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Original Article

Thrombin-activatable fibrinolysis inhibitor (TAFI) is a potential biomarker for cerebral hemorrhage patients

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Abstract: Cerebral hemorrhage is the most common human cerebrovascular disease and frequently leads to paralysis, a vegetative state, and even death. Previous studies have indicated that thrombin-activatable fibrinolysis inhibitor (TAFI) is associated with chronic thromboembolic pulmonary hypertension and cerebral hemorrhage. The purpose of this study was to investigate the correlation between serum levels of TAFI and inflammatory markers in cerebral hemorrhage patients. A total of 138 patients diagnosed with cerebral hemorrhage were enrolled in this clinical study, and 100 healthy volunteers were recruited as controls. Serum levels of IL-1β, IL-6, IL-17, and TNF-α were detected by ELISA. Serum levels of TAFI, procalcitonin (PCT), and C-reactive protein (CRP) were analyzed by colloidal gold test strip. We showed that serum levels of inflammatory cytokines IL-1β, IL-6, IL-17, and TNF-α were markedly increased in cerebral hemorrhage patients compared to healthy volunteers ($p < 0.01$). Serum levels of TAFI, PCT, and CRP were also remarkably enhanced in cerebral hemorrhage patients compared to healthy volunteers ($p < 0.01$). Correlation analysis demonstrated that there was a significant correlation between serum TAFI concentration and illness degree of hemorrhage patients but not in healthy volunteers. We also found that serum TAFI concentration was positively correlated with IL-1β, IL-6, IL-17, and TNF-α in the cerebral hemorrhage patients but not in healthy volunteers. In conclusion, these outcomes indicate that TAFI and inflammatory cytokines are up-regulated and correlate with illness degree in patients with cerebral hemorrhage, suggesting that TAFI may have potential ability to be considered as a biomarker for cerebral hemorrhage diagnosis or risk prediction.

Keywords: Cerebral hemorrhage, TAFI, inflammation, biomarker

Introduction

Cerebral hemorrhage is one of the most common human cerebrovascular diseases and patients with cerebral hemorrhage often present higher mortality during periods of disease [1, 2]. The most common manifestations in cerebral hemorrhage patients are cerebral arteriosclerosis, hypertension, and intracranial vascular malformations [3, 4]. Pathological studies have shown that many factors can induce cerebral hemorrhage, and patients with cerebral hemorrhage are usually complicated with severe dysfunction of the cerebral nervous system and loss of social working and self-care ability, which further increase the burden on the family [5-7]. A comprehensive review of basic and clinical studies describes the current therapeutic drugs against cerebral vasospasm after subarachnoid hemorrhage [8]. However, the morbidity and mortality rate of patients with cerebral hemorrhage still remain high in the clinic.

Thrombin activatable fibrinolysis inhibitor (TAFI) plays an important role in the progression of human cerebral hemorrhage, by contributing to inhibition of both thrombosis formation and inflammation [9, 10]. TAFI can inhibit fibrinolysis and it remains to be elucidated whether TAFI is directly involved in the pathogenesis of human cerebral hemorrhage [11]. In addition, TAFI is regarded as a plasma procarboxypeptidase that is activated by the thrombin-thrombomodulin complex on the vascular endothelial surface, which further regulates tissue inflammation [12]. Furthermore, the role of TAFI in hemodialysis patients linking inflammation and hypofibrinolysis to cardiovascular events has been investigated in a previous study [13]. Therefore, a potential correlation between TAFI and inflammation in the progression of cerebral hemorrhage and more molecular mechanisms to elaborate the disease network are urgently needed.

