VASCULAR ENDOTHELIAL GROWTH FACTOR IS ASSOCIATED WITH BLOOD BRAIN BARRIER DYSFUNCTION IN EOSINOPHILIC MENINGITIS CAUSED BY ANGIOSTRONGYLUS CANTONENSIS INFECTION

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Abstract. Vascular endothelial growth factor (VEGF) is a potent vascular permeability factor and a mediator of brain edema. To assess the role of vascular endothelial growth factor in eosinophilic meningitis, vascular endothelial growth factor was measured in the cerebrospinal fluid (CSF) and blood of 9 patients with eosinophilic meningitis in a cohort study. VEGF_{CSF} was detected in 8 (90%) of 9 eosinophilic meningitis patients (range, 45–2190 pg/mL) at presentation. The mean VEGF_{CSF} at presentation, 1 week, and 2 weeks after admission was 568 pg/mL, 751 pg/mL, and 1031 pg/mL, respectively. There was an association between VEGF_{CSF}. CSF protein, white cell count, and eosinophil counts. The VEGF_{SERUM} fluctuated during the 6-month follow-up period. These results indicate that vascular endothelial growth factor may be associated with blood-brain barrier disruption in patients with eosinophilic meningitis.

INTRODUCTION

Angiostrongylus cantonensis, also known as the rat lungworm, is the most common cause of eosinophilic meningitis in the Pacific Islands and Southeast Asia. Rats serve as the definitive host of the nematode. If an infection occurs in non-permissive hosts, including humans and mice, the development of the parasites will terminate at the young–adult worm stage in the brain and cause eosinophilic meningitis or meningoencephalitis.\(^\text{1-4}\) Several indices have been developed to assess the blood–brain barrier (BBB) integrity in an individual with a central nervous system infection. It was reported that most human immunodeficiency virus (HIV)-infected patients have pathologic cerebrospinal fluid (CSF) findings for at least one parameter, such as an elevated CSF total protein level, CSF white blood cell count, or CSF serum Immunoglobulin G ratio.\(^\text{5}\) In a mouse animal model of eosinophilic meningitis caused by \(A.\) cantonensis infection, researchers showed that dysfunction of the BBB occurred in mice infected with \(A.\) cantonensis, evidenced by the high concentrations of protein and albumin, high leukocyte counts in CSF, high ratio of CSF/serum protein and albumin, and high permeability of BBB.\(^\text{6}\) Infection of the BBB causes a severe inflammatory reaction, mediated by pathogen products and host cytokines. This inflammatory reaction compromises the function of BBB, resulting in the exudation of plasma proteins and development of vasogenic brain edema, which contributes to cerebral dysfunction and brain damage.\(^\text{7}\) Vascular endothelial growth factor (VEGF), a 46-kDa glycosylated homodimeric protein, is a regulator of angiogenesis and a potent inducer of vascular permeability.\(^\text{8}\) VEGF is implicated in the pathogenesis of brain edema related to ischemia, trauma, bacterial meningitis, tuberculous meningitis, and tumors.\(^\text{9,10}\) To assess the role of VEGF in the pathophysiology of eosinophilic meningitis, dynamic VEGF levels were measured in CSF and serum of patients with eosinophilic meningitis in our cohort study.

PATIENTS

A case of eosinophilic meningitis was clinically defined as presentation with an acute onset of headache, eosinophil pleocytosis in the blood/CSF, accompanied by at least one of the following: fever, ataxia, visual disturbances, pho-thophobia, nuchal rigidity, neck pain, hyperesthesias, or pares-thesias.\(^\text{11}\) Three outbreaks of eosinophilic meningitis, caused by \(A.\) cantonensis occurred in Kaohsiung, Taiwan, in 1998, 1999, and 2001.\(^\text{11,13}\) Most of the patients (77%) were adult, male. Thai laborers who had eaten raw gold apple snails within 3 weeks. Study subjects were derived from the second outbreak in 1999.\(^\text{12,13}\) All of the 9 Thai laborers received only analgesics or glycerol as treatment (or both). Each patient underwent a physical, neurologic, and ophthalmic examination. Laboratory tests were performed at the time of admission and spinal taps were performed on all patients. CSF analysis included cell count, glucose and protein levels, gram and acid-fast stains, India ink preparation, wet mount preparations for larvae, and measurement of cryptococcal antigen. The patients were observed daily during their hospital course. CSF was examined weekly until discharge. Blood was obtained weekly for the first 2 months, every other week for the next 2 months, and monthly thereafter for as long as 6 months. Blood and CSF samples were centrifuged (1700 g at 4°C) immediately, aliquoted, and stored at −70°C.

