Early High Cerebrospinal Fluid Glutamate: A Potential Predictor for Delayed Cerebral Ischemia after Aneurysmal Subarachnoid Hemorrhage

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ABSTRACT: Delayed cerebral ischemia (DCI) is an important complication after aneurysmal subarachnoid hemorrhage (aSAH). Early identification of cerebrospinal fluid (CSF) markers is helpful for warning of impending DCI. This study assessed whether early high CSF glutamate levels can be observed in aSAH patients who later developed DCI. In this prospective clinical study, patients with normal pressure hydrocephalus or aSAH were enrolled. We found that the early CSF levels of glutamate were significantly elevated in aSAH patients compared to patients with normal pressure hydrocephalus. There was a significant difference in early CSF levels of glutamate between aSAH patients without DCI and with DCI. The early CSF levels of glutamate are significantly related to the Hunt and Hess grade, the World Federation of Neurological Surgeons (WFNS) grade, and the modified Fisher score on admission and occurrence of DCI in aSAH patients. Preliminary evidence of this study suggests that early high CSF glutamate levels are correlated with DCI in aSAH patients.

1. INTRODUCTION

Subarachnoid hemorrhage (SAH) is caused by rupture of intracranial aneurysm, which leads to devastating outcomes.1 SAH is a life-threatening disease, accounts for about 8% of all strokes, but the case fatality is around 40%. After the patient’s ruptured aneurysm was treated by clipping or coiling, they are still at risk due to severe complications. One of the most feared complications is delayed cerebral ischemia (DCI), which occurs in around 33% of aneurysmal SAH and is defined as a new focal neurologic deficit, an acute decrease in the level of mental status, or appearance of a new infarct on computed tomography (CT) imaging or magnetic resonance imaging (MRI).3,4 Severe neurologic deficits and deaths that occur in 30–40 percent of aneurysmal SAH patients are caused by DCI, and early management of DCI is critical to improving SAH patients’ outcome and survival.5 Possible causes of DCI are cerebral vasospasm that is narrowing of cerebral arteries leading to brain tissue ischemia and microthrombosis that is a result of the impaired fibrinolytic activity and the activation and amplification of coagulation cascade.5–7 Detection of early warning signals of impending DCI would be ideal to prevent DCI at the bedside. One strategy is to identify biochemical markers in the cerebrospinal fluid (CSF), which is helpful for early warning of impending DCI and thus provides an opportunity of an early window for intervention. Glutamate is the major excitatory neurotransmitter in the brain, which is released from the presynaptic vesicles, activates postsynaptic ionotropic glutamate receptors to regulate fast synaptic transmission, and activates metabotropic glutamate receptors that modulate the postsynaptic response and the synapse activity as well as the glutamate release.8–10 Excessive glutamate overactivates glutamate receptors, causes intracellular Ca2+ overload, and leads to excitotoxicity, which is associated with stroke damage.11,12 Clinical studies showed that interstitial glutamate concentration is increased significantly in aneurysmal subarachnoid hemorrhage (aSAH) patients with ischemic neurologic deficits13,14 and is related to compromized energy metabolism and correlated positively with lactate.15,16 The glutamate level is also increased significantly in the cerebrospinal fluid of aSAH patients17,18 and is correlated with the occurrence of cerebral vasospasm and edema.17,19 However, the relationship between early CSF glutamate and DCI is unclear. The objective of this study was to investigate whether early high CSF glutamate could differentiate between aSAH patients who develop DCI and who do not.
2. RESULTS AND DISCUSSION

Eight patients with normal pressure hydrocephalus served as a control group, and 61 patients with aSAH were enrolled in the study. The control patients were of a mean age of (57.2 ± 5.1 years), and 62.5% (31/61) were females. In the aSAH group, their age was (54.9 ± 5.7 years), and 59.0% (36/61) of them were females (Table 1). There was no significant significance in age and sex distribution (p > 0.05).

| Table 1. Characteristics of aSAH Patients |
|-----------------------------------------|
| overall (n = 61) | without DCI (n = 42) | with DCI (n = 19) | p value |
| Demographics |
| age, years | 54.9 ± 5.7 | 55.5 ± 5.9 | 53.8 ± 6.5 | 0.395 |
| gender, female | 36 (59.0) | 25 (59.5) | 11 (57.8) | 0.079 |
| hypertension | 27 (44.2) | 18 (42.8) | 9 (47.3) | 0.635 |
| Clinical Status on Admission |
| Hunt and Hess grade | 17 (27.8) | 7 (16.7) | 10 (52.6) | <0.001 |
| WFNS grade IV–V | 15 (24.5) | 6 (14.3) | 9 (47.3) | <0.001 |
| Fisher score 3–4 | 19 (31.1) | 10 (23.8) | 9 (47.3) | 0.005 |
| hydrocephalus | 5 (8.2) | 3 (7.1) | 2 (10.5) | 0.417 |
| Aneurysm Location |
| internal carotid artery | 28 (45.9) | 20 (47.6) | 8 (42.1) | 0.561 |
| internal carotid artery | 10 (16.4) | 6 (14.3) | 4 (21.0) | 0.259 |
| anterior communicating artery | 23 (37.7) | 16 (38.1) | 7 (36.9) | 0.890 |
| Aneurysm Treatment |
| coiling | 38 (62.2) | 26 (61.9) | 12 (63.2) | 0.905 |
| clipping | 23 (37.7) | 16 (38.1) | 7 (36.8) | 0.879 |

