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Value of serum OPN levels in patients with acute cerebral hemorrhage for assessment of nerve function impairment

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ABSTRACT

Objective: To study the value of serum OPN levels in patients with acute cerebral hemorrhage for assessment of neurological impairment.
Methods: A total of 48 patients with cerebral hemorrhage admitted to the Emergency Department of our hospital from April 2014 to August 2015 were selected as the cerebral hemorrhage group, and 50 cases who took health examination were selected as the control group. Then, their clinical data were collected and the contents of OPN, S-100β, NSE, SOD, T-AOC and MDA were detected.
Results: The contents of serum OPN [(24.52 ± 2.85) *vs.* (10.38 ± 1.25) pg/mL], S-100β [(1.77 ± 0.20) *vs.* (0.59 ± 0.07) pg/mL] and NSE [(24.52 ± 2.85) *vs.* (10.38 ± 1.25) pg/mL] in the cerebral hemorrhage group were significantly higher than those of the control group, while its SOD [(42.31 ± 5.45) *vs.* (67.65 ± 7.26) IU/mL] and T-AOC [(12.16 ± 1.42) *vs.* (20.35 ± 2.35) IU/mL] were all distinctly lower than those of the control group. The greater the amount of hemorrhage was, the more the contents of OPN [(5.75 ± 0.67) *vs.* (7.92 ± 0.91) *vs.* (10.36 ± 1.16) ng/mL], S-100β [(1.03 ± 0.12) *vs.* (1.79 ± 0.20) *vs.* (2.85 ± 0.30) pg/mL], NSE [(17.86 ± 2.14) *vs.* (23.94 ± 2.61) *vs.* (30.14 ± 3.48) pg/mL], MDA [(4.03 ± 0.51) *vs.* (6.18 ± 0.81) *vs.* (9.59 ± 1.05) nmol/mL] were and the less the contents of SOD [(52.44 ± 5.94) *vs.* (41.39 ± 5.26) *vs.* (25.52 ± 3.12) IU/mL] and T-AOC [(16.59 ± 1.83) *vs.* (13.04 ± 1.63) *vs.* (8.39 ± 0.92) IU/mL] became. The contents of serum OPN [(13.23 ± 1.63) *vs.* (6.95 ± 0.81) ng/mL], S-100β [(3.24 ± 0.52) *vs.* (0.91 ± 0.10) pg/mL], NSE [(41.32 ± 5.14) *vs.* (16.61 ± 1.89) pg/mL] and MDA [(11.18 ± 1.26) *vs.* (4.28 ± 0.54) nmol/mL] in patients with early deterioration were obviously higher than those in patients without early deterioration, while their SOD [(15.25 ± 1.79) *vs.* (40.59 ± 5.33) IU/mL] and T-AOC [(5.25 ± 0.77) *vs.* (16.25 ± 1.92) IU/mL] contents were all evidently lower. The content of serum OPN was positively associated with S-100β, NSE and MDA, but negatively correlated with SOD and T-AOC.
Conclusions: The level of serum OPN in patients with acute cerebral hemorrhage increased significantly. The level of serum OPN could estimate the bleeding volume and the severity of nerve function impairment for patients with acute cerebral hemorrhage.

1. Introduction

In recent years, the incidence rate of stroke in our country is rising constantly and exceeds cardiovascular disease, which has become a leading cause for death and disability. Stroke was divided into cerebral ischemic stroke and hemorrhagic stroke. Although the incidence rate of cerebral ischemic stroke is relatively high, hemorrhagic stroke causes more dangers and injuries the nerve functions rapidly which harms patients greatly[1,2]. After cerebral hemorrhage, symptoms such as headache, dizziness, vomit and so on would appear and then develop to limbs dysfunction, unconsciousness quickly. In clinical practice, the establishment of therapeutic regimen relies on the accurate judgment of the disease[3,4]. However, most cerebral hemorrhage patients remain in a coma when they are treated, and they will stay in a coma for a period of time after they receive evacuation of hematoma, which makes it difficult to

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The study protocol was performed according to the Helsinki declaration and approved by Theory Committee of the hospital. Informed written consent was obtained from all subjects.