In this study, we analyzed the changes of TAFI and inflammatory cytokines in patients with

Table 1. Characteristic of patients with cerebral hemorrhage

Characteristics	Patients	Health
Number	138	100
Female	65	48
Male	73	52
Age	36.6-66.5	40.5-68.7
Clinical stage		
CCH	34	0
ESCH	19	0
SLCH	21	0
ACH	24	0
HICH	27	0

cerebral hemorrhage compared to healthy volunteers. Our findings report that TAFI and inflammatory cytokines are up-regulated and correlate with the degree of illness in patients with cerebral hemorrhage. This study also investigated serum levels of PCT and CRP as well as the correlation with serum levels of TAFI in patients with cerebral hemorrhage.

Materials and methods

Patients and healthy volunteers

A total of 138 patients with cerebral hemorrhage and 100 healthy volunteers were recruited for analysis of the association of serum TAFI and inflammatory cytokines. The numbers of men and women patients were approximate equal. All participants were eligible to finish this clinical investigation. The characteristics of cerebral hemorrhage patients are summarized in **Table 1**.

ELISA

Serum levels of IL-1β (NO: MAF10524), IL-6 (NO: MAF12783), IL-17 (NO: MAF15283), TNF-α (NO: MAF7392), TAFI (NO: MAF7202), PCT (NO: MAF7710) and CRP (NO: MAB40283) were detected in patients with arrhythmia using ELISA kit (Bio-Techne, R&D Systems, USA) according to the manufacturer's instruction. The serum concentrations of inflammatory cytokines, TAFI, PCT, and CRP were also measured by an enzyme micro-plate reader at 570 nm.

Regression analysis

The serum levels of bilirubin and uric acid in the detective data (*Y*) were analyzed by regression analysis in different clinical stages and persistent auricular fibrillation patients with arrhythmia were analyzed using the least square convergence [14]. The predicted curve that resulted in the lowest sum of squares was the best fit. If the fit was robust, then the parameters of the observed curve could be inferred from those of the predicted.

Statistical analysis

For each experiment, the mean and standard error were determined. Statistical differences between groups were assessed by means of analysis of variance (ANOVA) from 6 replicate experiments with the post-hoc Dunnett's test. Statistical significance was considered at $P < 0.05$.

Results

Serum levels of inflammatory cytokines IL-1β, IL-6, IL-17, and TNF-α in patients with cerebral hemorrhage

We first analyzed serum levels of inflammatory cytokines IL-1β, IL-6, IL-17, and TNF-α in patients with cerebral hemorrhage and healthy volunteers. As shown in **Figure 1A**, **1B**, we show that serum levels of IL-1β and IL-6 were increased in patients with cerebral hemorrhage compared to healthy volunteers. Serum levels of IL-17 and TNF-α were significantly up-regulated in patients with cerebral hemorrhage (**Figure 1C**, **1D**). These results indicate that patients with cerebral hemorrhage presented with higher serum levels of inflammatory cytokines IL-1β, IL-6, IL-17, and TNF-α.

Serum levels of TAFI, PCT, and CRP in patients with cerebral hemorrhage

Inflammatory cytokines TAFI, PCT, and CRP were analyzed in patients with cerebral hemorrhage. As shown in **Figure 2A**, TAFI serum concentration levels were significantly up-regulated in patients with cerebral hemorrhage compared to those in the healthy volunteers. We observed that the serum levels of PCT and CRP were also increased in patients with cerebral hemorrhage compared to those in the healthy control (**Figure 2B** and **2C**). These results indicated that serum levels of TAFI, PCT, and CRP were up-regulated in patients with cerebral hemorrhage.

