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COMPARISON OF BIOCHEMICAL MARKERS IN CEREBROSPINAL FLUID AND SERUM BETWEEN ENDOVASCULAR THERAPY AND SURGERY FOR PATIENTS WITH SUBARACHNOID HEMORRHAGE; NEURON-SPECIFIC ENOLASE, S-100B PROTEIN, BASIC FIBROBLAST GROWTH FACTOR, AND VASCULAR ENDOTHELIAL GROWTH FACTOR

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SUMMARY

PURPOSE: The outcome of patients with subarachnoid hemorrhage (SAH) and the incidence of symptomatic vasospasm in endovascular therapy are well known to be significantly better than in surgical therapy. Some biochemical markers are known as possible indicators of brain damage. We tried to clarify the possible mechanism, which is related to the better prognosis of endovascular treatment than surgery by measuring biochemical markers. METHODS: We examined cerebrospinal fluid (CSF) levels of neuron specific enolase (NSE), S-100B protein, basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and serum level of NSE in 21 patients with aneurysmal SAH, and compared the results of biochemical markers between endovascular therapy and surgical therapy. RESULTS: The incidence of symptomatic vasospasm was significantly higher ($p < 0.05$) in surgical group than in endovascular group. Repeated measures of NSE from serum in endovascular group were significantly lower ($p < 0.01$) than in surgical group. Repeated measures of NSE, S-100B, VEGF and bFGF from CSF were not significantly different between endovascular group and surgical group. CONCLUSION: The present data is not conclusive to explain the clinical difference between endovascular therapy and surgery from the aspect of biochemical marker levels.

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Abbreviations used: SAH, subarachnoid hemorrhage; CSF, cerebrospinal fluid; NSE, neuron-specific enolase; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor

The higher incidence of vasospasm in surgical group may not be related to the operative damages.

Key Words: subarachnoid hemorrhage; endovascular treatment; neuron-specific enolase; S-100B protein; vascular endothelial growth factor

INTRODUCTION

Endovascular coiling takes an important role for treatment of subarachnoid hemorrhage (SAH), though direct clipping has been used as well. Selection of treatment, coiling or clipping, is usually made based on the location, size, or shapes of aneurysms. Grading of the patients as expressed by Hunt and Kosnik or World Federation of Neurological Surgeons grade is also important factor. The clinical course of the endovascular therapy is known to be different from the surgical therapy (1). Some authors reported that endovascular therapy has better outcome of the patients and less symptomatic vasospasm than surgical therapy, despite the fact that chance of aneurysmal rerupture is more frequent in endovascular therapy than in surgical therapy (1). Clinical outcome of the SAH is well known to be based on the initial brain damage due to increased intracranial pressure (2) and cerebral ischemia due to vasospasm (3).

Some biochemical markers are used for prediction of vasospasm and brain damage (4–9). Among those neuron-specific enolase (NSE) and S-100B protein can be markers for brain damage (5, 6, 9, 10). Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) express in cerebral ischemia (11).

There is a possibility that measuring biochemical markers can be helpful for the postoperative therapy of SAH. And we tried to give some accounts of the better prognosis of endovascular treatment than surgery from this point of view. We examined CSF levels of NSE, S-100B protein, VEGF, bFGF, and we also detected serum level of NSE in 21 patients with aneurysmal SAH. We compared the changes of measurements between endovascular therapy and surgical therapy.

PATIENTS AND METHODS

Patients

During a period of 29 months (January 1998 to May 2000), consecutive 21 patients (Table 1) admitted to Nagoya City University Hospital with aneurysmal SAH and survived for 14 days after the onset were enrolled to this study. Patients treated by endovascular therapy as the first choice (endovascular group, n = 13), and the cases which determined as inadequate for endovascular therapy, thus cases with wide necked or irregular shaped aneurysms or with less than three millimeters sized aneurysms, were treated with craniotomy and clipping surgery (surgical group, n = 8). Three patients with intracerebral hematoma are included in the population but it had no affection to the selection of treat-

TABLE 1. Overview of Patients.

