The Change of GFAP or S100B Concentration in Serum Before and After Carotid Artery Stenting

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Research article

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Abstract

Background—This study explored the role of GFAP and S100B as cerebral biomarkers in the pre-operative evaluation and post-operative efficacy in monitoring of carotid stent implantation. **Method**—32 with unilateral carotid stenosis who underwent carotid artery stenting (CAS) enrolled in the CAS group. The blood samples of operation patients were collected on three different time points: T1: the day before operation; T2: 24 hours after operation; T3, 72 hours after operation. 32 who were excluded for carotid artery stenosis by Digital Subtraction Angiography (DSA) were selected as the control group. The blood samples of patients in control group were collected at D1 (before DSA) and D2 (24 hours after DSA). **Results**—(1). The serum concentrations of GFAP and S100B was higher in the CAS group before operation than those in DSA group— $P < 0.05$. (2). In the operation group, GFAP and S100B increased significantly on the first day after operation (T2) and decreased gradually on the third day after operation (T3) but increased compared with that before operation (T1) with statistical significance ($P < 0.05$). (3). For patients with symptomatic stenosis before operation, the concentrations of GFAP and S100B in serum were higher than those in patients without symptomatic stenosis ($P < 0.05$). **Conclusions**—The cerebral biochemical markers GFAP and S100B have a certain change trend after CAS, which can be used as a method to evaluate and monitor the curative effect before and after carotid artery stent implantation combined with imaging tools.

Background

Carotid Artery Stenting (CAS) has been the micro-surgical method advocated in the recent 20 years. Among patients with symptomatic or asymptomatic carotid stenosis, CAS and Carotid Endarterectomy (CEA) are preventive measures for ischemic cerebrovascular disease. The risk of the composite primary outcome of stroke, myocardial infarction, and death do not significantly differ in patient groups undergoing CAS or undergoing CEA [1]. However, CAS is easy to accept because of its minimally invasive operation and short postoperative hospitalization period [2].

In clinical work, the long-term effect monitoring of CAS patients can evaluate the improvement of post-operative stenosis by DSA, CTA, MRA and other imaging methods. On the one hand, it is not accurate to use only the diameter of vasodilation to express the effective perfusion after operation. On the other hand, for the short-term effect evaluation after CAS, the above imaging examination methods have the disadvantages of vulnerability to human body, poor repeatability, high cost and so on.

Cerebral biochemical markers are the research hotspots in the recent 20 years, mainly including GFAP, S100B, Neuron Specific Enolase (NSE) and Myelin Basic Protein (MBP), etc [3]. At present, it has been confirmed that the concentrations of GFAP, S100B, NSE, MBP in cerebrospinal fluid and serum are related to the size of cerebral infarction area, the deficit of neurological function and the pre- and post-recovery of function [3-6]. So the researchers advocate the use of cerebral biochemical markers in CSF and serum combined with imaging examination as an assisting tool for the diagnosis, disease assessment and prognosis monitoring of cerebral infarction or other brain injury.

Therefore, this study combined CAS and cerebral biochemical markers to explore the expression of GFAP and S100B before and after CAS. Although there are imaging tools as the examination methods for the improvement of the degree of vascular stenosis after CAS operation, this is the first time for cerebral biochemical markers as the evaluation method.

Materials And Methods

Research object: From April 2018 to August 2019, Digital Subtraction Angiography (DSA) was performed in the Department of Neurology of Sichuan Provincial People's Hospital to diagnose unilateral carotid stenosis, then patients underwent carotid stent implantation were assigned as the operation group. The inclusion criteria of the operation group were: (1) symptomatic stenosis $\geq 50\%$ and the clinical symptoms were consistent with the vascular area of stenosis; (2) asymptomatic stenosis $\geq 80\%$; (3) age > 18 years. Exclusion criteria of the operation group: (1) patients with brain injury within 6 months; (2) patients with nervous system related tumor diseases; (3) patients with infectious diseases of nervous system (such as encephalitis, meningitis, myelitis, etc.); (4) patients with demyelination and degenerative diseases of nervous system (such as multiple sclerosis, optic neuromyelitis, Parkinson's disease, Alzheimer's disease, etc.); (5) patients with severe heart, liver, kidney Lung disease could not tolerate surgery. At the same time, the patients who were excluded from intracranial and extracranial vascular stenosis by DSA in neurology department were selected as the control group. This study was reviewed by the ethics committee of Sichuan Provincial People's Hospital, and all patients signed informed consent.