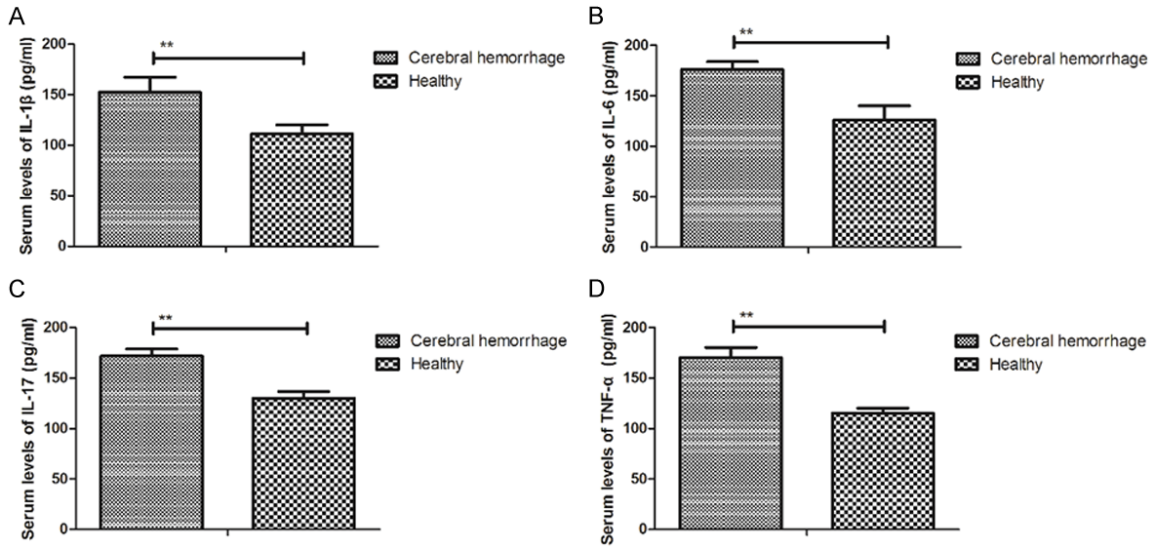

Figure 1. Serum levels of inflammatory cytokines IL-1β, IL-6, IL-17,and TNF-α in patients with cerebral hemorrhage. (A-B) Serum levels of IL-1β (A) and IL-6 (B) were increased in patients with cerebral hemorrhage compared to healthy volunteers. (C-D) Serum levels of IL-17 (C) and TNF-α (D) were significantly up-regulated in patients with cerebral hemorrhage.

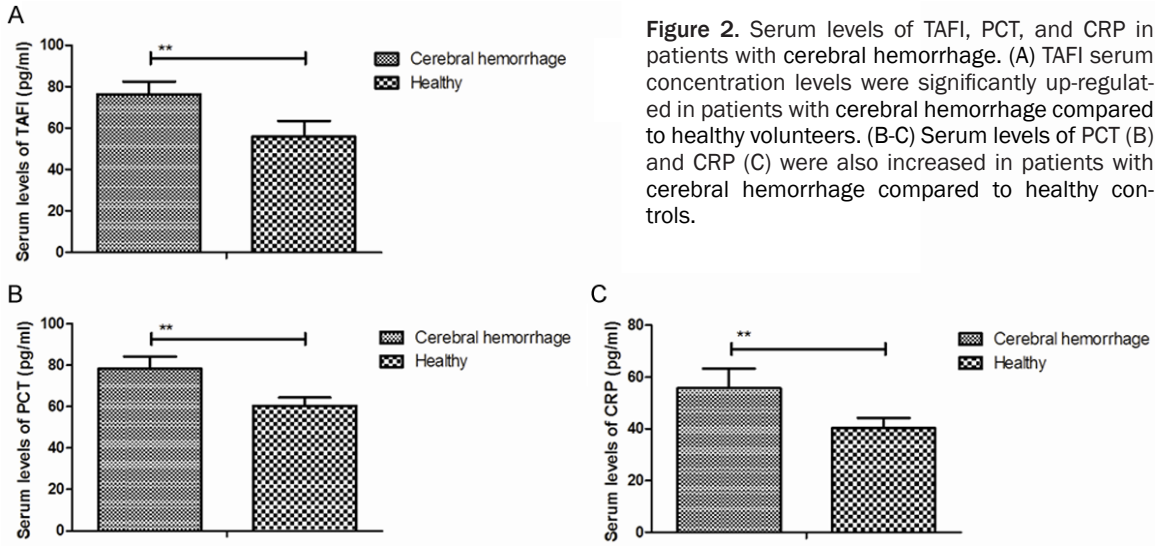

Figure 2. Serum levels of TAFI, PCT, and CRP in patients with cerebral hemorrhage. (A) TAFI serum concentration levels were significantly up-regulated in patients with cerebral hemorrhage compared to healthy volunteers. (B-C) Serum levels of PCT (B) and CRP (C) were also increased in patients with cerebral hemorrhage compared to healthy controls.

