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Laboratory Investigation

Increased Vascular Endothelial Growth Factor in the Ventricular Cerebrospinal Fluid as a Predictive Marker for Subsequent Ventriculoperitoneal Shunt Infection : A Comparison Study among Hydrocephalic Patients

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Objective : The aim of this study is to determine the association between the cerebrospinal fluid (CSF) biomarkers and inflammation, and the predictive value of these CSF biomarkers for subsequent shunt associated infection.
Methods : We obtained CSF samples from the patients with hydrocephalus during ventriculoperitoneal (VP) shunt operations. Twenty-two patients were enrolled for this study and divided into 3 groups: subarachnoid hemorrhage (SAH)-induced hydrocephalus, idiopathic normal pressure hydrocephalus (INPH) and hydrocephalus with a subsequent shunt infection. We analyzed the transforming growth factor-β1, tumor necrosis factor-α, vascular endothelial growth factor (VEGF) and total tau in the CSF by performing enzyme-linked immunosorbent assay. The subsequent development of shunt infection was confirmed by the clinical presentations, the CSF parameters and CSF culture from the shunt devices.
Results : The mean VEGF concentration (±standard deviation) in the CSF of the SAH-induced hydrocephalus, INPH and shunt infection groups was 236±138, 237±80 and 627±391 pg/mL, respectively. There was a significant difference among the three groups (p=0.01). Between the SAH-induced hydrocephalus and infection groups and between the INPH and infection groups, there was a significant difference of the VEGF levels (p<0.01). However, the other marker levels did not differ among them.
Conclusion : The present study showed that only the CSF VEGF levels are associated with the subsequent development of shunt infection. Our results suggest that increased CSF VEGF could provide a good condition for bacteria that are introduced at the time of surgery to grow in the brain, rather than reflecting a sequel of bacterial infection before VP shunt.

Key Words : Cerebrospinal fluid · Shunt infection · Biomarkers · Vascular endothelial growth factor · Hydrocephalus.

INTRODUCTION

Hydrocephalus occurs if the production of cerebrospinal fluid (CSF) exceeds its resorption, and this leads to elevated intracranial pressure. It often requires surgical treatments such as repetitive lumbar punctures or a ventriculoperitoneal (VP) shunt to reduce the intracranial pressure and avoid compression of the periventricular white matter and cerebral arteries. However, infection is a major complication after shunt insertion, with the infection rates ranging from as little as 1% to as much as 25%[1)]. Most infections in shunt systems originate from bacterial contamination introduced at the time of surgery and most manifest by 3 to 4 weeks postoperatively[28)]. Use of a CSF culture is the gold standard for diagnosing shunt infection[6)]. Clinically, the CSF parameters (white blood cell and red blood cell counts, the glucose level and the protein levels) are frequently used to aid in diagnosing shunt infection in patients with unreliable CSF cultures[19)]. However, the utility of the CSF parameters for

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patients with a VP shunt is known to be limited due to the poor diagnostic sensitivity and specificity[19]. Moreover, abnormal CSF parameters also imply that an infection process may already be underway. Thus, novel and precise CSF biological markers are needed to predict shunt-associated infection.

A number of biochemical products are exchanged with the central nervous system (CNS) through the CSF, which is the main component of the CNS's extracellular fluid. CSF may reflect the pathophysiology of various neurological disorders occurring in the CNS as well as healthy conditions because CSF plays a critical role for physiological control in the brain to maintain a stable CNS condition[3,20]. Various neuropeptides, neurotransmitters, proteins, enzymes and metabolic by-products have been assayed in the CSF of patients with psychiatric, neurochemical, dementing, neuroinflammatory and traumatic disorders[3]. Among them, transforming growth factor (TGF)-β[5,11] and vascular endothelial growth factor (VEGF)[11] are increased in the CSF of patients with posthemorrhagic hydrocephalus, whereas tau[18] and tumor necrosis factor (TNF)-α[30] are increased the CSF of patients with normal pressure hydrocephalus (NPH). These findings suggest that they may be the promising markers of chronic hydrocephalus[31]. However, the diagnositic utility of these biomarkers in patients with hydrocephalus as a infection indicator after shunt placement is currently unknown. Thus, the aim of this study is to determine the association between the CSF biomarkers and inflammation, and the predictive value of these CSF biomarkers for the subsequent development of shunt associated infection.