	No. of Pt.	age	sex (M/F)	W.F.N.S.	H&K	ICH	NPH	outcome
endovascular	13	66.9 ± 9.4	4 / 9	2 (1–5)	3 (1–5)	2	5	GR : 10 MD : 2 SD : 1
surgical	8	55.1 ± 14.9	2 / 6	2 (1–3)	3 (2–3)	1	4	GR : 6 MD : 0 SD : 2

No. of Pt. = Number of patients. age: Mean ± standard deviation. sex (M/F): male/female, number of patients. W. F. N. S.: World Federation of Neurological Surgeons grade. Median with range. H&K: Hunt and Kosnik grade. Median with range. ICH: Number of patients with intracerebral hematoma. NPH: Number of patients with postoperative normal pressure hydrocephalus. outcome : Number of patients scaled by Glasgow Outcome Scale. GR = Good Recovery. MD = Moderate Disability. SD = Severe Disability.

ments. They contains with the case of internal carotid artery aneurysm in nine, anterior communicating artery aneurysm in five, middle cerebral artery aneurysm in five and of basilar artery aneurysm in two. There were six men and 15 women, with age ranging from 27 to 77 (62.4 ± 12.9: mean ± S. D.). We scored the SAH grading as the World Federation of Neurosurgical Society Grade and the Hunt and Kosnik grade. Presence of postoperative normal pressure hydrocephalus were evaluated, and outcome of patients was classified on the Glasgow Outcome Scale (12). Patients with reversible neurological deficits or irreversible brain infarctions during14days of SAH were determined as the cases with "symptomatic vasospasm".

CSF Sampling

Serial CSF samples were obtained from lumbar drainage system or lateral ventricle drainage system on the day of 1, 3, 5, 7, 9, 11, 14 after the treatment. Each samples were frozen as soon as possible and stored at −80℃ until assayed.

Measurement of each biochemical markers

NSE levels were determined with commercial enzyme immunoradiometric assay kit (Ab beads NSE, EIKEN Chemical Co.,Ltd., Tokyo, Japan) (13). S-100B protein levels were determined with commercial enzyme immunoradiometric assay kit (Sangtec® 100 IRMA, BROMMA, Sweden) (7). VEGF levels were determined with commercial enzyme immunoradiometric assay kit (QUANTIKINE™ VEGF Immunoassay, R&D SYSTEMS, Minneapolis, MN) (14). Basic FGF levels were determined with commercial enzyme immunoradiometric assay kit (QUANTIKINE™ Human FGF basic Immunoassay, R&D SYSTEMS, Minneapolis, MN) (14).

Statistical Analysis

The significance of differences between two groups was determined by the two tailed multiple t-test with Bonferroni correction following ANOVA. Significant differences between non-parametric two groups were analyzed using the chi-square test. Data were analyzed using the SPSS statistical pro-

gram (SPSS,Inc., Chicago, IL). A probability of 0.05 or less was considered statistically significant.

RESULTS

Patients' clinical course

In the endovascular group, two of 13 patients suffered from symptomatic vasospasm, whereas in the surgical group, six of eight patients showed signs of vasospasm. There was a significant difference ($p<0.05$) between the endovascular and the surgical groups in the incidence of symptomatic vasospasm (Fig. 1). Normal pressure hydrocephalus was presented for nine patients (endovascular: five, surgery: four) and there was no difference in the incidence of hydrocephalus between endovascular group and surgical group. There were no difference in age, sex, existence of intracerebral hematoma on admission, the Hunt and Kosnik grading or the World Federation of Neurosurgical Society Grading between the endovascular and the surgical groups.

Neuron-specific enolase

Repeated measures of serum NSE in endovascular group were significantly lower ($p<0.01$) than in surgical group using multivariate analysis. On day three ($p<0.05$), five ($p<0.05$), and seven ($p<0.05$), the levels of NSE in endovascular group were significantly lower than in surgical group. On day one and11, no significant difference was found but there was also tendency for the low levels of NSE in endovascular group with each time points (Fig. 2).