Correlation analysis between TAFI and inflammatory cytokines IL-1β, IL-6, IL-17, and TNF-α in patients with cerebral hemorrhage

Associations were analyzed between TAFI and inflammatory cytokines IL-1β, IL-6, IL-17, and TNF-α in patients with cerebral hemorrhage as determined by regression analysis. We show that TAFI concentration is positively correlated with IL-1β in cerebral hemorrhage patients but not in healthy volunteers (**Figure 3A**). Results present serum levels of IL-6 and IL-17 that were positively correlated with TAFI serum concentration levels in cerebral hemorrhage patients (**Figure 3B** and **3C**). We also found that TAFI was positively correlated with TNF-α serum levels in patients with cerebral hemorrhage (**Figure 3D**). These results indicated that serum levels of TAFI are positively correlated with the inflammatory cytokines IL-1β, IL-6, IL-17, and TNF-α in patients with cerebral hemorrhage.

Correlation analysis between TAFI and PCT, CRP in patients with cerebral hemorrhage

The associations between TAFI and PCT, CRP were respectively analyzed in patients with cerebral hemorrhage and in the healthy con-

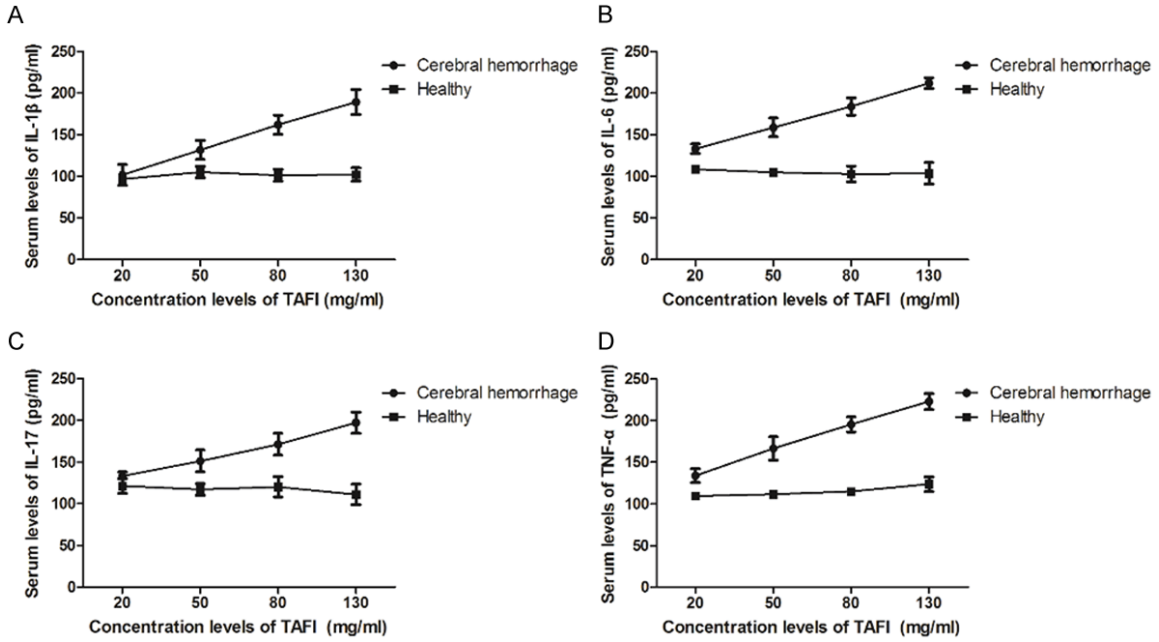