MATERIALS AND METHODS

We obtained CSF from seventy-six patients with hydrocephalus during their VP shunt operations from 2008 to 2009. Of the seventy-six patients, a total seven patients suffered from subsequent shunt infection. Twenty-two patients including these seven patients who presented with subsequent shunt infection were enrolled for this study. All the participants signed a consent form with regards to the aims of the study, and this study was approved by the University Hospital Ethics Committee.

Patient selection

INPH was confirmed with radiologic studies and by the hydrocephalus induced-signs and symptoms such as gait disturbance, cognitive dysfunction and urinary incontinence, and the response after external lumbar drainage. SAH-induced hydrocephalus was defined as clinical and radiologically diagnosed hydrocephalus that required treatment, and the hydrocephalus was demonstrable at least two weeks after SAH as either the progression of acute hydrocephalus or the development of chronic hydrocephalus de novo. Post-traumatic or -spontaneous intracerebral hemorrhage hydrocephalus was also defined as clinical and radiologically diagnosed hydrocephalus that developed during the next few weeks after resolution of the hemorrhage and the hydrocephalus required treatment.

Shunt infection

The subsequent development of shunt infection was confirmed by the clinical findings such as fever and/or meningeal irritation signs, laboratory studies that included the hematological and CSF parameters (the white blood cell and red blood cell counts, the glucose level and the protein levels) and CSF culture from the shunt devices. Antibiotics were started according to the results of CSF culture and all the shunt devices were removed when shunt infection was confirmed.

Cerebrospinal fluid samples

All the samples were taken through intraventricular catheterization during a VP shunt operation for treating the patients with hydrocephalus. Approximately 10 mL CSF was collected from a proximal catheter inserted into the ventricles before connecting the proximal catheter and the shunt valve. CSF analysis and culture were then performed in a routine fashion on these collected CSF samples. The CSF samples were centrifuged at 800 g for 10 minutes to sediment both the hematogenous cells and other cells contaminating the sample, and the supernatant was aliquoted and stored at -80°C until analysis.

VEGF, TGF-β1, TNF-α and total tau assays

We detected and analyzed the specific biomarkers in the CSF by way of enzyme-linked immunosorbent assay (ELISA). The biomarkers were TGF-β and TNF-α for inflammation, VEGF for angiogenesis and tau protein for neurodegeneration. The VEGF, TGF-β1 and TNF-α concentrations were determined using a commercially available sandwich ELISA (Bender MedSystems GmbH, Vienna, Austria), and the total tau concentration was determined using a commercially available sandwich ELISA (Invitrogen, CA, USA) according to the manufacturer's instructions. The sensitivity of the assay was 7.9 pg/mL for VEGF, 9.0 pg/mL for TGF-β1, 1.65 pg/mL for TNF-α and 12.0 pg/mL for total tau. The intra-assay coefficient of variation was 6.2% (mean : 571 pg/mL, n=8) for VEGF; the interassay coefficient of variation was 5.1% (mean : 14346 pg/mL, n=8) for TGF-β1; the interassay coefficient of variation was 6.0% (mean : 295 pg/mL, n=7) for TNF-α : the interassay coefficient of variation was 4.4% (mean : 578 pg/mL, n=3) for total tau. All the assays were carried out in duplicate. All the ELISA 96-well microtiter plates were analyzed using a microplate photometer with the maximum absorbance at 470 nm. (SpectraMax® M2e Microplate Reader, CA, USA).

Statistical analysis

The twenty-two patients were divided into 3 groups : SAH-induced hydrocephalus (n=9), INPH (n=6) and hydrocephalus with a subsequent shunt infection (n=7).

