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常壓性水腦病人腦脊髓液中血管內皮生長素及胎盤生長素於引流術前後之變化及其臨床意義

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共同主持人：廖漢文
計畫參與人員：賴達明

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Cerebral Ischemia and CSF Placenta Growth Factor or Vascular Endothelial Growth Factor in Idiopathic Normal Pressure Hydrocephalus Patients

Dar-Ming Lai [1], Yi-Ning Su[2], Hung Li [4], Chien-Nan Lee [5], Fon-Jou Hsieh [7], Yong-Kwang Tu [1]

[1]Department of Surgery, National Taiwan University Hospital, Taipei, Taiwan

[2]Departments of Medical Genetics, National Taiwan University Hospital, Taipei, Taiwan

[3] Institute of Biomedical Engineering, National Taiwan University, Taipei, Taiwan

[4]Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan

[5]Department of Obstetrics and Gynecology, National Taiwan University Hospital, Taipei, Taiwan

[6]Department of Forensic Medicine, Medical Center, National Taiwan University, Taipei, Taiwan

[7]Graduate Institute of Clinical Medicine, National Taiwan University, Taipei, Taiwan

Address correspondence to Dr Tu, Division of Neurosurgery, Department of Surgery,National Taiwan University, No. 7, Chung-San South Road, Taipei, Taiwan E-mail: yktu@ha.mc.ntu.edu.tw tel:886-2-25078228; Fax 886-2-25078228

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Abstract

Ideopathic normal pressure hydrocephalus is a common disease causing dementia, gait disturbance or incontinence. Cerebral ischemia at the periventricular region, aside from cerebrospinal fluid stagnation, was found to be a major pathophysiologic factor. Vascular endothelial cell growth factor and placenta growth factor was found to be elevated in the ventricular cerebrospinal fluid. Material and Methods Correlation was done on PlGF and VEGF with xenon-computed tomography study and albumin level or age of the patients. Results PlGF and VEGF are linearly corellated. Excluding age factor, ventricular PlGF was related to frontal part periventricular cerebral blood flow, yet not with ventricular albumin level. Ventricular albumin level was inversely related to the clinical good outcome after shunting (8 l vs 21 μ g/d). Conclusion Ongoing periventricular ischemia is one of the major factors in pathogenesis of INPH and ventricular PlGF can reflect that. However, stagnation of the cerebrospinal fluid outflow is probably more important factor.

Introduction

Normal pressure hydrocephalus (NPH), first described by Hakim and Adams in 1965, is often diagnosed based on symptomatic triad— gait disturbance, memory impairment and incontinence[1, 2]. On the computed tomography (CT) or magnetic resonance imaging (MRI) examination, NPH is characterized by disproportional enlargement of all four ventricles and frequently periventricular lucency. Patients receiving lumbar tap or ventricular tap usually show normal intracranial pressure. NPH can be categorized into two types, one being idiopathic NPH (INPH) and the other being NPH with known cause (after head injury, subarachnoid hemorrhage, meningitis etc)[3]. Treatment of either type of NPH is frequently by ventriculo-peritoneal shunt or sometimes third ventriculostomy[4]. The improvement rate following shunting is about 59- 73% and the complication rate of shunting is about 6-18%[4, 5]. The reason for low response rate comes from the nature of inhomogeneiety of the patients suspected of having INPH, making it difficult to select those who would most likely respond to shunting[5].

The pathophysiology of the NPH is thought to be two fold—decreased cerebrospinal fluid (CSF) turnover or periventricular ischemia[6-10]. Concerning CSF hydrodynamics and turnover, NPH patients usually showed increase resistance of CSF

outflow by spinal infusion test[11]. CSF outflow resistance was different in groups of patients who were responsive or nonresponsive to shunting, and patients with CSF outflow resistance over 20 mmHg/ml/mins were all responsive to shunt surgery[12, 13]. On the other hand, demyelination of periventricular white matters with microinfarcts and moderate to severe arteriosclerosis were found in patients with normal pressure hydrocephalus[14]. Concerning cerebral blood flow study by MRI or xenon-CT, NPH patients were found to show regional decrease of the cerebral blood flow on the periventricular area (especially frontal) or dysfunction of vasomotor tone after diazolamide infusion[6, 7, 9]. Whether ischemia induces ventriculomegaly or CSF stagnation resulted in periventricular distortion or vasculoinsufficiency remained unclear[15].