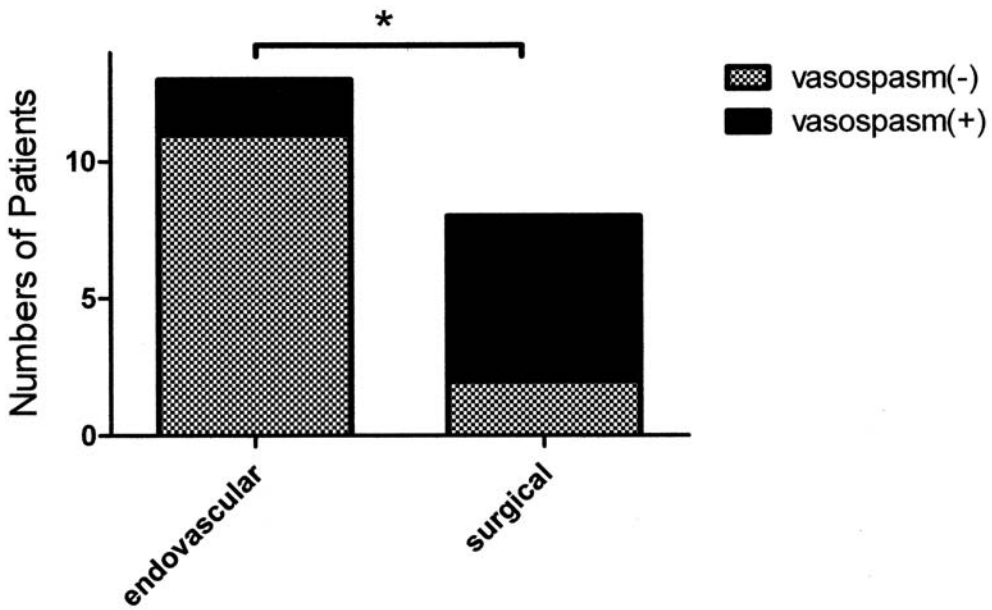

FIG. 1. Number of patients and incidence of symptomatic vasospasm.
* = significant difference ($p<0.05$).

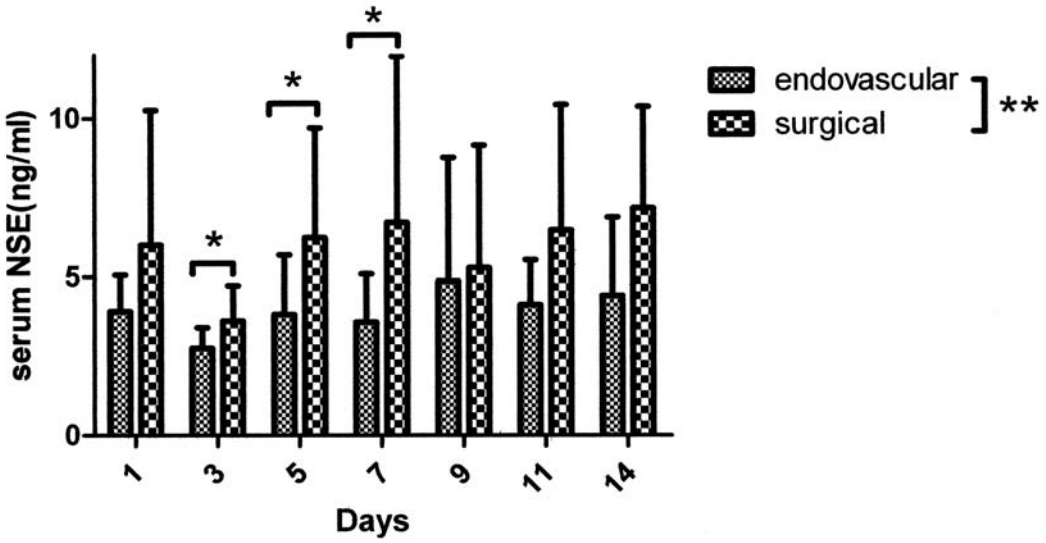

FIG. 2. Changes of NSE levels in serum after SAH. Mean+SD. NSE=neuron-specific enolase. SAH = subarachnoid hemorrhage. SD=standard deviation. * = significant difference ($p<0.05$). ** = significant difference ($p<0.01$).