The significance of differences of each biomarker level among the SAH-induced hydrocephalus, INPH and shunt infection groups was established by the Kruskal-Wallis test with asymptotic Sig. (p) values, as the distribution of the values was non-Gauss-

ian. The significance of differences of the VEGF level between the SAH-induced hydrocephalus and INPH groups, the SAH-induced hydrocephalus and shunt infection groups and the INPH and shunt infection groups was established by the Mann-Whitney U test with two-sided *p* values, as the distribution of the values was non-Gaussian. The statistical analyses were processed by the use of statistical software SPSS 12.0 for Windows. A probability value of $p<0.05$ was set as being statistically significant. All the values are given as means (±standard deviation).

Table 1. Clinical features of the study population

Case	Sex	Age (yrs)	Cause	Symptom duration	Procedure prior to shunt	Infection
1	F	70	SAH	72 ds	Clipping	
2	F	67	SAH	50 ds	Clipping	
3	F	70	SAH	138 ds	Clipping	
4	F	63	SAH	90 ds	Clipping	
5	M	69	SAH	120 ds	Clipping	O
6	F	63	SAH	95 ds	Clipping	
7	M	46	SAH	50 ds	Clipping	
8	F	79	SAH	128 ds	-	
9	F	63	SAH	73 ds	Coil embolization	
10	F	72	SAH	26 ds	Coil embolization	
11	F	59	INPH	3 yrs	-	
12	M	80	INPH	3 yrs	-	
13	F	71	INPH	1 yr		O
14	F	63	INPH	6 mos	-	
15	F	61	INPH	5 mos	-	
16	F	54	INPH	2 yrs	-	
17	M	74	INPH	1 yr	-	
18	M	11	Trauma	106 ds	Craniectomy	O
19	M	57	Trauma	84 ds	Craniectomy	O
20	F	49	SICH	180 ds	Craniotomy	O
21	F	34	SICH	120 ds	Craniotomy	O
22	F	16	Congenital	N.A.	VP shunt	O

SAH : subarachnoid hemorrhage, INPH : idiopathic normal pressure hydrocephalus, SICH : spontaneous intracerebral hemorrhage, N.A. : not available, VP shunt : ventriculoperitoneal shunt

Table 2. The initial CSF parameters and VEGF levels in the infection group

Case	CSF parameters* [RBC , WBC (/μL), Protein, Glucose (mg/dL)]	VEGF* (pg/mL)	Organisms†
5	0, 0, 23, 59	264	*Staphylococcus hominis*
13	3, 0, 13, 81	304	*Staphylococcus aureus*
18	98, 9, 42, 61	525	*Staphylococcus aureus*
19	3, 1, 69, 50	874	*Staphylococcus epidermdis*
20	18, 0, 32, 58	1393	*Staphylococcus epidermidis*
21	0, 0, 40, 67	544	*Staphylococcus aureus*
22	7250, 3, 125, 66	487	*Bacteroides fragilis*
			Serratia fonticola

*The CSF parameters and VEGF levels were analyzed in the CSF samples from the intraventricular catheterization during shunt operations. †The organisms grew in the CSF samples from the shunt devices when shunt infection was diagnosed. CSF : cerebrospinal fluid, VEGF : vascular endothelial growth factor

RESULTS

Patient clinical profiles

Of the enrolled twenty-two patients, one patient suffered from congenital hydrocephalus, two had post-traumatic cerebral hemorrhage hydrocephalus, two patients had hydrocephalus after moyamoya-induced hemorrhage, seven were INPH and ten were SAH-induced chronic hydrocephalus. The mean symptom duration of INPH was 18.7 months (range : 5 months to 3 years). The mean duration from SAH to VP shunt and from other intracerebral hemorrhages to VP shunt was 84.2 days (range : 26 to 138 days) and 122.5 days (range : 84 to 180 days), respectively. The patient with congenital hydrocephalus underwent shunt reoperation due to shunt failure with a non-infectious cause (Table 1).