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estimate the severity of patient's conditions and the degree of nerve function impairment correctly by routine neurological function evaluation method[5,6].

The pathophysiological procedure of acute cerebral hemorrhage is very complicated. Intracranial hematoma can not only cause mechanical press and damage the brain tissue, but also affect the function of neurons simultaneously by multiple humoral factors such as inflammation and oxidative stress[7,8]. In the development of cerebral hemorrhage, changes of the above humoral factors will lead to changes of the contents of multiple molecular in serum. Changes of the disease can be evaluated by detecting the contents of the corresponding molecular in serum. Osteopontin (OPN) is a kind of secretory extracellular matrix protein participating in the regulation processes such as immunological and inflammatory reaction, atherosclerotic plaque formation *etc*. There were researches reporting that the content of serum OPN in patients with cerebrovascular diseases, such as cerebral infarction and myocardial infarction, increased significantly and matched with the severity of the disease[9,10]. Nevertheless, researches whether OPN has participated in the occurrence and development of acute cerebral hemorrhage or not have not been reported. In the following study, we investigate the value of serum OPN levels in patients with acute cerebral hemorrhage for assessment of nerve function impairment.

2. Materials and methods

2.1. Study subjects

A prospective study was conducted. All study subjects were selected in accordance with the following criteria and grouped into the cerebral hemorrhage group and control group. The study was approved by the Theory Committee of the hospital and all subjects signed an informed consent. The inclusion criteria of subjects in the cerebral hemorrhage group were described as follows. Patients who were treated in the Emergency Department of our hospital from April 2012 to August 2015, met the diagnosis standards of cerebral hemorrhage made by the Fourth National Cerebrovascular Diseases Conference, admitted to hospital within 12 h after the onset of the disease and diagnosed with cerebral hemorrhage by CT examination were included in the cerebral hemorrhage group, while patients with tumor, trauma, vascular malformation caused cerebral hemorrhage, subarachnoid hemorrhage, intracranial tumor, history of antiplatelet or anticoagulant drugs and accompanied with heart failure or liver and renal diseases were excluded. Subjects of the control group were healthy people whose gender, age, BMI and were matched with those of patients in the cerebral hemorrhage group. They were all from the Physical Examination Center of our hospital and proved to be without cranial vascular diseases and liver and kidney dysfunction.

2.2. Clinical materials collection

According to their medical records or examination reports, the clinical materials of those study subjects were collected and typed into computers. The concrete materials included their gender, age, height, weight, blood pressure, body temperature and number of combined hypertension, diabetes and hyperlipidemia. Patients with cerebral hemorrhage would be given a CT examination after admission and the hematoma volume would be tested and calculated in accordance with Tada formula: hematoma volume = $\pi \times$ length (cm) $\times$ width (cm) $\times$ height (cm)/6. It could be defined as mild hemorrhage when the hematoma volume was less than 20 mL, moderate hemorrhage when it was 20–40 mL and severe hemorrhage when it exceeded 40 mL. Patients were all received NIHSS scores when admitted to hospital and 2 days after admission. Patients with an increase of four or more sores within 2 days were diagnosed with early neurological deterioration.

2.3. Serum samples collection and indexes detection

As soon as cerebral hemorrhage patients admitted to hospital, 10 mL peripheral venous blood of them were collected immediately, while the same amount of peripheral venous blood of subjects in the control group were collected during their physical examination, placed still for 10–15 min and centrifuged with a centrifugal force of 3 000 g for 10 min to gather the upper serum. ELISA kits were used to determine the contents of OPN, S-100β protein, neurone specific enolase (NSE), superoxide dismutase (SOD), total antioxidant capacity (T-AOC) and malonaldehyde (MDA).