Antibodies to \(A.\) cantonensis were detected in serum and CSF by a microenzyme-linked immunosorbent assay (ELISA) using young-adult worm antigen, with a molecular weight 204kD, and puriﬁed by monoclonal antibody.\(^\text{14}\)

Vascular endothelial growth factor measurements. Levels of VEGF in the serum and CSF (VEGF_{SERUM} and VEGF_{CSF}, respectively) were measured by enzyme immunoassay (Neogen Corporation, Lansing, MI). This Neogen’s Sandwich Human VEGF is a sandwich enzyme immunoassay (EIA), which measures the free forms of the cytokine VEGF_{tot}. Mouse monoclonal antibodies generated against human VEGF were used to capture human VEGF in a sample. Simultaneously, biotinylated rabbit anti-human VEGF polyclonal antibodies detected VEGF in the sample. The assay was visualized using a streptavidin alkaline phosphatase conjugate and an ensuring chromogenic substrate reaction. The assay sensitivity was 26.6 pg/ml, the range of de-

MATERIALS AND METHODS

INTRODUCTION

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The association between VEGF and CSF A. cantonensis value < 0.05 was considered statistically significant. The mean VEGF level, an arbitrary level of 25 pg/ml was used for statistical purpose.

**Statistical analysis.** The association between VEGF and CSF laboratory abnormalities was analyzed with Pearson correlation test. A P value < 0.05 was considered statistically significant.

**RESULT**

All 9 patients in the cohort study were young Thai men. The source of epidemic was ingestion of raw snails seasoned with lemon juice and red pepper. Patients in this outbreak in 1999 only received supportive therapy. A total of 25 lumbar punctures were performed in these 9 patients. Nine lumbar punctures (5 and 4, respectively) were done in two patients due to recurrent headache. Headache, stiff neck, transient right facial palsy, ataxia, and diplopia recurred in 1 patient 24 days after treatment. A spinal tap revealed an elevated opening pressure of 250 mm H₂O, a white cell count of 578 × 10⁶ cells per µL with 24% eosinophils, a protein level of 165 mg/dL, and a glucose level of 43 mg/dL. (initial CSF white cell count 1270 × 10⁶ cells per µL at admission). He was treated with intravenous glycerol for 7 days and recovered in a week. The serial VEGF₃ levels in this patient were 2190 pg/mL, 2200 pg/mL, 1550 pg/mL, 3640 pg/mL, and 45 pg/mL. Vomiting and headache developed in a second patient 15 days after treatment. A spinal tap revealed an elevated opening pressure of 240 mm H₂O, a white cell count of 1110 × 10⁶ cells per µL with 74% eosinophils, a protein level of 107 mg/dL, and a glucose level of 46 mg/dL (initial CSF white cell count 1390 × 10⁶ cells per µL at admission). He recovered without treatment in about a week. The serial changes of VEGF₃ were 595 pg/mL at presentation, 160 pg/mL, and 3250 pg/mL one and two weeks after admission, and 3640 pg/mL at recurrence of headache. VEGF₃ was detected in 8 (90%) of 9 eosinophilic meningitis patients (range, 45–2190 pg/mL) at presentation. The mean VEGF₃ at presentation, 1 week, and 2 weeks after admission was 568 pg/mL, 751 pg/mL, and 1031 pg/mL, respectively. The VEGF₃, serum, fluctuated during the 6-month follow-up. The mean CSF protein was 131 mg/dL at presentation and dropped to 81 mg/dL 2 weeks later. One patient had a rebound CSF protein level (141 mg/dL) on fifth lumbar puncture done 4 weeks later. CSF white cell count showed a time-dependent decrease, with mean levels of 618 cumm/ml, 320 cumm/ml, and 433 cumm/ml at presentation, 1 week, and 2 weeks after admission, respectively. The CSF white cell counts during the fourth and fifth lumbar puncture in the two patients with recurrent headache were 369 cumm/ml and 667 cumm/ml, respectively. There was an association between VEGF₃, total CSF protein concentrations, white cell counts, and eosinophil counts (Table 1).

**DISCUSSION**

In this study, we showed that VEGF₃ was detected in 8 (90%) of 9 eosinophilic meningitis patients (range, 45–2190 pg/mL) at presentation. There was an association between VEGF₃, CSF protein, white cell count, and eosinophil counts. These results indicate that VEGF may play a role in the pathophysiology of eosinophilic meningitis. An increase in leukocyte count can be observed in the CSF of patients with central nervous system infection due to BBB damage.15,16 Our result showed that CSF white cell count had a time-dependent decrease, with mean levels of 618 cumm/ml, 320 cumm/ml, and 433 cumm/ml at presentation, 1 week, and 2 weeks after admission, respectively. The high white cell count in the CSF represented that the transmigration of leukocytes from the peripheral blood to the CSF in human infected with A. cantonensis becomes easier due to the presence of BBB dysfunction. The time-dependent decrease in CSF white cell count is compatible with the study by Dorta-Contreras and others,17 which showed that at the time of early clinical recovery, the blood–CSF barrier dysfunction was normalized in 75% of the patients. The CSF protein was elevated in our patients with eosinophilic meningitis. In the study of Yiu,4 CSF protein was higher in patients with A. cantonensis-induced eosinophilic meningitis than the normal human. The explanation for an increase in CSF protein concentrations include a decreasing CSF flow rate18 and appearance of plasma proteins in the CSF due to presumed or overt disruption of blood–CSF barrier.19 Furthermore, Dorta-Contreras and others17 demonstrated that during the first 3 days of acute phase of eosinophilic meningoencephalitis, a blood–CSF barrier dysfunction occurred, usually due to a reduced CSF flow rate. Taken together, VEGF is associated with blood–brain barrier dysfunction in patients with eosinophilic meningitis.