Of the seventy-six patients who underwent VP shunt, a total seven patients suffered from shunt infection and they all were enrolled for this study. Of them, one patient each had congenital hydrocephalus, INPH and SAH-induced hydrocephalus, respectively. There were 2 patients with post-traumatic cerebral hemorrhage hydrocephalus and post-moyamoya-induced hemorrhage hydrocephalus, respectively (Table 1). The mean duration from the shunt operation to shunt infection was 46 days (range : 20 to 68 days). A routine CSF analysis and culture during VP shunt showed that there was no sign of infection at the moment that the shunt operation was performed. The mean red and white blood cell count of the CSF (±SD) was 1053±2733 (range : 0-7250)/μL and 1.9±3.3 (range : 0-9)/μL, respectively. The mean protein and glucose level of the CSF (±SD) was 49±38 (range : 13-125) and 63±10 (range : 50-81) mg/dL, respectively. There was no growth of pathogenic organisms on the culture study with these CSF samples, even though at the time of the subsequent development of shunt infection, some bacteria grew in the CSF samples from the shunt devices. The main pathogenic organisms were *Staphylococcus aureus* (3 patients) and *Staphylococcus epidermidis* (2 patients). One patient had a *Staphylococcus hominis* infection and one patient had a mixed

infection-*Bacteroides fragilis* and *Serratia fonticola* (Table 2). Table 1 summarized the characteristics of the enrolled patients with hydrocephalus and Table 2 demonstrated the results of CSF analysis and culture before and after shunt infection in the infection group.

Comparison of CSF biomarkers

There was no difference in the red and white blood cell counts and the protein and glucose levels among the CSF samples (data was not shown). The mean VEGF concentration (±SD) in the CSF of the SAH-induced hydrocephalus, INPH and shunt infection groups was 236±138 (median : 201), 237±80 (median : 224) and 627±391 (median : 525) pg/mL, respectively. There was a significant difference among the three groups (p=0.01). The mean total tau concentration (±SD) in the CSF of the SAH-induced hydrocephalus, INPH and shunt infection groups was 1247±1983 (median : 352), 195±151 (median : 158) and 326±182 (median : 282) pg/mL, respectively. There was no significant difference among the three groups. The mean TNF-α concentration (±SD) in the CSF of the SAH-induced hydrocephalus, INPH and shunt infection groups was 112±35 (median : 121), 141±41 (median : 139) and 152±38 (median : 144) pg/mL, respectively. There was no significant difference among the three groups. The mean TGF-β1 concentration (±SD) in the CSF of the SAH-induced hydrocephalus, INPH and shunt infection groups was 991±439 (median : 895), 918±498 (median : 763) and 2407±2210 (median : 949) pg/mL, respectively. There was no significant difference among the three groups.

We statistically analyzed the CSF VEGF between the SAH-induced hydrocephalus and infection groups and between the INPH and infection groups by the Mann-Whitney U test. There was a significant difference between the SAH-induced hydrocephalus and infection groups, and between the INPH and infection groups, respectively (p<0.01), whereas there was no significant difference between the SAH-induced hydrocephalsus and INPH groups. Table 3 shows each level of the CSF biomarkers of the study groups.

DISCUSSION

The present study demonstrated that only VEGF in the CSF of the patient group with the subsequent development of shunt infection after VP shunt was significantly increased compared to that of the patient groups with SAH-induced chronic hydrocephalus and INPH without infections. There was no significant difference of the concentration of other biomarkers such as total tau, TNF-α and TGF-β1 among the three groups. Of interest, analysis of VEGF showed that there was a significant difference between the SAH-induced hydrocephalus and infection groups, and between the INPH and infection groups (p<0.01), whereas there was no significant difference between the SAH-induced hydrocephalus and INPH groups. Although Koehne et al.[15] reported that the VEGF concentration of the non-hydrocephalic CSF controls was less than 1 pg/mL, the normal ranges of the above biomarkers in the CSF are unclear. In our study, the increased VEGF in the infection group should be considered to be relative (compared to that of the SAH-induced hydrocephalus and INPH groups) because we did not compare the biomarkers of the study groups with the CSF biomarkers of healthy people.

