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Identification of plasma adrenomedullin as a possible prognostic biomarker for aneurysmal subarachnoid hemorrhage

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ABSTRACT

Increased plasma adrenomedullin levels have been reported in critically ill patients. This study tested the hypothesis that plasma adrenomedullin levels are significantly increased in patients with acute spontaneous aneurysmal subarachnoid hemorrhage, and are predictive of clinical outcomes. Plasma adrenomedullin levels from 120 adult patients with spontaneous aneurysmal subarachnoid hemorrhage and 120 healthy volunteers during the study period were evaluated. Mortality and poor long-term outcome (Glasgow Outcome Scale score of 1–3) at 6 months were recorded. Data showed that circulating plasma adrenomedullin levels significantly increased in patients on admission compared with the volunteers. In patients who died or had poor outcome at 6 months, plasma adrenomedullin levels were significantly higher compared with survivors and patients with good outcome. Plasma adrenomedullin levels on presentation were highly associated with clinical severity assessed using World Federation of Neurological Surgeons score and Fisher score, emerged as the independent risk factor of 6-month mortality and poor outcome, and possessed similar predictive value to World Federation of Neurological Surgeons score and Fisher score based on receiver operating characteristic curves. A combined logistic-regression model did not demonstrate the additive benefit of adrenomedullin to World Federation of Neurological Surgeons score and Fisher score. Thus, higher plasma adrenomedullin levels on presentation are associated with clinical severity and worse outcomes in patients with acute spontaneous aneurysmal subarachnoid hemorrhage.

Introduction

Intracranial aneurysms are the most common cause of spontaneous subarachnoid hemorrhage. Global incidence of aneurysmal subarachnoid hemorrhage (aSAH) is estimated at approximately 10 per 100,000. Despite comprising only 5% of strokes, aSAH accounts for a significant proportion of stroke-related morbidity and mortality [23]. The World Federation of Neurological Surgeons (WFNS) grade and Fisher grade are commonly used to assess the severity and the amount of bleeding of aSAH [15]. However, prediction of outcome remains difficult and complicates decision-making for active treatment in aSAH [22].

Adrenomedullin (AM) is a 52-amino-acid peptide belonging to the calcitonin gene-related peptide family and identified as a multifunctional peptide which exerts, through an autocrine/paracrine mode of action, multiple biological effects [4], including the regulation of blood pressure, cell growth and differentiation, modulation of hormone secretion, central nervous system functions and the potentiation of host defences against microbes [6,12]. Its gene expression is promoted by various stimuli, including inflammation, hypoxia, oxidative stress, mechanical stress and activation of the renin–angiotensin and sympathetic nervous systems [26,31]. AM appears to be a promising therapeutic tool for human diseases including ischemic stroke [7,24], traumatic brain injury [1], myocardial infarction [16] and inflammatory bowel disease [2]. Blood levels of AM were independently correlated with prognosis of ischemic or hemorrhagic stroke [30,33] and traumatic brain injury [5]. Cerebrospinal fluid AM concentration correlates with delayed ischemic neurological deficits after aSAH [19,20]. However, plasma AM concentrations are not associated with angiographic vasospasm, but reflect the severity of hemorrhage [17]. Thus, in this study, we aimed to find out the relationship between AM and clinical outcome by determining the plasma levels of AM in samples obtained from patients with aSAH and healthy volunteers.

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Materials and methods

Study population

This prospective observatory study initially assessed the patients with first-ever non-traumatic SAH admitted to the Department of Neurosurgery, Central Hospital of Wenzhou City, China during the period of January 2011–May 2013. Only patients who had clinical history of SAH within the last 24 h before admission, suffered from aSAH confirmed by computerized tomography (CT) angiography with or without digital subtraction angiography and received the treatment by surgery or coiling within the 48 h after admission were include in this study. Patients were excluded if they had (1) rebleeding after admission, (2) less than 18 years of age, (3) previous head trauma, (4) previous neurological disease, (5) previous use of antiplatelet or anticoagulant medication, and (6) other prior systemic diseases including uremia, liver cirrhosis, malignancy, chronic heart disease, chronic lung disease, diabetes mellitus and hypertension. We also excluded patients with (1) unavailable biomarker measurements, (2) refusal of participation and (3) loss of follow-up. Finally, One hundred and twenty aSAH patients were enrolled in this study. The mean age of the patients with aSAH, a group consisting of 71 men and 49 women, was 41.1 ± 11.5 years. A control group consisted of 120 healthy subjects. The healthy volunteers had a mean age of 42.1 ± 13.5 years and included 65 men and 55 women. Intergroup differences in sex and age did not appear statistically significant. The study was conducted in accordance with the guidelines approved by the Human Research Ethics Committee at our hospital. Written informed consent was obtained from the patients or their relatives.

Clinical and radiological assessment

At admission, we collected information on demographic, clinical, radiological and outcome data for all patients. The radiological severity of SAH on admission was classified according to the Fisher grading system [10]. Clinical severity of SAH was classified according to the WFNS system [8]. Whenever clinical deterioration occurred, CT was performed to search for secondary complications such as hydrocephalus or ischemia. Clinical onset of cerebral vasospasm was defined as the acute onset of a focal neurologic deficit or a change in the Glasgow Coma Scale score of 2 or more points. All suspected cases of cerebral vasospasms were confirmed by CT angiography. All CT scans were performed according to the neuroradiology department protocol. Investigators who read them were blinded to clinical information. Outcome at 6 months was classified according to the Glasgow Outcome Scale, as previously reported [9]: Scores 1–3, poor outcome; Scores 4–5, favorable outcome. For follow-up, structure telephone interviews were performed by one doctor who was blinded to clinical information and AM levels.