2.4. Statistical methods

Version SPSS 19.0 of the software was applied to record and analyze data. Measurement data were presented as mean ± SD. Analysis between groups were determined by *t*-test. Enumeration data were expressed by frequency and analyzed by *Chi*-square test. The collection between two variables was tested by Pearson's correlation analysis. Differences were statistical significant when $P < 0.05$.

3. Results

3.1. Clinical data of subjects of the two groups

In the 48 cases of the cerebral hemorrhage group, there were 30 males and 18 females with ages of (52.4 ± 6.4) years, BMI of (22.1 ± 2.5) kg/m^2, and 27 cased were combined with hypertension, 11 with diabetes and 13 with hyperlipidemia. In the control group, there were 33 males and 17 females with ages of (53.1 ± 5.9) years and BMI (22.5 ± 2.3) kg/m^2, and 9 cases were combined with hypertension, 4 with diabetes and 11 with hyperlipidemia. The results of statistic analysis showed no differences in gender, age, BMI and the combined hyperlipidemia between the two groups, while the combined hypertension and diabetes cases of the cerebral hemorrhage group were obviously higher than those of the control group. The serum OPN [(8.14 ± 0.95) *vs.* (3.37 ± 0.42) ng/mL], S-100β [(1.77 ± 0.20) *vs.* (0.59 ± 0.07) pg/mL] and NSE [(24.52 ± 2.85) *vs.* (10.38 ± 1.25) pg/mL] of the cerebral hemorrhage group were all significantly higher than those of the control group, while its SOD [(42.31 ± 5.45) *vs.* (67.65 ± 7.26) IU/mL] and T-AOC [(12.16 ± 1.42) *vs.* (20.35 ± 2.35) IU/mL] were evidently lower than those of the control group (Table 1).

Table 1
Clinical data of subjects of the two groups.

Parameter	Cerebral hemorrhage group (n = 48)	Control group (n = 50)	P
Gender (male/female)	30/18	33/17	> 0.05
Age (year)	52.4 ± 6.4	53.1 ± 5.9	> 0.05
BMI (kg/m^2)	22.1 ± 2.5	22.5 ± 2.3	> 0.05
Hypertension	27 (56.25%)	9 (18%)	< 0.05
Diabetes	11 (22.92%)	4 (8%)	< 0.05
Hyperlipidemia	13 (27.08%)	11 (22%)	> 0.05
OPN (ng/mL)	8.14 ± 0.95	3.37 ± 0.42	< 0.05
S-100β (pg/mL)	1.77 ± 0.20	0.59 ± 0.07	< 0.05
NSE (pg/mL)	24.52 ± 2.85	10.38 ± 1.25	< 0.05
SOD (IU/mL)	42.31 ± 5.45	67.65 ± 7.26	< 0.05
T-AOC (IU/mL)	12.16 ± 1.42	20.35 ± 2.35	< 0.05
MDA (nmol/mL)	6.33 ± 0.69	2.14 ± 0.28	< 0.05

BMI: Body mass index; S-100β: S-100β protein.

3.2. Serum biochemical indexes of cerebral hemorrhage patients with different bleeding volume

The contents of OPN, S-100β, NSE, SOD, T-AOC and MDA of cerebral hemorrhage patients with different bleeding volume were different. The greater the amount of bleeding volume was, the more the contents of OPN [(5.75 ± 0.67) *vs.* (7.92 ± 0.91) *vs.* (10.36 ± 1.16) ng/mL], S-100β [(1.03 ± 0.12) *vs.* (1.79 ± 0.20) *vs.* (2.85 ± 0.30) pg/mL], NSE [(17.86 ± 2.14) *vs.* (23.94 ± 2.61) *vs.* (30.14 ± 3.48) pg/mL] and MDA [(4.03 ± 0.51) *vs.* (6.18 ± 0.81) *vs.* (9.59 ± 1.05) nmol/mL] were and the less the contents of SOD [(52.44 ± 5.94) *vs.* (41.39 ± 5.26) *vs.* (25.52 ± 3.12) IU/mL] and T-AOC [(16.59 ± 1.83) *vs.* (13.04 ± 1.63) *vs.* (8.39 ± 0.92) IU/mL] (Table 2).