Previous studies have reported that VEGF is increased in the CSF of patients with posthemorrhagic hydrocephalus[11] or chronic obstructive hydrocephalus[34]. Chronic hydrocephalus probably increases the intracranial pressure, and increased intracranial pressure may decrease the cerebral blood flow and induce chronic tissue hypoxia. Finally, such conditions may induce VEGF secretion from the chroid plexus or migration of VEGF from the surrounding brain tissue[11,34]. The choroid plexus, which is involved in CSF production and it secretes numerous growth factors[4,29], normally has high levels of VEGF[21]. In the CSF, the VEGF levels may reflect choroid plexus-secreted ligand or migration of VEGF from the surrounding brain tissue and this plays an active role in brain tissue angiogenesis[34]. On the contrary, the origin of tau is probably damaged or degenerative neuronal cells in the subependymal region of the dilated cerebral ventricles[18], and TNF-α may result from the activated microglial or inflammatory cells in the brain during the development of chronic hydrocephalus[30]. TGF-β1 is expressed from endothelial, hematopoietic and connective tissue cells in response to tissue injury for wound healing or fibrosis[13], whereas following hemorrhage, it is released from astrocytes[17] and platelets[5,11] into the CSF.

It is unclear why the patients suffering from hydrocephalus with increased VEGF in the CSF more often developed shunt infection. VEGF is mainly expressed from endothelial cells. It increases peripheral oxygen delivery by promoting angiogenesis, and it is involved in endothelial cell migration, proliferation

Table 3. ELISA results for the VEGF, total tau, TNF-α and TGF-β1 levels in the CSF samples of the study groups

	SAH-induced hydrocephalus (n=9)	INPH (n=6)	Infection (n=7)	p^*
VEGF	236±138 (54-550)	237±80 (140-374)	627± 392 (264-1393)	0.011
Tau	1247±1983 (95-5696)	195±151 (52-463)	326± 182 (144-632)	0.148
TNF-α	112±35 (49-170)	141±41 (102-214)	152± 38 (114-227)	0.095
TGF-β1	991±439 (347-1843)	918±498 (538-1912)	2407± 2210 (553-5954)	0.577

Values are means±SDs and ranges (pg/mL). *A probability value of p<0.05 was set as being statistically significant by the Kruskal-Wallis test. ELISA : enzyme-linked immunosorbent assay, SAH : subarachnoid hemorrhage, INPH : idiopathic normal pressure hydrocephalus, VEGF : vascular endothelial growth factor, TNF : tumor necrosis factor, TGF : transforming growth factor

and differentiation, as well as proteolysis of the extracellular matrix[2,14]. The expression of VEGF after acute hypoxia is highly sensitive[26]. Enhanced levels of VEGF were found in animal brains after hypoxic insults[22,24,25] and in human patients after acute ischemic stroke[12]. Under normal conditions, there is only a diffuse expression of VEGF in the brain, with the exception of some specialized cells such as those of the epithelium in the choroid plexus[22]. In contrast, under local or systemic hypoxia, the neurons, astrocytes and microglial cells all show an enhanced VEGF expression[10,24,27]. Additionally, VEGF is also expressed in response to inflammatory stimuli from preformed granules that are present in neutrophils and platelets, and this is independent of hypoxia[15]. VEGF has been shown to be elevated in the CSF of children and adults with bacterial meningitis.[33] Koehne et al.[16] reported that the VEGF concentrations were significantly elevated in the hydrocephalus CSF samples regardless of the causes compared with those VEGF concentrations in routine diganostic lumbar punctures for unrelated reasons. Interestingly, they observed higher VEGF concentrations in the CSF samples that grew *Staphylococcus epidermidis* compared to the VEGF concentrations in those CSF samples of any other hydrocephalic patients, although the small number of samples precluded demonstarting a statistically significant association between the VEGF concentrations and bacterial infection. Koehne et al.[15] assumed that a high VEGF level in the CSF may reflect a sequel of inflammation. We also routinely performed a CSF analysis and culture from the intraventricular catheterization during shunt operations. Interestingly, only the CSF VEGF levels of the patients with a subsequent shunt infection were significantly higher than those CSF VEGF levels of the non-infection groups, whereas the other biomarkers and the CSF parameters such as the red and white blood cell counts and the protein and glucose levels in the infection group were not different from those of the non-infection groups and any bacteria did not grow in all the CSF samples.