Repeated measures of NSE in CSF were not significantly different ($p=0.17$) between endovascular group and surgical group, but there was tendency for the higher levels in endovascular group. Using multiple comparison, the level of NSE in CSF was significantly higher on day three than on day one ($p<0.05$), day 11 ($p<0.01$) or day 14 ($p<0.01$). On day three the levels of NSE reached its peak and declined thereafter (Fig. 3).

S-100B protein

There were no significant differences ($p>0.05$) in the levels of S-100B protein between endovascular group and surgical group. In each group, the levels of S-100B protein peak on day one and declined gradually (Fig. 4).

Vascular endothelial growth factor

There were no significant differences ($p>0.05$) in the levels of VEGF between endovascular group and surgical group. In surgical group, the levels of VEGF tend to increase after day seven (Fig. 5).

Basic fibroblast growth factor

There were no significant difference ($p>0.05$) in the levels of bFGF between endovascular group and surgical group. In each group, the levels tend to show two peaks, which are on day one and on day nine (Fig. 6).

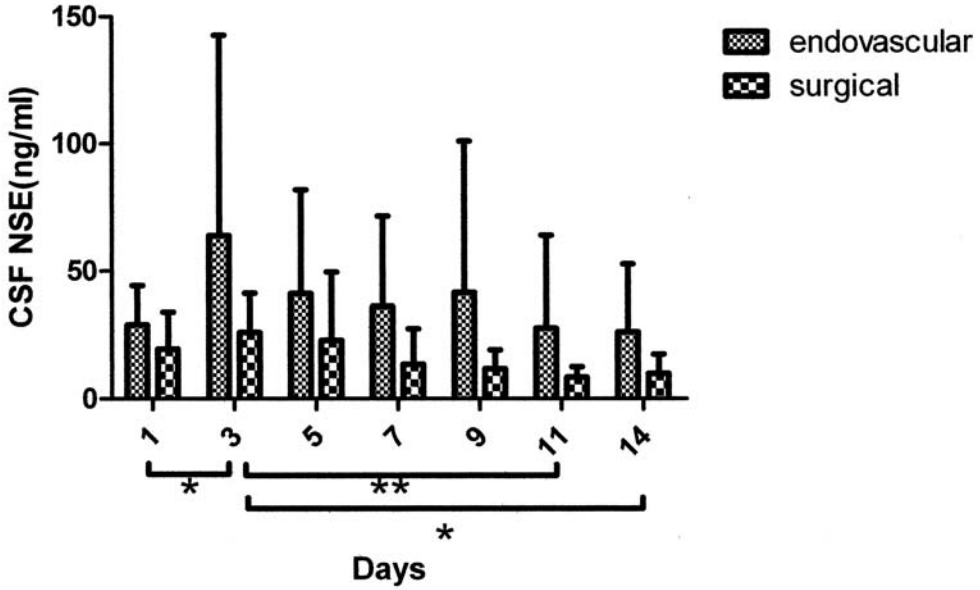

FIG. 3. Changes of NSE levels in CSF after SAH. Mean+SD. NSE=neuron-specific enolase. SAH = subarachnoid hemorrhage. SD=standard deviation. * = significant difference ($p<0.05$). ** = significant difference ($p<0.01$).

