CORRELATION BETWEEN SERUM LEVELS OF HMGB1 AND AB WITH DISEASE SEVERITY IN PATIENTS WITH ACUTE ISCHEMIC STROKE

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ABSTRACT

Objective: *To explore the correlation between serum levels of HMGB1 and Aβ and disease severity in patients with acute ischemic stroke (AIS).*

Methods: *The data of 100 patients with AIS admitted to our hospital from February 2020 to February 2021 were selected for retrospective analysis. According to the National Institutes of Health Stroke Scale (NIHSS) score within 24 hours of admission, the patients were divided into the light group (LG), moderate group (MG) and severe group (SG), and 30 cases with health examination were the control group (CG). After taking the serum of patients, the levels of HMGB1 and Aβ were measured to compare their differences among different groups and analyze the correlation between the levels of HMGB1 and Aβ and the degree of neurological deficits.*

Results: *The NIHSS score in the LG was overtly lower than the MG and SG (P<0.001), and the NIHSS score in the MG was visibly lower than the SG (P<0.001). The levels of HMGB1 and Aβ in the LG were clearly lower than the MG and SG (P<0.001), and higher than the CG (P<0.001). The preliminary analysis of scatter diagram showed a trend of linear correlation between serum levels of HMGB1 and Aβ and NIHSS, with no obvious outlier. According to r-analysis, the correlation between serum HMGB1 level and NIHSS showed r=0.930, r^2=0.865, t=25.007 and Sig<0.001, and the correlation between Aβ level and NIHSS showed r=0.970, r^2=0.941, t=39.488 and Sig<0.001.*

Conclusion: *The serum levels of HMGB1 and Aβ in patients with AIS were positively correlated with the disease severity. In other words, the higher the serum levels of HMGB1 and Aβ, the more severe the AIS.*

Keywords: *Acute ischemic stroke, HMGB1, Aβ, correlation.*

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Introduction

Acute ischemic stroke (AIS), with cerebrovascular atherosclerosis as basic pathological feature, is the most common type of stroke, accounting for about 70.0% of all acute cerebrovascular diseases, whose mortality is as high as 40.0% and disability rate is as high as 80.0%[1-3], seriously endangering the lives and health of Chinese residents. At present, with the help of C-reactive protein and leukocyte count, the patients' condition was analyzed to give them early diagnosis and treatment as much as possible, but such indicators cannot effectively assess disease progression and reduce patient mortality and disability rate[4]. In recent years, domestic and foreign literature have shown that some serum chemical indicators can evaluate the condition of ischemic stroke by reflecting the important pathological links of ischemic stroke[5-6]. For example, high mobility group box-1 protein (HMGB1), a nuclear protein in mononuclear macrophages, has a wide expression in eukaryotic cells and belongs to a delayed inflammatory mediator, which can regulate the production of oxygen free radicals and affect

oxidative stress response. Amyloid beta protein (Aβ) affects the oxidative stress response[7], and is produced by β-secretase and γ-secretase via catalytic pyrolysis of amyloid precursor protein, which has strong neurotoxicity, exerting an important role in inducing neuronal apoptosis and the occurrence and development of atherosclerosis.

HMGB1 and Aβ may have ideal value in evaluating the severity of patients with AIS, which is helpful to evaluate the development of patients and provide objective basis for treatment.

Therefore, this article will use the National Institutes of Health Stroke Scale (NIHSS) score as a medium to analyze the correlation between the levels of HMGB1 and Aβ and NIHSS, then analyzing the actual value of NHMGB1 and Aβ, with the results reported as follows.

Materials and methods

Study design

As a retrospective study, this study was conducted in our hospital from February 2020 to February 2021, aiming to explore the correlation between serum levels of HMGB1 and Aβ and disease severity in patients with AIS.

This study was in accordance with the principles of Declaration of Helsinki (2013)[8], and the patients and their family who were aware of the purpose, significance, content and confidentiality signed the informed content.

Inclusion and exclusion criteria

From February 2020 to February 2021, the data of 100 patients with AIS in our hospital were selected for retrospective analysis, with the inclusion criteria as follows:

• Patients met the diagnostic criteria for AIS established by the fourth national academic meeting for cerebrovascular disease in 1995;

• The results of imaging examination in patients were in line with AIS;

• The time from first onset to admission was within 24 hours.