Generally, most infections in shunt systems originate from bacterial contamination introduced at the time of surgery and most manifest by 3 to 4 weeks postoperatively.[28] On the contrary, the mean duration from the shunt operation to the shunt infection was 46 days in our study. We have used an antibiotic-coated shunt system for VP shunt operation since 2008. We think that this system probably delays the implantation of organisms into the CSF space. Thus, our results suggest that increased CSF VEGF probably provides a good condition for bacteria, which are introduced at the time of surgery, to grow in the brain, rather than being a a sequel of subclinical bacterial infection before VP shunt. VEGF has been shown to play a major role in angiogenesis and increasing vascular permeability.[23] VEGF-mediated neovascularization may enhance the oxygen supply. Besides, VEGF that is produced intrathecally may contribute to disruption of the blood-brain barrier (BBB)[9,32]. Thus, the bacteria introduced at the monent of VP shunt surgery may easily break into the brain tissue and vascular channels through the disrupted BBB and the increased vascular networks probably provide nutrients and oxygen to the bacteria. Taken together, these circumstances induced by increased VEGF may make the external bacteria adhere to the brain tissue and shunt devices and grow better. By contrast, the CSF parameters and culture results are probably not predictive factors for shunt associated infection, but rather, they are markers for a present infection only.

This study has some limitations. As mentioned above, because we did not obtain the CSF biomarkers of nonhydrocephalic controls, we could not compare the CSF biomarkers between normal controls and the hydrocephalus patients. Moreover, there is still no reference values of the biochemicals in the CSF of healthy subjects, and we do not know the age-matched reference values for the evaluation of these biomarkers among the study groups. However, because the aim of this study is to compare CSF biomarkers between the subsequent shunt infection and non-infection groups with hydrocephalus, we did not consider a normal control group and age-related variables. Another limitation is the small number of patients in each group even though this is a pilot study. This may lead to statistical misinterpretation. Moreover, there may be a selection bias for the shunt-related infection. Of the seventy-six patients who underwent VP shunt during the study period, a total seven patients suffered from shunt infection and they all were enrolled for this study because the number of shunt-related infection was very few. Finally, we obtained CSF samples only one time during ventricular catheterization for a shunt operation. It has been demonstrated that the levels of certain markers in the CSF might fluctuate over time, so a sampling at one time point might be of limited use[7]. Moreover, the effect of the gravity may influence the concentration of the some biomarkers in the samples (lumbar versus ventricle)[8]. Thus, in future studies, the corrleation of the CSF VEGF levels between lumbar and ventricular CSF samples should be confirmed. Further, repeated ventricular punctures should be limited due to ethical issues and technical problems if the monitoring of CSF VEGF is necessary for making decision when a VP shunt operation is performed.

CONCLUSION

Our data suggests that the levels of VEGF in the CSF, which is taken during a shunt operation, could be relevant to the subsequent development of shunt infection. In future, a comparison study among a healthy group and a hydrocephalic group might be needed to determine the normal reference values of the CSF biomarkers. Prospective studies are also needed to determine the longitudinal profile of the CSF biomarkers, and especially the VEGF levels, from the occurrence of hydrocephalus until a VP shunt operation and to test whether CSF VEGF can be used to predict a shunt infection. More refined assay techniques and further understanding of the pathophysiology of shunt infection will probably lead to tests of the CSF composition that are indeed useful for the clinical management of hydrocephalic patients.

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