

Early elevated levels of soluble triggering receptor expressed on myeloid cells-1 in subarachnoid hemorrhage patients

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Abstract Early brain injury (EBI) contributes to poor prognosis of subarachnoid hemorrhage (SAH). This study aimed to clarify whether triggering receptor expressed on myeloid cells-1 (TREM-1) was implicated in the inflammatory mechanisms of EBI. The cerebrospinal fluid (CSF) levels of soluble TREM-1 (sTREM-1), tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) as well as plasma levels of white blood cells (WBC) count and C-reactive protein in 17 SAH patients at early stage (within the EBI period) and 9 volunteers were observed. Also World Federation of Neurosurgical Societies (WFNS) scale of SAH patients was calculated on admission. Compared to controls, increased CSF levels of sTREM-1 ($t = 5.66$, $P < 0.001$), TNF- α ($t = 5.41$, $P < 0.001$) and IL-6 ($t = 2.98$, $P = 0.007$) as well as elevated plasma WBC counts ($t = 7.61$, $P < 0.001$) and C-reactive protein levels ($t = 3.91$, $P = 0.001$) were found in SAH patients. Considering the increased WBC counts in SAH group, covariate analysis was also performed when comparing patients' sTREM-1 levels with respect to controls and no

obvious difference was found ($F = 0.982$, $P = 0.332$). For SAH group, early CSF concentrations of sTREM-1 were correlated with those of both TNF- α ($r = 0.582$, $P = 0.014$) and IL-6 ($r = 0.593$, $P = 0.012$). Also the CSF sTREM-1 levels were positively correlated with WBC counts ($r = 0.629$, $P = 0.007$) and C-reactive protein levels ($r = 0.804$, $P < 0.001$) as well as WFNS scale ($r = 0.835$, $P < 0.001$). This study showed an early increased sTREM-1 CSF level in SAH patients, which correlated with inflammation intensity post-SAH and clinical severity, indicating that TREM-1 may participate in the inflammatory mechanisms of EBI.

Keywords Subarachnoid hemorrhage · sTREM-1 · TNF- α · IL-6 · Inflammation

Introduction

Subarachnoid hemorrhage (SAH) is a devastating disease that carries a poor prognosis frequently [1]. Recent studies have demonstrated that early brain injury (EBI) within 3 days post-SAH mainly leads to the poor prognosis [2], and inflammatory response is the main contributor to EBI [3, 4]. A number of investigations have clarified that triggering receptor expressed on myeloid cells-1 (TREM-1) is a critical amplifier of inflammatory signaling in various diseases [5]. However, whether TREM-1 is implicated in the inflammatory mechanisms of EBI has not been explored before. Thus, in this study, the early level of soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) within the period of EBI (within 3 days post-SAH) in cerebrospinal fluid (CSF) of SAH patients was observed, and its association with the inflammatory response intensity post-SAH and clinical severity on admission was analyzed. The aim of this study

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was to explore TREM-1's potential role in the inflammatory mechanisms of EBI.

Materials and methods

Patients and control subjects

The Ethics Review Board of our institution examined and approved this research protocol in accordance with the Declaration of Helsinki before the study. In this study, 17 patients with aneurysm SAH (SAH group) from the neurosurgery departments of Shanxi People's Hospital and The Second Hospital Affiliated to Shanxi Medical University were recruited from September 1 to November 30th 2015, and 9 healthy volunteers were enrolled as control (control group). The inclusion criteria of SAH patients were: age 18 years and above, aneurysm SAH confirmed by cerebral computed tomography and digital subtraction angiography, treated with endovascular coiling or surgical clipping within 3 days after SAH, received lumbar puncture or lumbar drain within 3 days after SAH. Cases who had meningitis, infectious aneurysm, brain tumors, hematological disorders, head trauma or other major injuries were excluded.

CSF sTREM-1 levels analyzed

For SAH patients, CSF specimens were collected through lumbar puncture or lumbar drain within 3 days after SAH. Also CSF samples were taken from the 9 healthy subjects as control. After being collected, the CSF specimens were centrifuged at 1500g within 2 h and 4 °C for 10 min, and the supernatant fluids were stored at –80 °C until required for analysis. The CSF levels of sTREM-1 were determined with an established available enzyme-linked immunosorbent assay kit (R&D Systems, Inc., Minneapolis, MN, USA) specific for human, and the operation was carried out according to the specification.

Inflammatory response intensity and clinical severity analyzed

For SAH patients, the inflammatory response intensity was calculated by the CSF levels of TNF- α and IL-6 within 3 days post-SAH with enzyme-linked immunosorbent assay kit as mentioned above, as well as white blood cells (WBC) counts and C-reactive protein level in peripheral blood on admission. The same examinations were performed for the healthy subjects as control. World Federation of Neurosurgical Societies (WFNS) scale was calculated on admission to evaluate SAH patients' clinical severity for clinical deterioration. Then, the association

between patients' CSF sTREM-1 levels with the inflammatory response intensity and clinical severity was analyzed.