Immunoassay methods

The informed consents were obtained from all participants or their legal representatives before the blood were collected. Venous blood of patients was drawn on admission, and those of control group were drawn at study entry. Samples were placed on ice, centrifuged at 3000 × *g*, and plasma aliquoted and frozen at −70 °C. Plasma AM concentration was analyzed by enzyme-linked immunosorbent assay using commercial kits (R&D Systems, Heidelberg, Germany) in accordance with the manufactures' instructions. The blood samples were run in duplicate. Researchers running enzyme-linked immunosorbent assays were blinded to all patient details.

Statistical analysis

All statistical analyses were performed with the use of computer software SPSS 19.0 (SPSS Inc., Chicago, IL, USA). The results were reported as counts (percentage) for the categorical variables and mean ± standard deviation for the continuous variables. Comparisons were made using Chi-square test or Fisher exact test for categorical data, and unpaired Student's *t*-test for the continuous variables. Correlations of AM with WFNS score and Fisher score were assessed by Spearman's or Pearson's correlation coefficient and followed by a multivariate linear regression. A logistic-regression model was constructed to identify independent outcome predictors. The logistic regression results were presented as odds ratio (OR) and 95% confidence interval (CI). A receiver operating characteristic (ROC) curve was configured to determine the cutoff point of the AM concentration to differentiate poor outcome. The results were estimated with calculated area under curve (AUC) and 95% CI. A combined logistic-regression model was configured

Table 1
The characteristics in patients with aneurysmal subarachnoid hemorrhage and the factors associated with 6-month clinical outcomes.

	Total	6-Month mortality			6-Month functional outcome		
		Non-survivors	Survivors	*P* value	Poor outcome	Good outcome	*P* value
Cases	120	16	104		35	85	
Male	71 (59.2%)	9 (56.3%)	62 (59.6%)	0.799	20 (57.1%)	51 (60.0%)	0.772
Age (year)	41.1 ± 11.5	41.9 ± 11.8	40.9 ± 11.5	0.741	41.3 ± 10.7	40.9 ± 11.9	0.858
WFNS score on admission	2.6 ± 1.1	3.9 ± 0.7	2.4 ± 1.0	<0.001	3.6 ± 0.7	2.2 ± 1.0	<0.001
Fisher score on admission	2.8 ± 0.9	3.9 ± 0.6	2.6 ± 0.8	<0.001	3.6 ± 0.8	2.5 ± 0.7	<0.001
Surgery	68 (56.7%)	10 (62.5%)	58 (55.8%)	0.613	22 (62.9%)	46 (54.1%)	0.380
Acute hydrocephalus	26 (21.7%)	8 (50.0%)	18 (17.3%)	0.003	13 (37.1%)	13 (15.3%)	0.008
Intraventricular hemorrhage	23 (19.2%)	7 (43.8%)	16 (15.4%)	0.007	12 (34.3%)	11 (12.9%)	0.007
External ventricular drain	30 (25.0%)	8 (50.0%)	22 (21.2%)	0.026	14 (40.0%)	16 (18.8%)	0.015
Vasospasm	36 (30.0%)	9 (56.3%)	27 (26.0%)	0.020	16 (45.7%)	20 (23.5%)	0.016
Computed tomography ischemia	15 (12.5%)	6 (37.5%)	9 (8.7%)	0.005	9 (25.7%)	6 (7.1%)	0.012
Admission time (h)	6.4 ± 4.1	6.3 ± 6.0	6.4 ± 3.8	0.964	5.6 ± 4.3	6.7 ± 4.0	0.170
Plasma-sampling time (h)	8.2 ± 4.5	8.0 ± 6.1	8.3 ± 4.3	0.880	7.5 ± 4.5	8.5 ± 4.5	0.238
Systolic arterial pressure (mmHg)	130.7 ± 23.7	137.5 ± 18.8	129.7 ± 24.2	0.220	134.7 ± 21.6	129.1 ± 24.4	0.244
Diastolic arterial pressure (mmHg)	80.7 ± 13.6	85.4 ± 10.9	79.9 ± 13.8	0.137	80.8 ± 12.3	80.6 ± 14.1	0.945
Blood glucose level (mmol/L)	13.2 ± 4.8	16.5 ± 4.7	12.8 ± 4.6	0.003	15.0 ± 4.9	12.5 ± 4.5	0.009
Plasma CRP level (mg/L)	11.1 ± 3.3	13.7 ± 3.3	10.8 ± 3.1	0.001	12.6 ± 3.3	10.5 ± 3.1	0.002
Plasma adrenomedullin level (pg/mL)	109.1 ± 34.1	146.4 ± 32.6	103.4 ± 30.6	<0.001	135.4 ± 30.4	98.3 ± 29.4	<0.001

Numerical variables were presented as mean ± standard deviation. Categorical variables were expressed as counts (percentage). Numerical variables were analyzed by unpaired Student's *t*-test. Categorical variables were analyzed by chi-square test or Fisher exact test. WFNS indicates World Federation of Neurological Surgeons; CRP, C-reactive protein.