Exclusion criteria:

• Patients with intracranial hemorrhage;

• Patients with cranial trauma;

• Patients with cerebral infarction due to the vascular malformations;

• Patients with takayasu arteritis and cerebral infarction caused by autoimmune disease;

• Patients with coagulation disorders;

• Patients with hematological diseases;

• Patients with dysfunction in vital organs such as heart, liver and kidney.

Methods and observational criteria

According to the NIHSS score[9] within 24 hours of admission, the patients were divided into the light group (LG), moderate group (MG) and severe group (SG), and 30 cases with health examination were the control group (CG). The NIHSS score was used to evaluate the degree of neurological deficits before and after treatment, including consciousness, gaze, visual field, facial paralysis, upper limb motion, lower limb motion, ataxia, sensation, language expression ability, dysarthria and neglect, with the total score as 42 points.

The mild neurologic impairment was less than 3 points, 4-15 points were moderate neurologic impairment, and severe neurologic impairment was more than 16 points. The fasting venous blood of the patients was taken to centrifuge at 3000 r/min for 5 min, and the supernatant was collected and stored at -20°C for test. After collecting the samples, the levels of HMGB1 and Aβ were measured via enzyme-linked immunosorbent assay (Beijing Kewei Clinical Diagnostic Reagent Inc.; NMPA approval No.: S20060028) to compare the level differences of HMGB1 and Aβ among different groups, and the correlation between the levels of HMGB1 and Aβ and the degree of neurological deficit was evaluated by r-analysis.

Statistical treatment

The software SPSS20.0 was used to analyze the experimental data, and GraphPad Prism 7 (GraphPad Software, San Diego, USA) was adopted to draw pictures of data. The enumeration data and measurement data in this study were tested by X^2 and t test. $P<0.05$ indicated a statistical significance in difference.

Results

Comparison of general information in patients

According to NIHSS score, there were 45 cases in the LG, 33 cases in the MG and 22 cases in the SG. Among them, there were 28 males and 17 females in the LG, with the average age of (55.33±5.24) years old and the body mass of (65.65±3.57) kg, the numbers in the MG were 20, 13, (54.30±5.29) years old and (65.74±3.50) kg, the numbers in the SG were 15, 7, (56.36±5.16) years old and (64.80±3.65) kg,

and the numbers in the CG were 19, 11, (55.37±5.20) years old and (65.77±3.45) kg, with no obvious difference in gender and age among the four groups (P>0.05).

Comparison of NIHSS scores in patients

The NIHSS score in the LG was overtly lower than the MG and SG (P<0.001), and the NIHSS score in the MG was visibly lower than the SG (P<0.001), as shown in Figure 1.

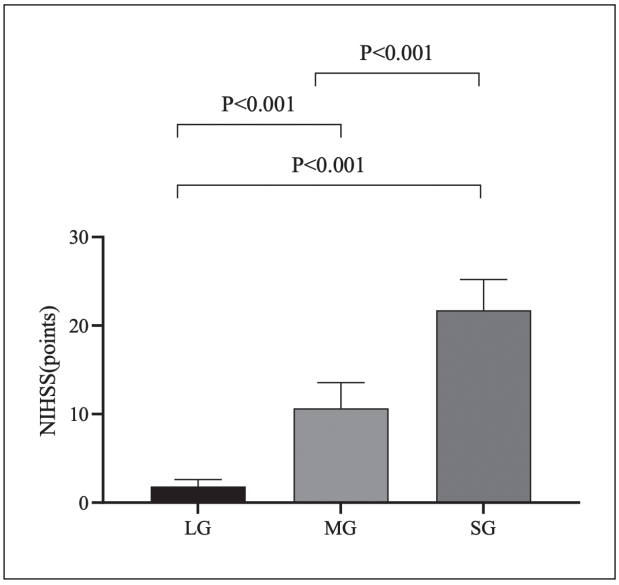

Figure 1: Comparison of NIHSS score in patients ($\bar{x}$±s, points).
Notes: The transverse axis showed the LG, MG and SG, and the vertical axis showed the NIHSS (points).

The NIHSS score in the LG was distinctly lower than the MG and SG (1.84±0.76 vs 10.67±2.88 vs 21.73±3.49, P<0.001).

Comparison of serum levels of HMGB1 and Aβ in patients

The levels of HMGB1 and Aβ in the LG were clearly lower than the MG and SG (P<0.001), and higher than the CG (P<0.001), as shown in Figure 2.