Statistics

Levels of CSF sTREM-1, TNF- α and IL-6 were expressed as the mean \pm standard deviation. Numerical and categorical variables between two groups were compared using the Student *t* tests and the Chi square tests, respectively. Covariate analysis was also performed when comparing patients' sTREM-1 levels with respect to controls in order to show whether elevated sTREM1 in SAH group can be justified by an increase in total number of WBC. Correlations were evaluated with the Pearson or Spearman rank test (double-sided). $P < 0.05$ was considered statistically significant. The statistical analyses were performed with SPSS 20.0 and Graphpad Prism 5.0.

Results

Characteristics of the study population

A total of 17 patients and 9 healthy volunteers were enrolled in this study. No patient died or was found to have rebleeding during the trial period. 10 patients were accompanied with fever and leukocytosis was found in 16 cases on admission in SAH group. The other characteristics of the studied population are detailed in Table 1.

sTREM-1, TNF- α and IL-6 levels in CSF

In comparison with that of the healthy controls, significantly increased mean level of sTREM-1 ($t = 5.66$, $P < 0.001$), TNF- α ($t = 5.41$, $P < 0.001$) and IL-6 ($t = 2.98$, $P = 0.007$) was detected in patients' CSF within 3 days post-SAH (Table 1). Considering the increased WBC counts in SAH group, covariate analysis was also performed when comparing patients' sTREM-1 levels with respect to controls and no obvious difference was found ($F = 0.982$, $P = 0.332$) (Table 1). For the SAH group, the CSF concentrations of sTREM-1 correlated statistically with that of both TNF- α ($r = 0.582$, $P = 0.014$) (Fig. 1) and IL-6 ($r = 0.593$, $P = 0.012$) (Fig. 2).

WFNS scale and peripheral blood examination

For the SAH group, the mean WFNS scale was 3.59 on admission. Compared to that of the volunteers, patients' mean plasma WBC count ($t = 7.61$, $P < 0.001$) and C-reactive protein ($t = 3.91$, $P = 0.001$) level examined

Table 1 Characteristics and clinical data of study population

	SAH group	Control group	<i>P</i>
Number	17	9	
Age (year)	56.59 ± 9.55	58.89 ± 10.23	0.57
Male (%)	6 (35.29%)	4 (44.44%)	0.69
Smoking index	115.29 ± 145.91	127.78 ± 152.79	0.84
Coil/clip	12/5	–	–
WFNS scale	3.59 ± 1.06 ^a	–	–
Count of WBC (×10 ⁹ /L)	15.07 ± 2.70 ^a	7.68 ± 1.44	<0.001
C-reactive protein (mg/L)	37.67 ± 24.81 ^a	5.0 ± 2.2	0.001
sTREM-1 (pg/mL)	265.22 ± 50.10 ^b	166.68 ± 18.13	<0.001, 0.332 ^c
TNF-α (pg/mL)	54.97 ± 26.70 ^b	6.13 ± 3.80	<0.001
IL-6 (pg/mL)	2689.10 ± 2135.17 ^b	548.00 ± 162.78	0.007

SAH subarachnoid hemorrhage, WFNS World Federation of Neurosurgical Societies, WBC white blood cells, sTREM-1 soluble triggering receptor expressed on myeloid cells-1, TNF-α tumor necrosis factor-α, IL-6 Interleukin-6

^a On admission, ^bwithin 3 days after SAH, ^ccovariate analysis

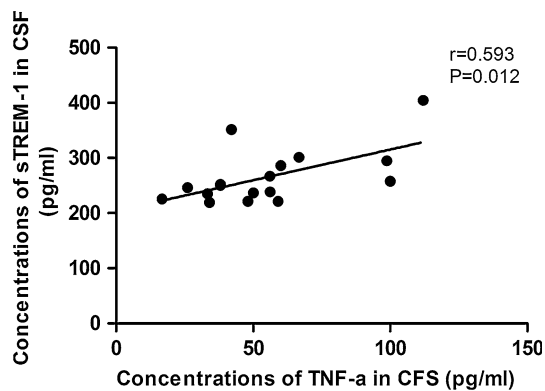


Fig. 1 Correlation between soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) concentrations and tumor necrosis factor-α (TNF-α) concentrations in cerebrospinal fluid (CSF) within 3 days after subarachnoid hemorrhage

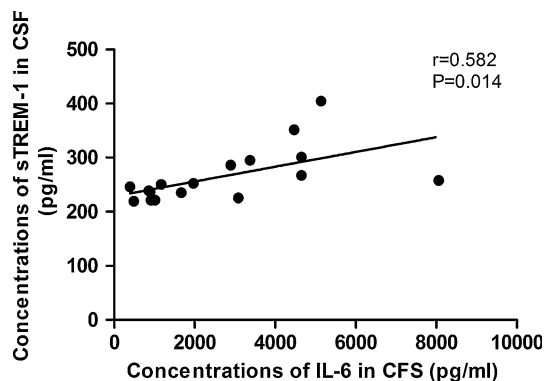


Fig. 2 Correlation between soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) concentrations and interleukin-6 (IL-6) concentrations in cerebrospinal fluid (CSF) within 3 days after subarachnoid hemorrhage

on admission were obviously increased (Table 1). The patients' CSF sTREM-1 levels at early stage were positively correlated with WBC counts ($r = 0.629$, $P = 0.007$) and C-reactive protein levels ($r = 0.804$, $P < 0.001$) in peripheral blood as well as the WFNS scale ($r = 0.835$, $P < 0.001$) performed on admission.

