWF, vWF-cp, sTM, ET-1 and D-D contents were statistically significant between two groups of subjects (P<0.05).

Analysis of serum vWF, vWF-cp, sTM, ET-1 and D-D contents between cerebral stroke patients with different sTLT-1 contents was as follows: serum vWF, vWF-cp, sTM, ET-1 and D-D contents of stroke patients with high sTLT-1 content were significantly higher than those of stroke patients with low sTLT-1 content. Differences in serum vWF, vWF-cp, sTM, ET-1 and D-D contents were statistically significant between stroke patients with different sTLT-1 contents (P<0.05).

Table 1.

Comparison of serum T-SOD, GSH-Px, 8-OHdG, LPO and NOS between two groups of subjects.

Groups	n	T-SOD	GSH-Px	8-OHdG	LPO	NOS
Stroke group	58	66.3±9.3	38.6±5.2	572.5±72.4	13.2±1.6	56.2±7.8
Control group	46	117.6±15.2	78.5±9.1	257.3±33.6	3.2±0.6	23.1±3.5
t		8.958	11.027	12.375	29.585	13.218
P		<0.05	<0.05	<0.05	<0.05	<0.05

Table 2.

Comparison of serum T-SOD, GSH-Px, 8-OHdG, LPO and NOS between stroke patients with different HMBG-1 contents.

HMBG-1 content	n	T-SOD	GSH-Px	8-OHdG	LPO	NOS
Low HMBG-1	29	85.2±11.4	49.3±6.8	394.5±52.6	8.3±1.1	40.2±5.7
High HMBG-1	29	47.6±5.7	27.1±3.6	760.2±93.5	18.7±2.3	74.1±8.9
t		9.181	8.795	8.138	12.385	9.382
P		<0.05	<0.05	<0.05	<0.05	<0.05

Table 3.

Comparison of serum endothelial injury indexes between two groups of subjects.

Groups	n	vWF	vWF-cp	sTM	ET-1	D-D
Stroke group	58	312.5±46.6	84.9±9.5	13.2±1.8	6.7±0.9	0.93±0.11
Control group	46	136.5±16.5	36.1±5.2	4.5±0.7	3.1±0.5	0.32±0.06
t		16.866	12.351	19.283	11.385	17.686
P		<0.05	<0.05	<0.05	<0.05	<0.05

Table 4.

Comparison of serum endothelial injury indexes between stroke patients with different sTLT-1 contents.

sTLT-1 content	n	vWF	vWF-cp	sTM	ET-1	D-D
Low sTLT-1	29	221.3±32.7	66.4±8.2	7.6±0.9	4.6±0.7	0.67±0.09
High sTLT-1	29	407.5±58.3	102.1±13.9	19.4±2.6	9.0±1.3	1.21±0.18
t		8.395	7.786	13.478	9.185	8.492
P		<0.05	<0.05	<0.05	<0.05	<0.05

4. Discussion

Oxidative stress is the important pathological change in the course of ischemic stroke, which not only participates in the atherosclerotic plaque formation and nature change, but is also closely related to brain tissue damage after ischemia hypoxia[5,6]. The increase of oxygen free radical generation is the characteristic of oxidative stress reaction activation, but it is not clear about the regulation mechanism of oxygen free radical generation in ischemic stroke. HMBG-1 is a highly conservative nucleoprotein in mononuclear macrophage. After stimulated by ischemia oxygen and so on, it can be massively released to outside the cells, and then be combined with cell membrane receptor to exert pro-inflammatory effect. The HMBG-1 in atherosclerotic plaques not only promotes the activation of inflammatory responses and affects the stability of plaque, but also increases the generation of oxygen free radicals and causes damage to ischemic local brain tissue[7,8]. In the study, analysis of HMBG-1 content in serum of patients with stroke showed that serum HMBG-1 of stroke group was significantly higher than that of control group. This indicates that the increase of HMBG-1 generation is involved in the occurrence of ischemic stroke, and activating the inflammatory response and oxidative stress may be the possible mechanism for HMBG-1 to be involved in the progression of ischemic stroke.

