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Serum matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 levels in patients with tick-borne encephalitis

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Summary *Objectives:* Matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) play important roles in the function of the blood–brain barrier (BBB). To investigate the function of the BBB during tick-borne encephalitis (TBE), the levels of MMP-9 and its common tissue inhibitor, TIMP-1, were measured in serum from patients with acute phase of TBE.
Methods: Serum MMP-9 and TIMP-1 levels were measured in 147 patients with TBE and 153 controls by ELISA.
Results: Serum MMP-9 levels and MMP-9/TIMP-1 ratios of TBE patients were significantly higher than controls ($p < 0.0001$ and $p < 0.005$, respectively). There were no significant differences in serum TIMP-1 levels between TBE patients and controls. Serum MMP-9 and TIMP-1 levels and MMP-9/TIMP-1 ratios were not associated with age of the patients. However, TBE-positive males with TBE had higher levels of MMP-9 than TBE-positive females ($p < 0.05$).
Conclusions: Our results suggest that the increased serum level of MMP-9 and MMP-9/TIMP-1 ratio is associated with the pathogenesis of TBE. Serum MMP-9 can serve as an indicator of breakdown of the BBB and inflammatory brain damage during TBE.

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Introduction

Tick-borne encephalitis (TBE) is considered an important health problem in Europe and Russia because of protracted course and in some cases severe illness resulting in long-lasting cognitive dysfunction and even persisting pareses of spinal nerves. Although mortality in Europe is relatively low (<1%), rates as high as 20% have been observed in Asia.[1] TBE is endemic in regions of 27 European countries, and new risk areas are discovered every year. In the 30 years between 1974 and 2003, a continuous increase in TBE morbidity reaching up to 400%, was observed in Europe.[2] From 2004 to 2006, another considerable increase was seen in some TBE countries, notably the Czech Republic, Germany, Poland, Slovenia, and Switzerland.[3] The etiologic agent, tick-borne encephalitis virus (TBEV), a member of the family *Flaviviridae*, genus Flavivirus, is an arbovirus that is transmitted to humans by infected ixodid tick vectors.

The pathophysiology of the development of encephalitis is unclear.[4] In particular, very little is known about the role of the blood–brain barrier (BBB) in the neuropathogenesis of TBE.[5] In this study, the function of the BBB during TBE was investigated by measurements the levels of matrix metalloproteinase-9 (MMP-9) and its common tissue inhibitor (TIMP-1) in serum from patients with acute phase of TBE. Various experimental studies demonstrated that MMP-9 might be a potential mediator for the disruption of BBB.[6–8] Matrix metalloproteinases (MMPs) represent a family of enzymes that are responsible for the degradation of extracellular matrix proteins. MMPs play important roles in normal and pathological processes, including inflammation.[9] MMP-9 is capable of degrading collagen IV, a major component of the basement membrane of the cerebral endothelium, and promotes the migration of cells through tissue or across the BBB.[9,10] The activity of MMPs is controlled by specific tissue inhibitors of metalloproteinases (TIMPs). TIMP-1 has a high affinity for MMP-9.[9,11] The levels of MMP-9, TIMP-1 and the ratio between these two proteins were extensively studied in various diseases of the central nervous system, including subacute sclerosing panecephalitis,[9,10] fungal or tuberculous meningoencephalitis,[12] HIV-associated neurological diseases,[13] herpes simplex virus encephalitis,[14] and influenza-associated encephalopathy.[15]

Here, we demonstrate that the development of TBE is associated with increase of MMP-9 levels and MMP-9/TIMP-1 ratio in serum. Our results suggest that the increased serum level of MMP-9 and MMP-9/TIMP-1 ratio is associated with the pathogenesis of TBE, and serum MMP-9 can serve as an indicator of inflammatory damage to the brain during TBE.