Figure 3. Correlation analysis between TAFI and inflammatory cytokines IL-1β, IL-6, IL-17, and TNF-α in patients with cerebral hemorrhage (A) TAFI concentration is positively correlated with IL-1β in cerebral hemorrhage patients but not in healthy volunteers. (B-C) Serum levels of IL-6 (B) and IL-17 (C) were positively correlated with TAFI serum concentration levels in cerebral hemorrhage patients. (D) Serum levels of TAFI are positively correlated with TNF-α serum levels in patients with cerebral hemorrhage.

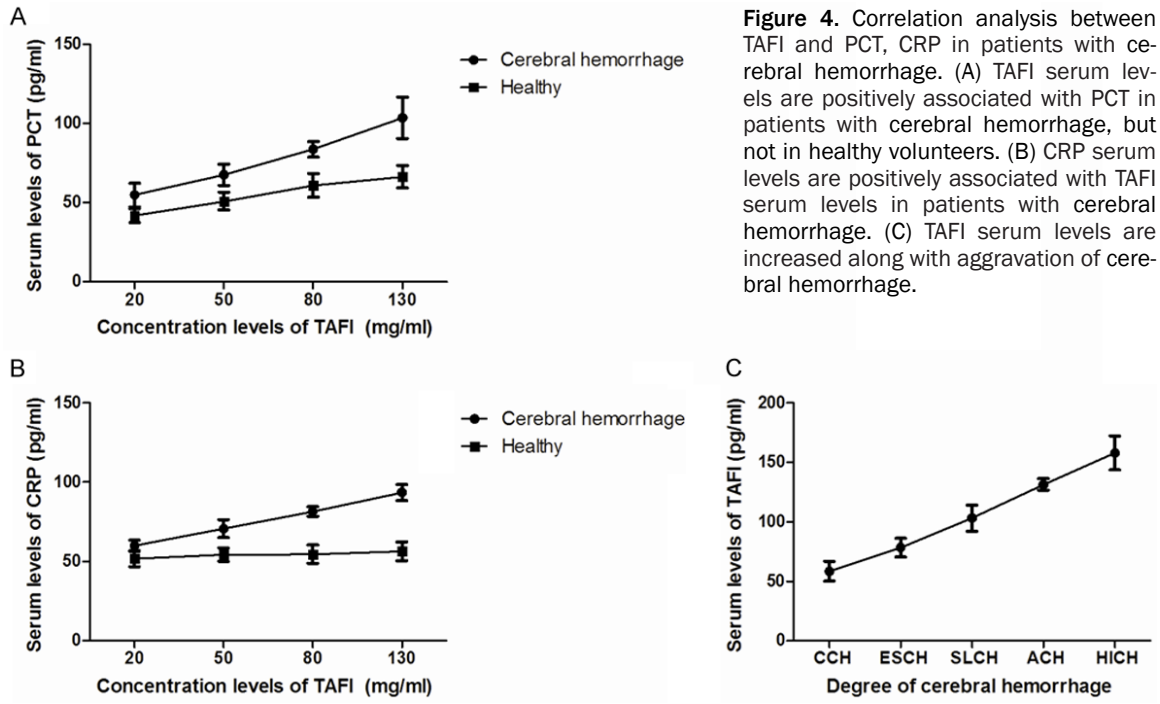

Figure 4. Correlation analysis between TAFI and PCT, CRP in patients with cerebral hemorrhage. (A) TAFI serum levels are positively associated with PCT in patients with cerebral hemorrhage, but not in healthy volunteers. (B) CRP serum levels are positively associated with TAFI serum levels in patients with cerebral hemorrhage. (C) TAFI serum levels are increased along with aggravation of cerebral hemorrhage.

trols. Outcomes showed that TAFI serum levels were associated with PCT in patients with cerebral hemorrhage, but not in healthy volunteers (**Figure 4A**). Results also revealed that CRP serum levels were positively associated with TAFI serum levels in patients with cerebral hemorrhage (**Figure 4B**). Importantly, TAFI serum levels were increased along with aggra-

vation of cerebral hemorrhage (**Figure 4C**). These results suggest that serum levels of TAFI are positively associated with serum levels of CT, CRP, and illness status of patients with cerebral hemorrhage.