After stroke, oxidative stress reaction activation and massive oxygen free radical generation can on the one hand, directly react with lipids and nucleic acids in cells to cause cellular structure damage and generate 8-OHdG, LPO and other oxidation products, and on the other hand, cause NOS activation and increase the release of NO. Excessive release of NO can produce neurotoxicity and cause cell damage[9,10]. At the same time, the continuous production of oxygen free radicals can cause the continuous consumption of antioxidant enzymes T-SOD and GSH-Px, resulting in the decrease of antioxidant capacity. In the study, analysis of the changes in serum the oxidative stress indexes in stroke patients indicated that serum T-SOD and GSH-Px contents of stroke group were significantly lower than those of control group while 8-OHdG, LPO and NOS contents were significantly higher than those of control group. This indicates that excessive activation of oxidative stress and the excessive consumption of antioxidant enzymes are closely related to the occurrence of ischemic stroke. Further analysis of the effect of HMBG-1 on oxidative stress in patients with ischemic stroke showed that serum T-SOD and GSH-Px contents of stroke patients with high HMBG-1 content were significantly lower than those of stroke patients with low HMBG-1 content while 8-OHdG, LPO and NOS contents were significantly higher than those of stroke patients with low HMBG-1 content. The results show that the abnormally increased HMBG-1 could result in the aggravation of oxidative stress response in the course of ischemic stroke, and lead to the increase in both the generation of oxygen free radicals and the consumption of antioxidant enzymes.

Endothelial injury is another important pathologic feature of ischemic stroke. The endothelial structure and function injury will on the one hand, promote the infiltration of inflammatory cells and lipids in the endometrium and accelerate the formation of foam cells and atheromatous plaque, and on the other hand, cause the subendothelial collagen exposure and platelet activation, and promote thrombosis[11,12]. In the process of endothelial injury, the activation of platelet is a vital link, but the specific regulation mechanism is not clear. TLT-1 is a protein stored in platelet granule, which can be combined with fibrous protein, moesin and so on to promote platelet to form pseudopod and increase platelet adhesion and aggregation[13]; sTLT-1 is a soluble form of platelet surface TLT-1. In the case of increased TLT-1 expression, oxidative stress and other external stimuli can lead to the increase of sTLT-1 shedding and generation[14]. In the study, analysis of sTLT-1 content in serum of stroke patients showed that serum sTLT-1 content of stroke group was significantly higher than that of control group. This indicates that the increase in sTLT-1 generation is involved in the occurrence of ischemic stroke, and activating endothelial injury may be a possible mechanism for sTLT-1 to participate in the progression

of ischemic stroke.

In the process of endothelial injury, multiple endothelial injury markers will be massively released into the blood circulation. vWF is the most important endothelial marker known at present, and vWF-cp can dissociate the vWF into segments without adhesion activity and inhibit platelet activation and adhesion[15]; TM is the glycoprotein which is distributed on the surface of endothelial cells and is involved in the regulation of thrombus formation and dissolution process; when endothelial cells are damaged, the TM on cell surface is cracked under the action of elastase, enters the blood circulation and becomes sTM[16]. ET-1 is a vasoconstrictor active peptide synthesized and secreted by endothelial cells. The secretion and release of ET-1 increase during stroke, which can cause vasoconstriction and aggravate cerebral ischemia[17]. D-D is a fibrinolytic product. In the process of endothelial injury and platelet activation, clots are formed continuously and will activate the fibrinolytic system and increase the generation of D-D. In the study, analysis of the changes in serum endothelial injury indexes in patients with stroke showed that serum vWF, vWF-cp, sTM, ET-1 and D-D contents of stroke group were significantly higher than those of control group. This indicates that there is excessive endothelial injury in the course of ischemic stroke. Further analysis of the effect of sTLT-1 on endothelial injury in patients with ischemic stroke showed that serum vWF, vWF-cp, sTM, ET-1 and D-D contents of stroke patients with high sTLT-1 content were significantly higher than those of stroke patients with low sTLT-1 content. Therefore, the elevated sTLT-1 can result in the aggravation of endothelial injury in the pathogenesis of ischemic stroke.

Serum HMGB1 and sTLT-1 contents increase remarkably in patients with ischemic stroke; the abnormally elevated HMGB1 can aggravate the oxidative stress response, and the abnormally elevated sTLT-1 can aggravate the endothelial injury.

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