3.3. Serum biochemical indexes of cerebral hemorrhage patients with different neurological function

The contents of OPN, S-100β, NSE, SOD, T-AOC and MDA of cerebral hemorrhage patients with different neurological function were different. The contents of serum OPN [(13.23 ± 1.63) *vs.* (6.95 ± 0.81) ng/mL], S-100β [(3.24 ± 0.52) *vs.* (0.91 ± 0.10) pg/mL], NSE [(41.32 ± 5.14) *vs.* (16.61 ± 1.89) pg/mL] and MDA [(11.18 ± 1.26) *vs.* (4.28 ± 0.54) nmol/mL] in patients with early deterioration were obviously higher than those in patients without early deterioration, while their SOD [(15.25 ± 1.79) *vs.* (40.59 ± 5.33) IU/mL] and T-AOC [(5.25 ± 0.77) *vs.* (16.25 ± 1.92) IU/mL] contents were all evidently lower (Table 3).

Table 2
Serum biochemical indexes of cerebral hemorrhage patients with different bleeding volume.

Parameter	Mild hemorrhage	Moderate hemorrhage	Severe hemorrhage	P
OPN (ng/mL)	5.75 ± 0.67	7.92 ± 0.91	10.36 ± 1.16	< 0.05
S-100β (pg/mL)	1.03 ± 0.12	1.79 ± 0.20	2.85 ± 0.30	< 0.05
NSE (pg/mL)	17.86 ± 2.14	23.94 ± 2.61	30.14 ± 3.48	< 0.05
SOD (IU/mL)	52.44 ± 5.94	41.39 ± 5.26	25.52 ± 3.12	< 0.05
T-AOC (IU/mL)	16.59 ± 1.83	13.04 ± 1.63	8.39 ± 0.92	< 0.05
MDA (nmol/mL)	4.03 ± 0.51	6.18 ± 0.81	9.59 ± 1.05	< 0.05

Table 3
Serum biochemical indexes of cerebral hemorrhage patients with different neurological function.

Parameter	Early deterioration	No early deterioration	P
OPN (ng/mL)	13.23 ± 1.63	6.95 ± 0.81	< 0.05
S-100β (pg/mL)	3.24 ± 0.52	0.91 ± 0.10	< 0.05
NSE (pg/mL)	41.32 ± 5.14	16.61 ± 1.89	< 0.05
SOD (IU/mL)	15.25 ± 1.79	40.59 ± 5.33	< 0.05
T-AOC (IU/mL)	5.25 ± 0.77	16.25 ± 1.92	< 0.05
MDA (nmol/mL)	11.18 ± 1.26	4.28 ± 0.54	< 0.05

3.4. The correlation between serum biochemical indexes

The content of OPN was positively correlated with S-100β, NSE and MDA, and the r values were 0.771, 0.715 and 0.664, respectively. Besides, it was negatively correlated with SOD and T-AOC, and the r values were −0.689 and −0.735, respectively.

4. Discussion

Acute cerebral hemorrhage is a serious emergency with poor prognosis. Its clinical symptoms develop rapidly and the neurological functions of patients deteriorate in a short period. Patients will then suffer from unconsciousness, hemiplegic limbs, which raise difficulties for the evaluation of the disease and the design of a treatment plan[11]. In clinical practices, evacuation of hematoma is the preferred way to treat cerebral hemorrhage, but the specific treatment plan depends on the accurate evaluation of the disease[12,13]. At present, evaluation of the conditions and neurological function of acute cerebral hemorrhage patients have reached no common understanding. There are many researches reporting the value of different serum molecular for the evaluation of the conditions of patients with cerebral hemorrhage[14–16].

