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Elevated cerebrospinal fluid levels of thrombospondin-1 correlate with adverse clinical outcome in patients with aneurysmal subarachnoid hemorrhage

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ABSTRACT

Background: Thrombospondin-1 (TSP-1) is a homotrimeric glycoprotein which modulates a wide range of biological functions. Elevated level of TSP-1 in plasma was reported to be correlated with intracerebral hemorrhage. Our study was designed to investigate the relationship between cerebrospinal fluid (CSF) TSP-1 levels and clinical outcomes in patients with aneurysmal subarachnoid hemorrhage (aSAH).
Methods: CSF TSP-1 levels were measured in 31 aSAH patients on days 1–3, days 5–7 and days 8–10 after aSAH onset using enzyme-linked immunosorbent assay. Patients were under a close follow-up until death or completion of three months after aSAH. Binary logistic regression analyses were performed to determine independent risk factors for the clinical outcomes.
Results: TSP-1 levels peaked on days 1–3 after aSAH, kept up high on days 5–7 and remained elevated until days 8–10 ($p < 0.05$). Significant elevation of CSF TSP-1 levels were found in patients both with and without vasospasm. Modified Rankin Scale at 3 months after aSAH showed a significant correlation with CSF TSP-1 levels on days 1–3 and days 5–7 (both $p < 0.01$). Binary logistic regression analysis showed that higher TSP-1 level on days 1–3 ($p < 0.05$) and on days 5–7 ($p < 0.05$) was a predictive marker of cerebrovasospasm and poor outcome of patient with aSAH.
Conclusions: Upregulation of TSP-1 may involve in the pathological process of aSAH and might be a risk factor of future adverse prognosis of aSAH.

1. Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) is a life-threatening disease of the central nervous system that kills or severely disables 70% of victims [1]. About 30% of cases suffered with delayed cerebral ischemia (DCI), which is the chief cause of mortality and morbidity [2]. Vasospasm developing mainly 3–7 days after rupture in 70% of patients has traditionally been regarded as the principal cause of DCI [3]. However, despite the high morbidity and mortality of aSAH, the means to evaluate the prognosis of patients are still limited. A readily measurable marker in early stage of aSAH which predicts poor outcomes may contribute to timely intervention. Accumulating evidence shows the use of biomarkers as potential adjuncts could help to assess aSAH severity and to provide an insight into the prognosis of patients [4].

Thrombospondin-1 (TSP-1) is a 450 kDa trimeric glycoprotein, which is one of the members of the thrombospondin gene family [5, 6], and exerts its diverse biological effects through binding to extracellular matrix (ECM) proteins and cell surface receptors, thereby regulating cell-cell and cell-matrix interactions. TSP-1 has received increased attention because of its participates in a wide range of physiological and pathological processes such as synaptogenesis, angiogenesis, platelet aggregation, inflammatory response and wound repair [6-11]. However, there is a paucity of data available on the changes of TSP-1 levels in cerebrospinal fluid (CSF) and the relationship between TSP-1 levels and clinical outcomes after aSAH. The present study was carried out to investigate the

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TSP-1 levels of CSF and whether the levels of CSF TSP-1 were associated with clinical outcomes in patients with aSAH.

2. Material and methods

2.1. Patients

Between August 2015 and December 2015, 35 patients with aSAH, confirmed by computerized tomography (CT) and digital subtraction angiography were admitted to the Neurosurgery Unit of the Jinling Hospital, Nanjing, China. All patients underwent detailed physical examination and routine blood chemistry analysis. Patients with a history of CNS disease (meningitis, stroke), and active systemic disease (diabetes mellitus, rheumatoid arthritis, malignancy, cirrhosis) were excluded from our study. The control group consisted of 15 patients without a history of cerebrovascular disease or contraindication for lumbar puncture, who underwent spinal anesthesia for arthroplasty. The study was approved by the local Ethics Committee, and all patients or next of kin were given written informed consent.

2.2. Sample collection and assays

In study group, CSF samples were collected through lumbar puncture on days 1–3, 5–7 and 8–10 after aSAH. In the control group, a single CSF sample was collected during spinal anesthesia before surgery. Immediately after collection, samples were centrifuged at 3000 rpm for 10 min and stored at −80 °C until assayed. The concentration of TSP-1 in CSF was analyzed by enzyme-linked immunosorbent assay (ELISA) using commercial kit 70-EK1822 (MultiSciences, Hangzhou, China), in accordance with manufacturer's instructions.

2.3. Assessment of vasospasm

Transcranial Doppler ultra sonography (TCD) was performed on days 1–3, 5–7 and 8–10 to assess the presence of vasospasm. The following three independent criteria just one time were defined as middle cerebral artery vasospasm: (1) velocity >200 cm/s, (2) a rapid rise (>50 cm/s) between serial TCD measurements, or (3) a Lindegaard Index >6. The Lindegaard Index refers to the ratio of velocities between the middle cerebral artery and the ipsilateral extracranial internal carotid artery [12].