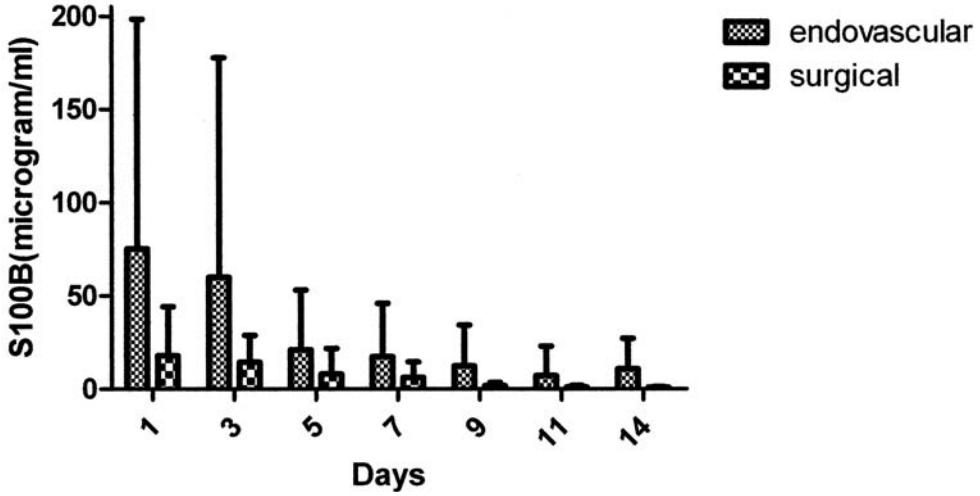

FIG. 4. Changes of S-100B protein levels in CSF after SAH. Mean+SD. SAH = subarachnoid hemorrhage. SD = standard deviation. *=significant difference ($p<0.05$). ** = significant difference ($p<0.01$).

DISCUSSION

Enolase is known as dimeric cytoplasmic enzyme in the glycolytic pathway. Three subunits, as α, β,γ, compose this enzyme, and enolase composed with γ subunit (αγ-enolase and γγ enolase) is called

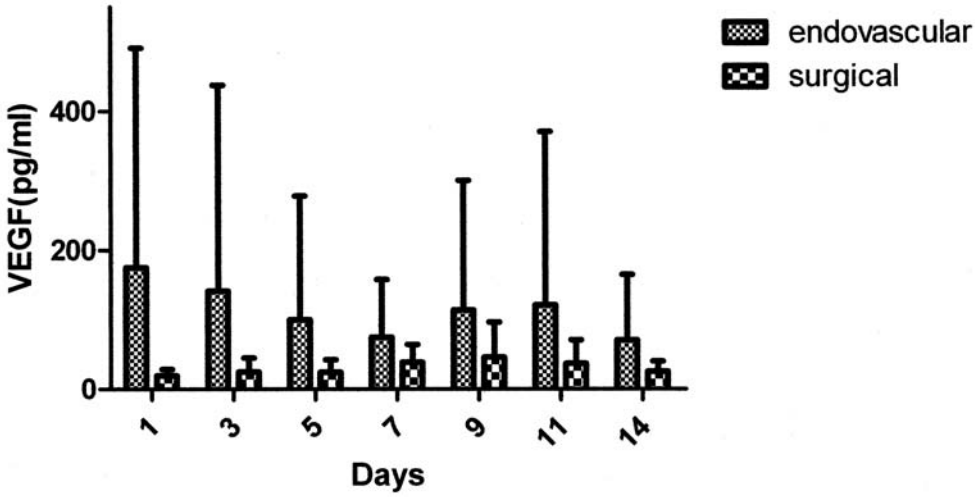

FIG. 5. Changes of VEGF levels in CSF after SAH. Mean + SD. VEGF = vascular endothelial growth factor. SAH = subarachnoid hemorrhage. SD = standard deviation.