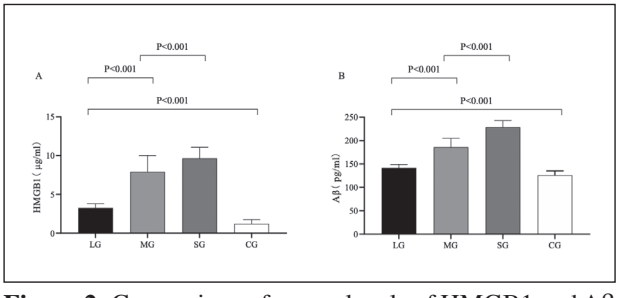

Figure 2: Comparison of serum levels of HMGB1 and Aβ in patients ($\bar{x}$±s).
Notes: Figure 2A showed HMGB1 (μg/ml) and Figure 2B showed Aβ (pg/ml).

The HMGB1 level in the LG was clearly lower than the MG and SG (3.25±0.55 vs 7.91±2.10 vs 9.66±1.41, P<0.001), and higher than the CG (3.25±0.55 vs 1.22±0.54, P<0.001), while the Aβ level in the LG was overtly lower than the MG and SG (141.80±7.30 vs 185.90±19.13 vs 228.67±14.06, P<0.001), and higher than the CG (141.80±7.30 vs 125.65±9.65, P<0.001).

Scatter diagram of correlation between serum levels of HMGB1 and Aβ and NIHSS

The preliminary analysis of scatter diagram showed a trend of linear correlation between serum levels of HMGB1 and Aβ and NIHSS, with no obvious outlier. See Figure 3 and Figure 4.

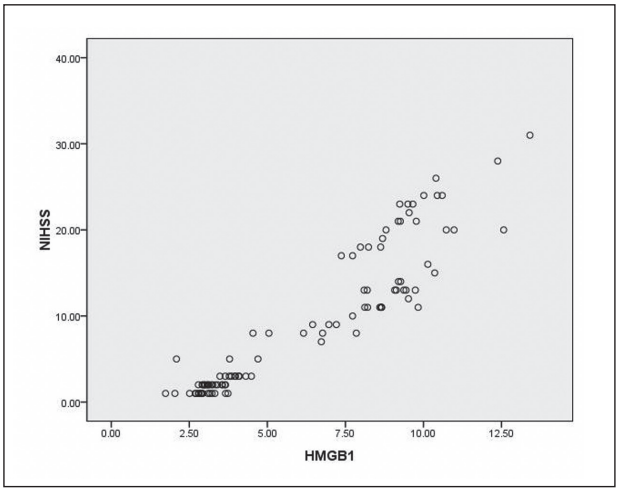

Figure 3: Scatter diagram of correlation between serum HMGB1 level and NIHSS.

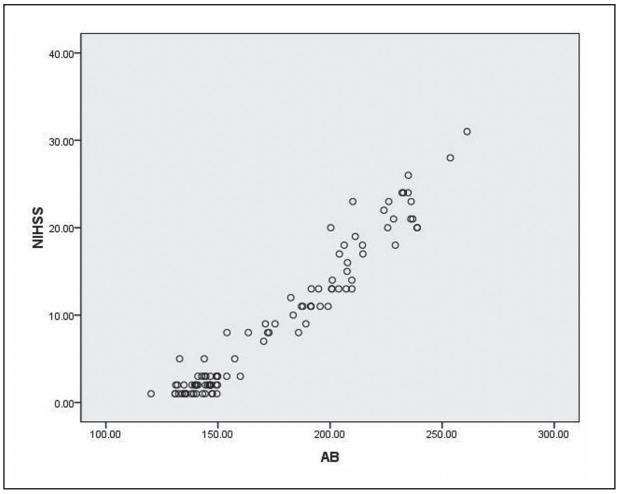

Figure 4: Scatter diagram of correlation between serum Aβ level and NIHSS.

R-analysis of correlation between serum levels of HMGB1 and Aβ and NIHSS

According to r-analysis, the correlation between serum HMGB1 level and NIHSS showed

r=0.930, r^2=0.865, t=25.007 and Sig<0.001, and the correlation between Aβ level and NIHSS showed r=0.970, r^2=0.941, t=39.488 and Sig<0.001.

