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Clinical Study

Increased cerebrospinal fluid concentrations of asymmetric dimethylarginine correlate with adverse clinical outcome in subarachnoid hemorrhage patients

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ABSTRACT

Elevated cerebrospinal fluid (CSF) concentrations of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, have been found in patients with subarachnoid hemorrhage (SAH). In addition, CSF levels of ADMA are associated with the severity of vasospasm. However, the relation between CSF ADMA levels and the clinical outcome of SAH patients is still unclear. We hypothesized that elevated ADMA levels in CSF might be related to the clinical outcome of SAH patients. CSF ADMA levels were measured in 20 SAH patients at days 3–5, days 7–9 and days 12–14 after SAH onset using high-performance liquid chromatography. Cerebral vasospasm was assessed by transcranial Doppler ultra sonography. Clinical outcome at 2 year follow-up was evaluated using the Karnofsky Performance Status scale (KPS). CSF ADMA concentrations in all SAH patients were significantly increased at days 3–5 ($p = 0.002$) after SAH, peaked on days 7–9 ($p < 0.001$) and remained elevated until days 12–14 ($p < 0.001$). In subgroup analysis, significant increases of CSF ADMA levels were found in patients both with and without vasospasm. The KPS scores significantly correlated with CSF levels of ADMA at days 7–9 (correlation coefficient = −0.55, $p = 0.012$; 95% confidence interval −0.80 to −0.14). Binary logistic regression analysis indicated that higher ADMA level at days 7–9 predicted a poor clinical outcome at 2 year follow-up after SAH (odds ratio = 1.722, $p = 0.039$, 95% confidence interval 1.029 to 2.882). ADMA may be directly involved in the pathological process and future adverse prognosis of SAH.

1. Introduction

Subarachnoid hemorrhage (SAH), especially aneurysmal SAH, is a life-threatening disease of the central nervous system. The incidence of SAH is about 22.5 cases per 100,000 people according to the World Health Organization [1]. Between 4 to 9 days after SAH, severe cerebral vasospasm develops in 30–70% of patients, resulting in delayed ischemic neurological deficits (DIND) in 25% of patients with vasospasm. Half of these patients suffer severe neurological dysfunction or death due to DIND [2]. However, despite substantial efforts, treatment strategies to reduce the incidence of vasospasm and to improve the prognosis of SAH patients are limited.

Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of nitric oxide synthase (NOS). Elevated concentrations of ADMA decrease the availability of nitric oxide (NO) and thus induces vasoconstriction [3,4]. During the past decade, studies have demonstrated that ADMA is not only a marker of endothelial dysfunction, but also an independent risk factor for cardiovascular and cerebrovascular diseases [5]. ADMA has also been shown to predict outcomes of cardiovascular diseases and ischemic stroke [6–10].

In SAH studies, cerebrospinal fluid (CSF) concentrations of ADMA have been found to be significantly increased after SAH, and elevated levels of ADMA are correlated with the course and degree of vasospasm as well as with the decrease of NO after SAH [11–13]. However, the relationship between CSF ADMA concentrations and the clinical outcome of SAH patients is still unknown. The present study was carried out to investigate whether levels of CSF ADMA were associated with clinical outcome in patients with SAH.

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2. Material and methods

2.1. Patients

Between December 2008 and March 2009, 25 consecutive patients diagnosed with SAH were prospectively recruited upon admission to the Neurosurgery Unit of the Jinling Hospital, Nanjing, China. The diagnosis of SAH was supported in every patient by axial CT scan. All patients underwent detailed physical examination and routine blood chemistry analysis. Those who had been previously diagnosed with hypertension, hyperlipidemia, diabetes mellitus, arterial atherosclerosis, chronic renal failure or peripheral vascular disease were excluded.

The control group consisted of 18 patients without a history of cerebrovascular disease or contraindication for lumbar puncture, who underwent spinal anesthesia for elective urogenital tract intervention or lower limb fracture surgery. The study was approved by the local Ethics Committee, and all patients gave written informed consent.

2.2. Patient management

All patients received intravenous nimodipine at a dose of 2 mg/hour from admission until at least day 14 after SAH onset. The type of surgical procedure (clipping or coiling) was decided according to both size and location of the aneurysm by the treating neurosurgeon and neuroradiologist. After clipping or coiling, those patients who had delayed ischemic neurological deficit or cerebral vasospasm were managed with "triple H" therapy (hypertension with a mean arterial pressure goal greater than 100 mmHg, hypervolemia and hemodilution with a goal hematocrit of 30). Clinical onset of vasospasm is defined below. Each vasospasm episode was treated with cerebral intra-arterial administration of nimodipine. This therapy was repeated if necessary. Balloon angioplasty was used as a second-line therapy when nimodipine was judged insufficient.