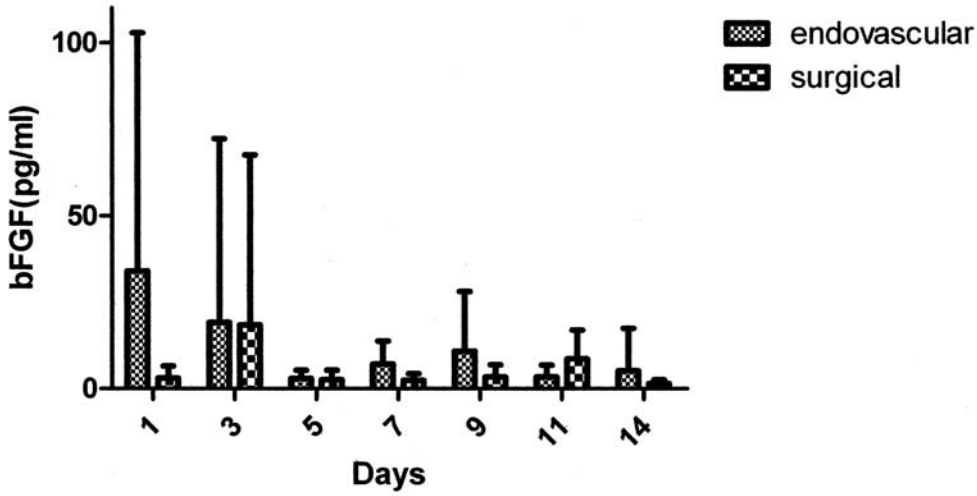

FIG. 6. Changes of bFGF levels in CSF after SAH. Mean + SD. bFGF = basic fibroblast growth factor. SAH = subarachnoid hemorrhage. SD = standard deviation.

neuron-specific enolase (NSE) (5, 6, 10, 15, 16). When central nervous system is injured, NSE is released into both CSF and serum. NSE obtained from serum is reported as a predicting factor of prognosis in SAH patients (6). In this study, we could not find any relation of NSE levels to the patient 'outcome, though the levels from serum in surgical group was higher than in endovascular group. We speculate this result relating to higher rate of symptomatic vasospasm in surgical groups. Another possibility of this result is insufficient flow of CSF in surgical group due to disruption of subarachnoid membrane integrity due to surgical resection can make stagnation of biochemical materials in the in-

tracranial 'pocket' for longer time. This makes some delay of washing out the biomarkers from CSF, and blood concentration of markers might be higher in later times. Erythrocytes (17) and platelets (18) contain NSE as well. Therefore, samples with hemolysis can make falsely high levels of NSE. Some authors reported NSE from CSF may be inadequate for evaluating effects of SAH (5), because of the hemolysis. Our data showed the highest level of NSE from CSF on day three. If delayed hemolysis makes the levels of NSE high on that timing, the level of NSE on day one can be used for the "true" indicator of brain damage and predictor of patients' outcome.

S-100B protein is a member of the calcium binding proteins family (7-10, 19-22). It is found often in the cytosol of glial cells. S-100B protein is also released into CSF and serum when severe brain damage occurred. The levels of S-100B protein from serum is well known as the predicting factor of prognosis for severe brain injury (19, 21), brain ischemia or infarction (10), and hemorrhagic brain diseases (10, 19). There were no significant differences in the levels of S-100B protein between endovascular group and surgical group. There was no difference in Hunt and Kosnik grade or World Federation of Neurological Surgeons grade, so the initial brain damage between the two groups seems to be equivalent, and it is quite compatible with former reports. The peak of the sequential S-100B level was identified on day one. We suppose S-100B, free from consequence of hemolysis, can be more accurate marker of initial brain damage than NSE.

VEGF and bFGF express at the time of tissue ischemia (23) and make strong stimulation for the proliferation of endothelial cells. Some authors reported that VEGF was expressed after the onset of SAH (4, 23-25), because of the hypoxia due to vasospasm. Basic FGF is considered to affect the clinical course of cerebral vasospasm (26). VEGF and bFGF are considered to act as protecting factors of central neural system exposed to ischemia. In surgical group, the levels of VEGF tended to describe upward curves after day seven, and in each group bFGF peaked twice, on day one and nine. It is compatible with usual duration of vasospasm after SAH. There is a possibility that VEGF and bFGF become possible indicators of brain ischemia after SAH.

The incidence of vasospasm was significantly different between the endovascular group and the surgical group. We aimed to make explanation for this by measuring biochemical markers obtained from CSF. The level of NSE and S-100B had no relation to the incidence of vasospasm. These facts strongly suggest that the cause of higher incidence of symptomatic vasospasm in surgical group is not the initial brain damage including operative manipulations.

Further study is needed to make the significance of measuring biochemical markers of patients with aneurysmal SAH obvious in anticipation of this usefulness for the therapy.

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