Discussion

SAH is an acute and severe cerebrovascular disease that is mainly caused by ruptured aneurysms [1]. It has been demonstrated that EBI, instead of cerebral vasospasm, is the most important cause of patients' poor outcome [2]. EBI is the immediate injury to the brain within 3 days after SAH onset and strongly determines patients' prognosis [6]. To present, the exact pathogenesis is still unclear, and this study aimed to explore TREM-1's potential role in the inflammatory mechanisms of EBI.

Large amounts of studies have highlighted that inflammation appears to be the key contributor to EBI [3, 4]. After SAH onset, the lysis of erythrocytes into subarachnoid space activates neutrophils and monocytes, triggering an inflammatory cascade similar to systemic inflammatory response syndrome [6]. This excessive inflammatory response induces apoptosis and leads to blood brain barrier disruption by increasing vessel diameter and endothelial permeability [7, 8]. It has been demonstrated that the plasma C-reactive protein and CSF levels of TNF-α and IL-6 act as monitoring biomarkers facilitated to assess the inflammatory response intensity after SAH [9, 10]. In addition, previous studies have indicated the critical role of neutrophils in the exacerbating inflammation after SAH

[11], which release pro-inflammatory mediators and chemotactic agents, enhancing the recruitment of further neutrophils and excessive inflammation [12], so neutrophils are among the first cells in the blood to respond after EBI [13, 14]. Thus, in this study, the plasma C-reactive protein level, WBC count and CSF levels of TNF- α and IL-6 were used to evaluate the inflammatory response intensity, and they were all obviously increased in the SAH group compared to that of the volunteers in accordance with previous studies, indicating the presence of systemic inflammation during EBI.

Various inflammatory mediators have been found to be involved in the pathogenesis of EBI [15–19]. However, no clinical availability of anti-inflammatory therapies has been proved. Thus, it is speculated that there are still unknown inflammatory mediators in EBI. TREM-1 was initially thought to be a critical amplifier of acute inflammatory signaling in response to microbial products [5, 20]. However, latter investigations showed that TREM-1 may also be involved in nonmicrobial inflammation, such as acute pancreatitis, inflammatory bowel disease, rheumatoid arthritis and systemic lupus erythematosus [21–24]. Moreover, the expression of TREM-1 was also significantly increased in cancers and gastritis unrelated to *H. Pylori*, indicating its potential role in the inflammatory mechanism of various diseases [25, 26]. In the present study, significantly increased sTREM-1 levels were found in SAH patients' CSF within 3 days after aneurysm rupturing. Based on previous reports and our study, we hypothesize that TREM-1 might mediate the inflammatory cascade after SAH and participate in the mechanisms of EBI. Considering the increased WBC counts in SAH group and the positive correlation between sTREM-1 levels and WBC counts, covariate analysis was also used to compare patients' sTREM-1 levels with respect to controls and no obvious difference was found. So the elevated values of sTREM-1 in SAH group may be justified by the increase in total number of WBC. This result further supported the fact that TREM-1 is mainly expressed on monocytes/macrophages and neutrophils [5, 20].

TREM-1 has been demonstrated to be a critical amplifier of inflammatory response [5, 20]. Upon binding to the ligand, a residue in the transmembrane region of TREM-1 mediates the association and phosphorylation of the transmembrane adapter protein DNA activating protein 12, then Syk family kinase is recruited and activates the downstream signaling molecules including extracellular signal-regulated kinase 1 and 2 and p38 mitogen-activated protein kinase that trigger NF- κ B, which activates the expression of inflammatory cytokines, such as IL-1 β , IL-6, and TNF- α [5, 27]. Moreover, TNF- α in turn leads to the regeneration of TREM-1, resulting in expanding or even loss control of inflammation [5, 27]. Accumulating evidence has demonstrated that the

downstream molecules of TREM-1 pathway, including ERK1/2, p38MAPK, IL-6, IL-1 β , TNF- α and NF- κ B, are all up-regulated in the early inflammatory cascade after SAH [15–19]. Based on these results, it is speculated that TREM-1 may act as a critical player to initiate and amplify the inflammatory mechanisms of EBI. Our current study observed that the early increased CSF sTREM-1 levels significantly correlated with the inflammatory response intensity after SAH, and patients with high levels of sTREM-1 had unfavorable WFNS scales for clinical deterioration. These results supported our speculation and further studies are needed to validate TREM-1' exact role in the inflammatory mechanisms of EBI.

Conclusion

In this study, we demonstrated an early increased sTREM-1 level in CSF of SAH patients, which significantly correlated with inflammatory response intensity post-SAH and clinical severity on admission, indicating that TREM-1 may play an important role in the inflammatory mechanisms of EBI.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no financial or other conflicts of interest in relation to this research and its publication.

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