Patients and methods

Serum samples were obtained from 147 patients (78 males and 69 females, aged from 5 to 91 years; median, 46 years) with serologically confirmed acute TBE (detection of specific anti-TBEV IgM and IgG antibodies by ELISA). The samples were collected in years 2011 and 2012. Patients (or their parents) signed an informed consent before sample collection. Then the samples were investigated anonymously. The study was approved by Institutional Ethical Committee (PARU ASCR No. 01/11).

The control subjects for the serum levels of MMP-9 were 239 and TIMP-1 were 153 healthy individuals of a corresponding age group (115 males and 124 females; aged from 4 to 83 years; median, 40 years).

The serum concentrations of MMP-9 and TIMP-1 were determined by sandwich-type ELISA kits (Human TIMP-1 and Human MMP-9, Invitrogen Corporation CA, USA). Assays were performed following the instructions of the manufacturer. The detection limits were 0.0235 ng/ml for MMP-9, and 1.56 ng/ml for TIMP-1.

The statistical differences in MIP-9 and TIMP-1 concentration in sera between groups were analyzed using the Mann–Whitney test. Differences with $p < 0.05$ were considered significant. Correlations were analyzed using Pearson's coefficient correlation. Analyses and calculations were performed using GraphPad Prism 5.04 (GraphPad Software, Inc., USA).

Results

The geometric means of serum MMP-9 and TIMP-1 levels, and MMP-9/TIMP-1 ratios of the controls were 251.8 ng/ml (range, 58.8–342.5 ng/ml), 742.8 ng/ml (range, 153.4–3635.7 ng/ml), and 0.5400 (range, 0.0004–2.1804), respectively. The geometric means of serum MMP-9 and TIMP-1 levels, and MMP-9/TIMP-1 ratios of the TBE patients were 403.1 ng/ml (range, 93.9–599.3 ng/ml), 749.3 ng/ml (range, 188.1–3384.1 ng/ml), and 0.6801 (range, 0.0302–2.0236), respectively (Fig. 1). Serum MMP-9 levels and MMP-9/TIMP-1 ratios of TBE patients were significantly higher than the controls ($p < 0.0001$ and $p < 0.005$, respectively). There were no significant differences in serum TIMP-1 levels between TBE patients and controls ($p = 0.7964$).

When patients and control subjects divided into two groups according to their age (<50 and ≥50 years), there were no statistical differences between the groups (Fig. 2). The geometric means of serum MMP-9, TIMP-1 levels, and MMP-9/TIMP-1 ratios of the TBE patients <50 years-old were 350.8 ng/ml (range, 65.62–599.26 ng/ml), 758.90 ng/ml (range, 202.00–3384.13 ng/ml), and 0.6766 (range, 0.0302–1.8104), respectively. The geometric means of serum MMP-9, TIMP-1 levels, and MMP-9/TIMP-1 ratios of the TBE patients ≥50 years-old were 359.40 ng/ml (range, 74.88–588.28 ng/ml), 736.00 ng/ml (range, 188.11–1902.71 ng/ml), and 0.6848 (range, 0.0532–2.0237), respectively. There was no correlation of serum MMP-9 and TIMP-1 levels and MMP-9/TIMP-1 ratios with age of the TBE patients as well as controls (Fig. 3).

The geometric means of serum MMP-9, TIMP-1 levels, and MMP-9/TIMP-1 ratios of the male TBE patients were 372.80 ng/ml (range, 74.88–599.26 ng/ml), 763.9 ng/ml (range, 188.1–3384.1 ng/ml), and 0.709 (range, 0.532–2.024), respectively. The geometric means of serum MMP-9, TIMP-1 levels, and MMP-9/TIMP-1 ratios of the female TBE patients were 333.8 ng/ml (range, 65.6–567.3 ng/ml), 732.70 ng/ml (range, 199.67–2751.42 ng/ml), and 0.65 (range, 0.03–1.81), respectively. In case of controls, no difference in serum MMP-9 and TIMP-1 levels, and MMP-9/TIMP-1 ratios was seen between males and females. Males