Surgical procedure: The patients in the operation group were given dual antiplatelet therapy (aspirin 100mg and clopidogrel 75mg) three days before operation. The operation was performed by more than two experienced neuroscientists. Under the guidance of the guiding wire, the finger guide tube was placed near the opening of the beginning of the internal carotid artery in the affected side, and the protective umbrella was placed at the distal end of the stenosis artery. Then the stent was placed along the guide wire in the stenosis area. After precise positioning, the stent was implanted. The operation was completed, after the angiography showed that the carotid artery was unobstructed, the stent was well attached to the wall, no obvious residual stenosis was found, and the anterior blood flow was normal (TICI Level 3) [7]. Aspirin was taken for life after operation, combined with clopidogrel for at least 6 months.

Evaluation of carotid stenosis: The degree of carotid stenosis was determined by more than two neurologists, and the criteria were calculated according to the European Carotid Surgery Study (ECST) [8] and North American symptomatic carotid endarterectomy (NASCET) [9]. That is, stenosis degree (%) = (1 - diameter of stenosis / diameter of distal normal lumen) $\times 100\%$. The classification included: normal / mild stenosis ($< 50\%$), moderate stenosis (50-69%), severe stenosis (70-99%), complete occlusion (100%).

Collection and measurement on serum samples: In the operation group, 3 ml of blood was collected in the morning before operation (T1), one day after operation (T2) and three days after operation (T3). In the control group, the blood was collected in the morning before DSA (D1) and one day after operation (D2).

The blood samples were stored at - 80 °C before measurement. The ELASA procedure for detecting concentrations of serum GFAP and S100B was as follows: either anti-GFAP (Human GFAP ELISA KIT, ZC-34594) or anti-S100B (Human S100B ELISA KIT, ZC-32056) antibodies were coated in 96 well microporous plates to make solid-phase carriers, and standards or samples are added to the micropores respectively. The GFAP and S100B are attached to their specific antibody binding on the solid-phase carriers, then after thorough washing, GFAP and S100B antibodies are added. After the unconjugated biotin antibodies are cleaned, HRP labeled avidin is added, and then they are washed thoroughly again. TMB substrate was added and converted to blue under the catalysis of peroxidase, and finally to yellow under the action of acid. The color depth was positively correlated with concentration of either GFAP or S100B protein. The absorbance (OD value) was measured at 450 nm wavelength with the microplate reader, and the sample concentrations were calculated.

Statistical analysis: SPSS 22.0 was used for statistical analysis. The measurement data was expressed by mean $\pm$ standard deviation ($\bar{x} \pm s$), and t test was used for the comparisons between groups. The counting data was expressed by frequency and percentage, and chi-square test was used for comparison. Statistical significance was defined as $P < 0.05$.

Results

Demographic data: Following the criteria, 32 patients were enrolled in the operation group. Among them, 2 male patients were excluded because of serious postoperative complications. In total 30 patients, there were 20 males and 10 females. Their ages were 42 to 77 years old with an average age of 69.05 ± 10.83 years. There were 30 patients in the control group, 16 males and 14 females, aged 38 to 81 years, with an average age of 67.34 ± 7.56 years. There was no statistical difference between the operation group and the control group ($P > 0.05$, Table 1).

Image analysis on patients in the operation group: There were 30 patients in the operation group, including 18 patients with symptomatic stenosis and 12 patients with asymptomatic stenosis. There were 10 cases of moderate stenosis, 17 cases of severe stenosis and 3 cases of complete occlusion. The results of cerebral angiography before and after shown in Figure 1A, 1B.

The changes of serum GFAP and S100B in different groups: The serum concentrations of GFAP and S100B in the operation group and the control group before and after operation are shown in Table 2. (1)The serum GFAP and S100B in the control group had no significant change before and after DSA ($P > 0.05$). The serum GFAP and S100B in the operation group were higher than those patients in the control group, and the difference was statistically significant ($P < 0.05$). The serum GFAP and S100B in the operation group were significantly higher on the first day after operation, and decreased on the third day after operation, but still higher than those before operation. Comparing to prior to operation, the level of both GFAP and S100B were statistically significantly increased at T1 and T3 ($P < 0.05$, Figure 2A and B).

(2)The serum concentrations of GFAP and S100B in patients with symptomatic stenosis before operation were higher than those in patients with asymptomatic stenosis, and the difference there was statistically

significant ($P < 0.05$), but there was no significant difference between both groups after operation ($P > 0.05$, Table 3 and 4).

(3) For patients with different degree of carotid artery stenosis, the serum GFAP and S100B levels had no change before and after operation ($P > 0.05$).