2.4. Observational follow-up

Subjects were followed until death or the completion of three months after aSAH. The primary outcome was the functional state after three months, and the secondary outcome was in-hospital mortality. The functional outcome was defined using the Modified Rankin Scale (MRS) [13]. Patients with the ability to complete activities of daily living (MRS ≤ 3) were classified as having a relatively good outcome. Those who could not take care of themselves or needed to be admitted to care (MRS ≥ 4) were classified as having a poor outcome.

2.5. Statistical analysis

All analyses were performed with the Statistical Package for the Social Sciences software, version 15 (SPSS, Chicago, IL, USA). Values are expressed as mean and standard deviations if numerical data, counts and percentage if categorical data. Pearson's correlation coefficient (CC) was used to evaluate a possible correlation in continuous variables. The influence of TSP-1 levels on 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 $p < 0.05$.

3. Results

3.1. Clinical characteristics and CSF TSP-1 levels after aSAH

There were 4 cases lost to follow up, the remaining 31 patients (14 men and 17 women, 53.46 ± 9.151 years old) were included in our analysis, CSF samples were collected on days 1–3, days 5–7 and days 8–10. Respectively, 13 patients (42%) developed vasospasm, while the rest showed no evidence of vasospasm. Clinical characteristics and follow-up of all patients are shown in Table 1. A low level of TSP-1 was identified in the control group (4.56 ± 3.42 ng/ml), compared with the control group, TSP-1 levels were peaked on days 1–3 after aSAH (42.65 ± 10.92 ng/ml, $p < 0.01$), kept up high on days 5–7 (31.86 ± 13.46 ng/ml, $p < 0.01$) and remained elevated until days 8–10 (19.81 ± 6.16 ng/ml, $p < 0.05$, as in Fig. 1).

3.2. Correlation analysis of CSF TSP-1 levels and clinical parameters

As shown in Table 2, CSF TSP-1 levels on days 1–3 were highly associated with Hunt-Hess grade, World Federation of Neurosurgical Societies (WFNS) scores, vasospasm, plasma C-reactive protein, partial thromboplastin time, plasma fibrinogen and plasma D-dimer. CSF TSP-1 levels on days 5–7 were highly associated with WFNS scores, vasospasm, plasma C-reactive protein, plasma fibrinogen and plasma D-dimer.

3.3. CSF TSP-1 levels in relation to vasospasm and three-month outcome

Patients with vasospasm or poor outcome (MRS 4–6) at 3 months had a higher CSF TSP-1 levels on days 1–3 and days 5–7 than those without vasospasm or with good outcome (MRS 1–3) , respectively (all $p < 0.05$, Fig. 2A–B). MRS at 3 months showed a significant correlation with CSF levels of TSP-1 on days 1–3 (CC = 0.666, p = 0.001; 95% CI 0.453–0.845) and days 5–7 (CC = 0.525, p = 0.006; 95% CI 0.331–0.764), but not on days 8–10 (CC = 0.368, p = 0.161; 95% CI 0.044–0.736; Fig. 2C–E). In binary logistic regression analysis, higher TSP-1 level at days 1–3 (odds radio = 1.112, p = 0.042, 95% CI 1.004–1.231) and on days 5–7 (odds radio = 1.087, p = 0.032, 95% CI 1.007–1.174) was a predictive marker of cerebrovasospasm; higher TSP-1 level at days 1–3 (odds radio = 1.481, p = 0.049, 95% CI 1.001–2.008) and on days 5–7 (odds radio = 1.124, p = 0.023, 95% CI 1.016 to 1.243) was a predictive marker of poor outcome (Table 3).

4. Discussion

The current study demonstrated that (1) CSF TSP-1 levels were significantly higher in aSAH patients than that in healthy controls and reached the peak on days 1–3; (2) TSP-1 was closely associated with Hunt-Hess grade, WFNS, vasospasm and plasma C-reactive protein using correlation analysis; (3) CSF TSP-1 levels on days 1–3 and 5–7 were obviously higher in patients with unfavorable outcome than that in patients with favorable outcome; (4) CSF TSP-1 levels on days 1–3 and 5–7 were demonstrated to be an independent predictor for vasospasm and three-month poor outcome using a binary logistic regression analysis.

TSP-1 is a multifunctional molecule, which is known as the first discovered and characterized endogenous inhibitor of angiogenesis and regulator of apoptosis [6, 10, 14, 15]. It is reported that various kinds of injury can induce the release of TSP-1 from multiple cells, including platelets, leukocytes and endothelial cells, and expression of TSP-1 is also upregulated in the extracellular matrix following tissue injury [10, 16]. The origin of CSF TSP-1 after SAH was not very clear, we supposed that it may release from the platelets, leukocytes in the bloody CSF, or from the endothelial cells of the impaired blood-brain barrier, even from the damaged brain tissue [17]. In vitro and in vivo studies have confirmed that astrocytes could express TSP-1. Moreover, TSP-1