Values were expressed as mean ± SD or numbers (% of total).

aSAH patients had 27.8% (17/61) Hunt and Hess grade 4–5, 24.5% (15/61) World Federation of Neurological Surgeons (WFNS) grade IV–V, 31.1% (19/61) modified Fisher score 3–4 on admission (Table 1). 19 aSAH patients developed DCI (31.1%, 19/61), 42 patients were classified as aSAH without DCI (68.9%, 42/61) according to their postoperative clinical course. There was no statistical significance between groups with respect to age, sex, hypertension, and aneurysm location and treatment (Table 1). As compared with aSAH patients without DCI, aSAH with DCI patients had a higher Hunt and Hess grade of 4–5 (52.6% vs 16.7%, p = 0.0006, p < 0.001), a WFNS grade of IV–V (47.3% vs 14.2%, p = 0.0008, p < 0.001), and a modified Fisher score of 3–4 (47.3% vs 23.8%, p = 0.005, p < 0.01).

The glutamate concentration in CSF significantly increased in aSAH patients compared to that in control patients (20.5 ± 12.9 vs 2.6 ± 1.1 μmol/L, p = 0.0003, p < 0.001, Figure 1A). aSAH without DCI patients had a higher CSF glutamate as compared with the control group (13.2 ± 5.5 vs 2.6 ± 1.1 μmol/L, p = 0.0004, p < 0.001, Figure 1B). Moreover, CSF glutamate significantly increased in aSAH with DCI patients when compared to aSAH without DCI patients (36.6 ± 9.5 vs 13.2 ± 5.5 μmol/L, p = 0.0005, p < 0.001, Figure 1B). Next, there was a significant difference in early CSF levels of glutamate between patients with Hunt and Hess grades of 1–3 vs 4–5 (14.9 ± 8.0 vs 34.7 ± 12.4 μmol/L, p = 0.0002, p < 0.001, Figure 2A), WFNS grades of I–III vs IV–V (15.5 ± 8.4 vs 35.3 ± 13.1 μmol/L, p = 0.0005, p < 0.001, Figure 2B), and modified Fisher scores of 1–2 vs 3–4 (15.3 ± 8.6 vs 31.8 ± 13.8 μmol/L, p = 0.0007, p < 0.001, Figure 2C). In separate logistic regression analysis, early CSF levels of glutamate were significantly associated with the occurrence of DCI, which remained significant after adjustments for confounders (Table 2).

In this prospective clinical study, we found that early CSF levels of glutamate are significantly elevated in aSAH patients with DCI and are associated with occurrence of DCI in aSAH patients. DCI encompasses symptomatic deterioration of neurological examination and radiographic evidence of new ischemia or infarction in patients. Clinically, a Hunt and Hess grade of 4–5, a WFNS grade of IV–V, and a high Fisher score are risk factors of DCI. Our results also showed that patients with DCI after aSAH have higher scores of the Hunt and Hess grade, the WFNS grade, and the Fisher score when compared to SAH patients without DCI, confirming the definition of DCI experience new ischemia or infarction while lacking concurrent neurological deterioration, and this phenomenon called “silent infarcts” occurs most commonly in comatose patients, which independently contributes to bad outcomes. The clinical diagnosis of DCI is limited because radiological detection of cerebral infarction is a retrospective observation, and this may lead to delayed diagnosis and insufficient treatment of ongoing ischemia. One strategy is to detect early warning markers of DCI in CSF, which will alert the occurrence of DCI early.
Glutamate is an excitatory amino acid and neurotransmitter. The level of interstitial glutamate increases within minutes and remain elevated for several days after aSAH, and this increase in interstitial fluid is related to delayed ischemic neurologic deficits and identified as the earliest marker for impending DCI. Because the patient’s interstitial fluid is difficult to obtain, the detection of the biomarker in the interstitial fluid is very limited in clinical application. Cerebrospinal fluid examination based on lumbar puncture has become the cornerstone in the diagnosis of aSAH, which has become the cornerstone in the diagnosis of aSAH. The early high CSF glutamate levels could differentiate between aSAH patients who develop DCI and who do not and maybe a potential predictor for diagnosis. Our study has several limitations. First, the number of patients enrolled in this study is relatively small, and a prospective clinical study employing a larger sample size needs further implementation. Secondly, inclusion criteria contain aneurysm treatment within 24 h and external ventricular drainage within 48 h, which limits the number of aSAH patients that can be enrolled. Thirdly, some aSAH patients are in a coma, which may underestimate the number of DCI. Despite these limitations above, to our knowledge, this study is the first to show that early high CSF glutamate levels can be observed in aSAH patients who later developed DCI.