Discussion

Studies have confirmed that the rate of symptomatic internal carotid artery stenosis is $>50\%$, the rate of asymptomatic internal carotid artery stenosis is $>80\%$, and at least one patient with high risk factors for CEA treatment, CAS efficacy is not inferior to CEA [10]. Three large international multicenter randomized controlled trials also failed to demonstrate that CAS was less effective than CEA [11-13]. In addition, there was no significant difference in primary endpoint (perioperative stroke, myocardial infarction, and ipsilateral stroke of responsible vessels within 4 years) in both symptomatic and asymptomatic carotid stenosis (CAS group $7.2\% \pm 0.8\%$, CEA group $6.8\% \pm 0.8\%$, $P = 0.51$); but in terms of health-related quality of life, due to the small injury of CAS and short postoperative hospitalization period, it will benefit more in the short term [14].

Astrocytes are the main glial cells in brain tissue, which interact with neuronal cells and function to regulate neurotransmitters, promote immune responses, regulate intracranial blood flow, ions and antioxidants [15]. GFAP and S100B are the main components and signature proteins of astrocytes, and their presence ensures the maintenance and functional function of astrocyte morphological structure [16-17]. The increase of GFAP and S100B in cerebrospinal fluid (CSF) [18-19] and blood [20] reflects the formation of astrocyte filaments in the central nervous system. High concentrations of GFAP and S100B suggest the destruction of acute brain tissue. A moderate increase suggests the astrogliosis, the formation of scars, and delayed ischemic tolerance [21], which plays an important role in promoting neuronal survival and repairing after brain injury [22]. In addition, Herrmann et al found that the release of GFAP and S100B were significantly correlated with the incidence of cerebral infarction. While for patients with lacunar or mild stroke, GFAP was found to be a more sensitive cerebral biochemical marker [23].

The results in this study show that the serum concentration of GFAP and S100B from the operation patients' serum is higher than that in the control group. DSA has confirmed that the patients in the operation group have different degrees of carotid artery stenosis, which makes the brain tissue in the state of chronic ischemia and hypoxia, and chronic cerebral ischemia will inevitably lead to brain tissue damage. Meanwhile, astrocytes are very sensitive to cerebral ischemia and hypoxia, which could cause the astrocytes produce excessive GFAP and S100B into the CSF. These proteins as biomarkers can be in turn released into the peripheral blood through impaired blood-brain barrier, so that the increases of GFAP and S100B could be detected in blood serum. In addition, for patients with symptomatic carotid stenosis, the increased level of GFAP and S100B in serum is more significant. After CAS operation, GFAP and S100B in serum increased first at T2 and then decreased at T3, but they both were higher than those before CAS operation. The long-term stenosis of carotid artery can cause brain tissue to establish a

certain collateral circulation. When the application of the CAS mechanically expands the blood vessels, the temporary relative balanced brain tissue perfusion established before operation can be ruined, so that the excessive release of GFAP and S100B by astrocytes may happen which could be a response of cerebral ischemia-reperfusion. However, with the release of the stenosis after CAS, the blood flow of the stenosis or occlusion vessels was reestablished, the cerebral ischemia and hypoxia injury caused by insufficient blood perfusion was gradually recovered, which was reflected in the decrease of production of both GFAP and S100B proteins locally. It has been confirmed that the moderate increase of GFAP and S100B is related to repairing after brain injury [22]. Therefore, the level of biochemical markers within 3 days after operation is still higher than that before operation, suggesting that brain tissue is still in the process of injury repairing after CAS. In addition, it is reported that the reactive gliocytosis has dual effects [23]. When it cannot be solved in the acute and early chronic phase after injury, the reactive gliosis can have negative impact or consequences to injury area. If the intervention measures are adjusted correctly in the best time window, new methods of treating nervous system injury may be developed. The data in our current study is consistent with the results observed by Wunderlich MT [24]. When alteplase was used to a thrombolysis to patients with middle cerebral artery occlusion, GFAP concentration can slightly decrease comparing to those in patients without thrombolysis, but it is still higher than normal control. For patients with different degrees of carotid artery stenosis, the serum concentrations of GFAP and S100B did not change significantly before and after operation. We considered that the cause of this result may be due to the mixed analysis of the patients with symptomatic and asymptomatic stenosis.