Previous reports showed that VEGF can induce endothelial changes during bacterial meningitis, including increased vesicle transport and separation of endothelial intercellular tight junctions. Exposure of normal rat brain to VEGF results in BBB disruption, and VEGF is implicated in the formation of cerebral edema.9,20 Additionally, dexamethasone, which is used as adjunctive therapy in bacterial meningitis, suppresses tumor-associated brain edema by a mechanism involving downregulation of VEGF expression.21,22 Corticosteroid is also used as an adjunctive therapy in eosinophilic meningitis. Whether it is also mediated by a mechanism involving downregulation of VEGF expression still unknown. In the clinical study of Chotmongkol and others,23 a 2-week course of prednisolone was beneficial in relieving the headache in patients with eosinophilic meningitis. In another study, researchers24 showed that a 1-week course of corticosteroid had the same beneficial effect as a 2-week course in increasing the number

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<th>VEGF₃1</th>
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<tbody>
<tr>
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<td>0.334</td>
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<tr>
<td>CSF protein</td>
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<td>CSF wbc1</td>
<td>0.505</td>
<td>0.166</td>
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<td>CSF wbc2</td>
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<td>0.577</td>
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<tr>
<td>CSF eosin</td>
<td>0.15</td>
<td>0.001*</td>
<td>0.806</td>
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* showed P < 0.05.
of patients who felt free from headache. In our study, VEGF$_{CSF}$ measured 1 and 2 weeks after presentation was significantly correlated with CSF abnormalities. The time course is consistent to the clinical treatment duration for 1 or 2 weeks, although we did not use any steroids in our patients.

One of our patients with recurrent headache received glucose as an adjuvant therapy. Osmotherapy with mannitol, glycerol, or hypertonic saline is often used in patients with severe brain edema after stroke or neurotrauma. It can result in massive osmotic diuresis and fluid and electrolytes imbalance. As a result of a disrupted blood–brain barrier, the osmotic agents may leak into the brain parenchyma, with further disturbance of the osmotic gradient as a result. Interestingly, osmotherapy may even enhance disturbance of the blood–brain barrier. Mannitol cause the shrinkage of cerebral capillaries resulting in the opening of endothelial tight junction. This osmotic stress can involve in the calcium influx, nitric oxide, and cytoskeletal change and probably influence the measurement of VEGF in CSF.

The finding that VEGF$_{CSF}$ could not be detected in the CSF of all our patients with eosinophilic meningitis may be explained in several ways. First, locally secreted VEGF may induce endothelial permeability via the luminal receptors of endothelial cells, and may not be reflected in the CSF level. Disruption of the BBB may be further aggravated by VEGF secreted in the CSF via the abluminal receptors of endothelial cells. Second, the timing of sampling may affect VEGF$_{CSF}$ detection because the in vivo half-life of VEGF$_{CSF}$ is unknown. Third, not every patient in our study had CSF abnormalities. Finally, BBB disruption during eosinophilic meningitis involves a complex interaction between eosinophils and mediators, such as interleukin-4, interleukin-5, platelet-activating factor, nitric oxide, and matrix metalloproteinases, which might induce BBB permeability, independent of VEGF.

We did not measure the serum/CSF albumin for VEGF index. VEGF levels were higher in the serum compared with the CSF, therefore the possibility of VEGF accumulation in CSF caused by passive influx instead of intrathecal production can not be excluded. However, it remains to be determined whether this is due to a fairly low production of VEGF by inflammatory cells, rapid degradation of immunoreactive VEGF, or binding of VEGF to soluble or cell-bound VEGF receptors.

Based on our small cases series, we found that patients with eosinophilic meningitis and presence of VEGF proteins in CSF could be associated with disruption of BBB. There was an association between VEGF$_{CSF}$, CSF protein, white cell count, and eosinophil counts. The VEGF$_{ERUM}$ fluctuated during the 6-month follow-up period. However, larger cases studies are needed to justify this conclusion.

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