Studied subjects collected for this study included acute cerebral hemorrhage patients and healthy people. In the cerebral hemorrhage group, the ratio of cases combined with hypertension and diabetes was significantly higher than that of the control group, which agreed with previous researches made by other domestic and overseas scholars. That was to say hypertension and diabetes were dangerous factors of acute cerebral hemorrhage, which indicated that the levels of blood pressure and blood glucose of patients should be controlled strictly so as to avoid further deterioration in the treatment process of the disease. It was found in the analysis of the content of OPN in cerebral hemorrhage patients and healthy people that the OPN content of acute cerebral hemorrhage patients increased obviously. OPN is a kind of phosphoglycoprotein which was firstly found in the bones. It plays a regulatory role in the process of bone formation and bone calcification. With the deep recognition of OPN in recent years, more and more researches claim that OPN participates in the regulation processes such as immunological and inflammatory reaction, atherosclerotic plaque formation and so on and it is also connected with the incidence of cerebrovascular diseases, such as myocardial infarction and cerebral infarction[17–19]. According to the analysis of the OPN content in this study, the increase of the OPN content was related to the incidence of acute cerebral hemorrhage.

There are researches reporting that OPN can serve as a kind of soluble cytokine to participate in the processes of the

activation of inflammation reaction, cascade amplification, aggregation of multiple inflammatory cells in vascular endothelial cell, smooth muscle cell and macrophage, so as to produce various inflammatory cytokines and accelerate the formation of foam cells and atherosclerosis[20–22]. Moreover, OPN can also serve as extracellular matrix proteins, which would increase the risks of the plaque rupture by causing the reconstruction of the components of atherosclerotic plaques and changes of the nature of the plaque[23,24]. In order to confirm the relationship between OPN content and the development of acute cerebral hemorrhage, we analyzed the changes of OPN contents in acute cerebral hemorrhage patients with different bleeding volume and degradation extent. The results showed that the more the bleeding volume were, the more seriously the neurological functions deteriorated and the more significantly the OPN content increased, which illustrated that the increase of the OPN content was closely related to the severity and growing trend of the disease.

The physiological and pathological process of acute cerebral hemorrhage is very complicated. Intracranial hematoma could damage neurons by various humoral factors such as inflammation and oxidative stress[25,26]. S100β protein is a kind of acidic binding protein existing in glial cells. NSE is a type of enzymes existing in many neuroendocrine cells and participating in the energy metabolism of cells. When nerve cell and neural glia cells were injured by the stress of intracranial hematoma, a large amount of S100β and NSE were released and entered cerebrospinal fluid and then accessed to blood circulation through blood–brain barrier. To a certain degree, the impairment severity of neurological function could be determined by testing the contents of serum S100β and NSE[27–29]. Oxidative stress reaction is an important mechanism of brain tissue injury post cerebral hemorrhage. Many oxygen radicals were produced under the effect of mechanical press and regional hypoxia, which, on the one hand, caused the peroxidation of tissue structure and the creation of MDA, and on the other hand, the antioxidant substance SOD was consumed constantly and the total antioxidant capacity was therefore weakened[30–32]. After analyzing the contents of the above nerve-injured molecules and oxidative stress molecules, it was found that the contents of serum NSE, S100β and MDA of patients with acute cerebral infarction increased significantly and their SOD and T-AOC contents decreased distinctly. Besides, they were all associated with the content of serum OPN. It could be concluded that the level of OPN has good consistency with the neurological impairment severity and oxidative stress level.

To sum up, the level of serum OPN in patients with acute cerebral hemorrhage increased significantly; the level of serum OPN could estimate the bleeding volume, outcome and the severity of nerve function impairment for patients with acute cerebral hemorrhage.

Conflict of interest statement

The authors report no conflict of interest.

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