2.3. Assessment of vasospasm

Transcranial Doppler ultra sonography (TCD) was performed daily to assess the presence of vasospasm. In accordance with a 2004 technology assessment by the American Academy of Neurology [14], the following three independent criteria were defined as middle cerebral artery vasospasm: (1) velocity greater than 200 cm/s, (2) a rapid rise (greater than 50 cm/s) between serial TCD measurements, or (3) a Lindegaard Index greater than 6. The Lindegaard Index refers to the ratio of velocities between the middle cerebral artery and the ipsilateral extracranial internal carotid artery.

2.4. Sample collection and preparation

In the study group, lumbar puncture was performed on days 3–5, 7–9 and 12–14 after SAH. In the control group, a single CSF sample was collected during spinal anesthesia before surgery. In total, approximately 15 ml of CSF was collected in polypropylene vials which were immediately centrifuged at 3000 rotations/minute for 15 minutes at 4 °C. Supernatants were stored at −80 °C until analysis.

2.5. Determination of CSF ADMA concentration

CSF concentrations of ADMA were determined simultaneously by high-performance liquid chromatography (HPLC) as previously described [15]. Briefly, HPLC was performed on a Shimadzu LC-10AD system equipped with a Shimadzu RF-10Axl fluorescence detector (both Shimadzu, Kyoto, Japan) for excitation at 338 nm and emission at 450 nm with a Hypersil ODS (C18) Column (4.6 mm × 150 mm) (Thermo Fisher Scientific, Waltham, MA, USA). All reagents and chemicals for the analysis of ADMA were from Sigma-Aldrich (St Louis, MO, USA).

2.6. Observational follow-up

Patients were clinically re-examined at 3 to 6 month intervals after hospital discharge. If the regular re-evaluations did not occur, personal telephone contact to the patients or their relatives was established. Clinical conditions were recorded semiannually until April 2011 using the Karnofsky Performance Status scale (KPS) [16]. During the follow-up period, one patient died during the second month after discharge due to cardiorespiratory failure, but all others were followed up for a period of 2 years. Outcome was adjudicated by two independent observers blinded to the patients' other clinical and laboratory data.

Patients with the ability to complete activities of daily living (KPS $\geqslant$ 60) were classified as having a relatively good outcome. Those who could not take care of themselves or those who needed to be admitted to care (KPS < 60) were classified as having a poor outcome.

2.7. Statistical analysis

Data are presented as mean value ± standard deviation. Chi-squared test was used for categorical variables and unpaired Student's *t*-test for continuous variables, to assess differences between the groups. Pearson's correlation coefficient (CC) was used to evaluate a possible correlation in continuous variables. The influence of ADMA levels on vasospasm and clinical outcome was assessed using binary logistic regression. Results are presented as odds ratios with the corresponding 95% confidence intervals (CI). Statistical significance was defined as $p < 0.05$. All analyses were performed with the Statistical Package for the Social Sciences software, version 15 (SPSS, Chicago, IL, USA).

3. Results

3.1. Clinical characteristics and ADMA concentrations

Five patients were excluded from the study because clinical or follow-up data were incomplete. The remaining 20 patients (six men and 14 women, age 52.35 ± 7.21 years) were included into the analysis. Of these patients, 15, 20 and 17 CSF samples were obtained on days 3–5, days 7–9 and days 12–14, respectively. Seven patients (35%) developed vasospasm, while the rest showed no evidence of vasospasm. Clinical characteristics and follow-up data are shown in Table 1.

A low level of ADMA was identified in the control group (2.06 ± 0.66 ug/l) while the level of ADMA increased significantly at days 3–5 after SAH (4.48 ± 2.41 ug/l, $p = 0.002$), peaked on days 7–9 (6.71 ± 3.25 ug/l, $p < 0.001$) and remained elevated until days 12–14 (5.61 ± 2.19 ug/l, $p < 0.001$) (Fig. 1A).

3.2. CSF ADMA levels in relation to cerebral vasospasm

The CSF concentration of ADMA significantly increased at days 3–5 (5.89 ± 3.32 ug/l, $p = 0.061$), days 7–9 (9.64 ± 2.62 ug/l, $p < 0.001$) and days 12–14 (6.89 ± 1.35 ug/l, $p < 0.001$) in patients who developed vasospasm. Patients without vasospasm also showed a significant increase in CSF ADMA level at days 3–5 (5.89 ± 3.32 ug/l, $p = 0.007$), days 7–9 (5.13 ± 2.37 ug/l, $p = 0.001$) and days 12–14 (4.92 ± 2.29 ug/l, $p = 0.002$). CSF ADMA level at