Discussion

AIS is caused by cerebral vascular atherosclerosis, and patients with intracranial vascular embolism and cerebral artery stenosis result in the reduction of cerebral blood flow and hypoxia and ischemia in blood supply of brain tissue, ultimately leading to acute brain dysfunction[10-11]. In the process of its development, oxidative stress reaction is an important pathological link, with the increase of oxygen free radicals as the main feature of oxidative stress activation, and it is not only involved in the formation of atherosclerotic plaques, but also participates in the brain tissue injury after hypoxia and ischemia[12-14], but its specific mechanism is not clear. Zhang Xiaopeng[15] has found that the levels of total superoxide dismutase and glutathione peroxidase in the serum of patients with AIS are signally lower than the healthy control group, while the levels of 8-hydroxydeoxyguanosine and lipid peroxide are markedly higher than the healthy control group, which confirms that the excessive activation of oxidative stress reaction is closely related to the occurrence of ischemic stroke.

The contents of total superoxide dismutase and glutathione peroxidase in stroke patients with high HMGB1 content were prominently decreased, while the contents of 8-hydroxydeoxyguanosine and lipid peroxide were notably increased, confirming that the abnormal expression of HMGB1 could aggravate oxidative stress, and accelerate the generation of oxygen free radicals and the consumption of antioxidant enzymes.

This study found that the HMGB1 level in the LG was notably lower than the MG and the SG (P<0.001), and higher than the CG (P<0.001) by analyzing the HMGB1 levels of patients with AIS and healthy controls, which suggests that the HMGB1 level was closely related to the severity of brain injury, speculating the mechanism is that HMGB1, as a highly conserved nuclear protein in mononuclear macrophages, is secreted slightly by nerve cells and glial cells in normal conditions, and its release frequency increases under the influence of hypoxia and ischemia[16-17]. Then it exerts a proinflammatory effect by combing with the membrane receptor to trigger a cascade reaction of systemic inflammatory, which affects the stability of the plate, thereby accelerating the formation of oxygen free radicals and aggravating brain tissue damage at the blood supply site[18]. Yang K et al.[19] have believed that HMGB1 is a inflammatory mediator in sepsis, rheumatoid arthritis and other inflammatory diseases, and excessive inflammatory response will aggravate craniocerebral injury. Liu Jing et al.[20] have reported that the HMGB1 level in patients with ischemic stroke was higher than the healthy subjects, and the HMGB1 level was positively correlated with NIHSS and mRS scores, suggesting that the HMGB1 level is related to the prognosis of patients with ischemic stroke.

The preliminary analysis of the scatter diagram in this paper showed that there was a trend of linear correlation between HMGB1 level and NIHSS, with no obvious outlier. According to r-analysis, the correlation between serum HMGB1 level and NIHSS showed r=0.930, r^2=0.865, t=25.007 and Sig<0.001, indicating that the NIHSS score was affected by HMGB1 level in 86.5% of condition, which confirms the correlation between them.

In addition to HMGB1, Aβ also has an important relationship with oxidative stress[21-22]. Aβ is produced by β-secretase and γ-secretase via catalytic pyrolysis of amyloid precursor protein, with a strong neurotoxicity in extracellular matrix deposition, which could indirectly or directly product the lipid peroxidation, causing oxidative stress, thus leading to multiple diseases in combination with enkephalinase and insulin-degrading enzymes[23]. A large number of studies have confirmed that the marker is related to the development of diseases such as cerebral amyloid angiopathy and spinal cord injury. In cerebral amyloid angiopathy, Aβ can directly inhibit the secretion of fibroblast growth factor-2, so that a large number of fibers are deposited around the artery wall, resulting in amyloidosis in vascular function and morphology. Zhang Tian et al.[24] have believed that the neurotoxicity of Aβ is closely connected with the occurrence of stroke, which may be the main influencing factor of early neurological deterioration in patients with AIS. Qin Xue et al.[25] have shown that Aβ also has a high expression in patients with AIS, indicating that Aβ level exerts an important value in evaluating the condition of patients with AIS.

This study found that the Aβ level in the LG was signally lower than the MG and SG (P<0.001), and higher than the CG (P<0.001). The preliminary analysis of the scatter diagram showed that there was a tend of linear correlation between Aβ level

and NIHSS. According to r-analysis, the correlation between serum Aβ level and NIHSS showed r=0.970, r^2=0.941, t=39.488 and Sig <0.001, which confirmed the value of Aβ in evaluating the severity of patients.

In conclusion, the serum levels of HMGB1 and Aβ in patients with AIS were positively correlated with the disease severity. In other words, the higher the serum levels of HMGB1 and Aβ, the more severe the AIS.

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