Discussion

Numerous studies have indicated that cerebral hemorrhage causes damage of neuronal cells and further aggravates brain damage, even resulting in contralateral limb dysfunction [15-17]. TAFI plays important roles in the progression and prognosis of cerebral hemorrhage [18-20]. In this study, we analyzed changes of serum levels of TAFI and the relationships between TAFI and cytokines, CT, CRP in patients with cerebral hemorrhage. Although the systematic review and meta-analysis analyzed genetic variations in thrombin-activatable fibrinolysis inhibitor gene and risk of cardiovascular disease [21], the relationships between TAFI and the severity of illness of cerebral hemorrhage have not been well understood. Findings in this study indicate that serum TAFI is up-regulated in cerebral hemorrhage patients compared to that in the healthy volunteers. Results also found inflammatory cytokines and serum TAFI level was positively correlated with inflammatory cytokines IL-1β, IL-6, IL-17, and TNF-α in patients with cerebral hemorrhage.

IL-1β is increased following hypertensive intracerebral hemorrhage (ICH), potentially related to neural damage by cerebral edema and correlation between serum IL-1beta levels and cerebral edema extent has been reported in a hypertensive intracerebral hemorrhage rat model [22]. Croci et al. have analyzed serum IL-6 changes in experimental rabbit subarachnoid hemorrhage and the results reveal a statistically significant correlation between IL-6 and ET-1 levels in the CSF [23]. Beeftink et al. reported a relationship between serum TNF-alpha and *TNF-alpha* genotype with delayed cerebral ischemia and outcome in subarachnoid hemorrhage [24]. Previous studies have showed that TAFI is increased in patients with cerebral hemorrhage [25-27]. However, the association between TAFI and inflammation cytokines IL-1β, IL-6, IL-17, and TNF-α remains poorly understood. Results in this study show that inflammatory cytokines IL-1β, IL-6, IL-17, and TNF-α serum levels are up-regulated and correlate with TAFI increasing in patients with cerebral hemorrhage.

Previous studies have shown that early systemic PCT levels are increased in patients with aneurysmal subarachnoid hemorrhage [28]. Festic et al. suggested that the utility of serum PCT of 0.2 ng/mL or greater was demonstrated to be very specific for sepsis among patients with aneurysmal subarachnoid hemorrhage [29]. Additionally, Srinivasan et al. suggested that CRP has a significant independent association with poor GOS score, indicating the preeminence of early cellular response in SAH pathophysiology [30]. Our results indicate that serum levels of PCT and CRP are up-regulated in patients with cerebral hemorrhage. Our findings suggest that serum TAFI is positively associated with serum levels of PCT and CRP in patients with cerebral hemorrhage. Importantly, the results indicated that TAFI serum levels can be responsible for aggravation of cerebral hemorrhage.

In conclusion, although oral anticoagulant or even surgical resection are often used as the mainstay treatment for cerebral hemorrhage in the clinic, effective biomarkers and the prognosis of cerebral hemorrhage are insufficient to predict therapeutic effects. Results in this study show that inflammatory cytokines IL-1β, IL-6, IL-17, and TNF-α and PCT and CRP are up-regulated in patients with cerebral hemorrhage. Interestingly, TAFI serum levels are increased and positively correlate with inflammatory cytokines, PCT, and CRP in patients with cerebral hemorrhage. These results may provide a clinical foundation for patients with cerebral hemorrhage.

Disclosure of conflict of interest

None.

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