Conclusion

For the evaluation of the short-term effect after CAS, the changes of GFAP and S100B in serum can be monitored to timely reflect the changes after operation; for the evaluation before operation and the long-term monitoring of the effect after operation, biochemical markers and imaging tools can be used as the evaluation means; at the same time, the detection of GFAP and S100B in serum has the advantages of simplicity, low price, repeatability and small damage. However, the critical serum values of GFAP and S100B with different degrees of stenosis, different stages of operation and normal human need further study. In addition, the correct intervention of GFAP and S100B in the best time window after carotid stent implantation may lead to the development of new methods for the treatment of nervous system injury.

Declarations

Ethics approval and consent to participate:

this study was approved by the ethics committee of Sichuan Provincial People's Hospital, and all patients signed informed consent.

Consent for publication:

Not applicable

Competing interests:

The authors declare that they have no competing interests.

Availability of data and materials:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

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Author's contributions:

Xiaofan Yuan and Jianhong Wang designed the study; Shu Yang and Fuqiang Guo operated the surgery; Xiaofan yuan and Lei Guo carried out the study; Duozi Wang and Jie Huang analyzed the results; Xiaofan Yuan and Fuqiang Guo wrote the manuscript.

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Tables

Table 1 Demographic data in the DSA and CAS patient groups

Category	DSA group	CAS group	T or χ^2	P value
Mean $\pm$ SD age(y)	67.34 $\pm$ 7.56	69.05 $\pm$ 10.83	-0.373	0.81
Sex(male)	16 (53.33)	20 (66.67)	1.286	0.43
Diabetes	13 (43.33)	2 (73.33)	0.689	0.76
Hypertension	16 (53.34)	21 (70.00)	0.694	0.63
Hyperlipidemia	12 (40.00)	17 (56.67)	0.068	0.20
Heart disease	8 (26.67)	9 (30.00)	0.424	0.34
Current smoker	5 (16.67)	7 (23.33)	1.494	0.29
Stroke history	5 (16.67)	18 (60.00)	3.320	0.43
HbA1c (%)	5.88 $\pm$ 0.40	6.04 $\pm$ 0.76	-2.000	0.11
Hyperuricemia	304.23 $\pm$ 47.30	325.44 $\pm$ 50.68	1.267	0.27
Obesity	12 (40.00)	15 (50.00)	0.546	0.51

Table 2 The serum concentrations of GFAP and S100B in patients at different time points

Group	GFAP μ g/ml	S100B μ g/ml
D1	20.059 $\pm$ 10.219	0.853 $\pm$ 0.162
D2	21.392 $\pm$ 09.022	0.909 $\pm$ 0.127
T1	25.392 $\pm$ 11.022	1.500 $\pm$ 0.804
T2	29.877 $\pm$ 14.979	1.974 $\pm$ 1.082
T3	27.038 $\pm$ 12.294	1.786 $\pm$ 0.975

Table 2: The serum concentrations of GFAP and S100B did not change significantly before and after DSA (D1 vs. D2 $\square$ P $\square$ 0.05). Both GFAP and S100B in the DSA group had lower concentrations when compared with the CAS group $\square$ D1/D2<T1/T2/T3 $\square$ D1 vs T1 $\square$ T1 vs T2 and T1 vs T3 $\square$ P<0.05).

Table 3 Serum concentration of GFAP in symptomatic and asymptomatic stenosis patients

Group	Symptomatic stenosis	asymptomatic stenosis	P value
T1	27.685±10.265	24.960±10.167*	0.031
T2	30.317±12.384	28.257±09.742**	0.102
T3	28.984±11.309	25.746±11.473**	0.298

*P<0.05 **P>0.05.

Table 4 Serum concentration of S100B in symptomatic and asymptomatic stenosis patients

Group	Symptomatic stenosis	asymptomatic stenosis	P value
T1	1.675±1.212	1.329±0.881*	0.048
T2	2.170±1.007	1.845±0.987**	0.376
T3	1.829±1.102	1.520±1.003**	0.769

Table 3-4: Before carotid artery stenting, the serum concentrations of GFAP and S100B in patients with symptomatic carotid stenosis were higher than patients with asymptomatic carotid stenosis (*P<0.05). However, there was no significant difference in patients with symptomatic or asymptomatic carotid stenosis after operation **P>0.05.

Figures

1A

1B

Figure 1

1A: The carotid sinus showed severe stenosis before carotid artery stenting. 1B: The stenosis in carotid sinus was significantly improved after carotid artery stenting and the reperfusion was restored.

Figure 2

After surgery, the serum concentrations of GFAP (2A) and S100B (2B) in the CAS group increased at T2 and then decreased at T3, but both proteins in T2 and T3 were higher than those at T1. T2 > T3 > T1; T1 vs T2 and T1 vs T3: $P < 0.05$.