3. CONCLUSIONS

In this study, we showed that early CSF glutamate was significantly elevated in aSAH patients compared to patients with normal pressure hydrocephalus. There was a significant difference in early CSF glutamate between aSAH patients who develop DCI and who do not. The early CSF glutamate is related to the Hunt and Hess grade, the WFNS grade, and the modified Fisher score on admission. The early high CSF glutamate level is a potential predictor of DCI in aSAH patients.

4. MATERIALS AND METHODS

4.1. Patients with aSAH or Normal Pressure Hydrocephalus. After approval by the ethical committee of Jining No. 1 People’s Hospital and Baotou Central Hospital, an observational study of CSF analysis in aSAH patients between January 2019 and October 2019 was performed. Patients enrolled in this study if they had been diagnosed with SAH according to the basis of the World Health Organization criteria and had a ruptured aneurysm documented by a head computed tomography (CT) angiography. Inclusion criteria were as follows: aneurysm treatment <24 h post rupture and external ventricular drainage <48 h post rupture. Exclusion criteria included: no aneurysm, radiologic evidence of DCI present on admission, and history of CNS disease. Clinical severity on admission was determined by the Hunt and Hess (HH) grade and the World Federation of Neurological Surgeons (WFNS) grade. The hemorrhage severity was defined by the modified Fisher grade according to radiological features. All patients were treated according to the guidelines set forth by the American Heart Association. The aneurysm treatment was decided by the consensus of a multidisciplinary team. DCI was defined as previously described: a focal neurologic deficit lasting for at least 1 h, a decrease of 2 points on the Glasgow Coma Scale, or an increase of 2 points on the National Institutes of Health Stroke Scale and (2) a new infarct on a head CT that was not visible on admission or within 24 h after aneurysm treatment. The CSF of patients with normal pressure hydrocephalus was used as a control.
because it is difficult to obtain the samples from normal healthy people.

4.2. CSF Sample Collection and Measurement of Glutamate Concentration. Using sterile procedures, a single CSF sample of a patient with normal pressure hydrocephalus was collected from the external ventricular drainage, and a single CSF sample of an aSAH patient was collected within 48 h after SAH from the external ventricular drainage or lumbar puncture as previously described.\(^\text{17,19}\) CSF samples were centrifuged, and the supernatants were stored at \(-80\) °C until the assay. The glutamate concentration in CSF was measured using the Glutamate Assay Kit according to the manufacturer’s instructions (MAK004, Sigma-Aldrich) and represented as \(\mu\)mol/L.

4.3. Statistical Analysis. All analyses were generated using SPSS 19.0 software. The normality of data distribution was evaluated using the Kolmogorov–Smirnov test. Data presented as mean \(\pm\) standard deviation (SD). Statistical analysis was performed using the two-tailed \(t\)-test and one-way ANOVA followed by Bonferroni’s multiple comparison test.

For the univariate analysis, categorical variables and continuous variables were assessed using Pearson’s \(\chi^2\) test and the Mann–Whitney \(U\)-test, respectively. Pearson’s correlation coefficient was performed to assess correlations. The influence of glutamate levels on DCI was assessed using binary logistic regression after adjusting for main variables of Table 1 related to the outcome in univariate analyses (enter approach, probability of entry \(p < 0.05\)). The results were expressed as adjusted odds ratios (ORs) with 95% confidence intervals (95% CI). Statistical significance was defined as \(p < 0.05\).

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**Author Contributions**

H.-B.W., Q.-J.W., and S.-j.Z. contributed equally to this work. B.-l.S. and Z.-y.Z. designed experiments. H.-B.W., Q.-J.W., S.-j.Z., Y.-j.H., H.-x.L., M.-i.Y., and B.M. performed the experiments. Z.-y.Z. and B.-l.S. analyzed the results. Z.-y.Z. wrote the manuscript with contribution from B.-l.S. All authors read and approved the final manuscript.

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All procedures performed involving human participants were in accordance with the ethical standards of the 1964 Helsinki Declaration and approved by the ethical committee of Jining No. 1 People’s Hospital and Baotou Central Hospital.

**Notes**

The authors declare no competing financial interest.

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