Vascular endothelial growth factor (VEGF) is a homodimeric angiogenic factor ubiquitously expressing in the brain by choroid plexus, astroytes and neurons [16, 17]. In brain hypoxia and ischemia, VEGF expression is induced through transcriptional activation of the hypoxia-inducing factor (HIF)-1 and HIF-2 [16, 18, 19]. Following traumatic brain injury or cerebral ischemia, VEGF showed early elevation as early as 3 hours after ischemia through the heat responsive factor 1 (HIF-1)[20, 21] Placental growth factor (PlGF), a VEGF family pro-angiogenic cytokine that act on VEGF receptor Flt-1, was a proangiogenic factor that was also elevated following cerebral

ischemia[20, 21]. In NPH patients, cerebrospinal fluid (CSF) VEGF has usually been shown to be elevated; However, the role of PlGF on the NPH cases has never been investigated[22, 23]. Since INPH is a ischemic cerebral disease it is very possible that VEGF and PlGF will be elevated in these patients and can reflect the degree of cerebral ischemia[24, 25].

CSF albumin, originally thought to reflect leakage of the blood brain barrier, is found to be mainly indicative of changing CSF flow rate or changing CSF turnover[26-28]. Protein concentration in CSF not only is influenced by CSF-blood barrier, CSF turnover, the origin that the substances are produced (blood, meninges or brain), but also the site of the pathology and the place from which the CSF is sampled[25, 26, 29-31]. Since that the main patholoy site in NPH is in the periventricular white matter, that CSF turnover (or CSF outflow obstruction) is slow and that no marked BBB breakdown is found in NPH patients, thus CSF albumin should reflects mainly outflow obstruction status. By correlation of preoperative ventricular CSF VEGF, PlGF and albumin level on shunt outcome or Xenon-computed tomography (XCT) cerebral blood flow study in INPH patients, we tried to understand the role of angiogenesis cytokine and its clinical implication. We hypothesized that in patients with INPH, CSF VEGF and PlGF could reflect periventricular ischemia. Yet in INPH patients, concerning clinical outcome after ventriculoperitoneal shunt, we found that

CSF outflow status (or albumin level) is more predictive than angiogenesis factors.

Material and Methods

Study Group

This study was performed at the National Taiwan University Hospital with the approval of the internal review board. Consecutive patients under the diagnosis of idiopathic normal pressure hydrocephalus who received ventriculo-peritoneal shunt were enrolled as the disease group in this study. Patients having history of major head injury, central nervous system infection, prior cerebral hemorrhage or other known causes that might cause hydrocephalus were excluded. Patients were also excluded when they had major organ dysfunction or systemic infection. Because of the ethical reason normal ventricular CSF were collected only from a small group of patients who received cranial surgery for small benign intracranial pathology without hydrocephalus on preoperative CT or MRI scan. These normal ventricular CSF were collected after craniotomy and before major neurological surgery. In these patients ventricular drainage was part of the surgical procedure to achieve brain slackness. The indication for ventriculo-peritoneal shunt surgery in INPH patients was based on the presence of symptoms (gait disturbance, mental deterioration, urinary incontinence) compatible with disproportionate ventriculomegaly relative to sulcal widening on CT scan or MRI image. In five patients, the indication for surgery was strengthened by the clinical improvement found after temporary lumbar CSF drainage. Because of the

facility limitation only Ninteen INPH patients received Xenon CT cerebral blood flow examination before shunting procedure. All the INPH patients underwent medium pressure shunting without anti-siphon devise. In addition, questionnaire regarding modified Larson scale was recorded before and 6 months after shunting surgery. Briefly, patients' symptom was scored according to his gait: 0 = normal, 1 = insecure, 2 = insecure (cane), 3 = bimanual support, 4 = aided, 5 = wheelchair; living condition: 0 = independent, 1 = at home with assistance, 2 = retirement home, 3 = nursing home, 4 = hospital; or urinary symptoms: 0 = nil, 1 = present.

In our study, intracranial pressure was not taken into account because it added little information and was beyond the scope of our theory. In addition, serum VEGF and PlGF were not included because they showed little difference despite significant elevation of CSF VEGF or PlGF in a variety of diseases in our pilot study (data not shown).

Cerebral blood flow (CBF) study with Xenon-CT scanning

All CBF studies were performed with a CT scanner (General Electric Medical Systems, Milwaukee, WI) equipped with a stable xenon gas delivery system. Xenon-CBF study was performed with mixture of 26% of Xe, 30% of O2 and 44% of room air. Six areas at frontal, middle, and posterior parts of the periventricular white

matter in each patient were calculated for cerebral blood flow. We obtained sequential CT images, while the patient inhaled 30% stable xenon gas in oxygen for 3 minutes and underwent a clearance period of 5 minutes. The CBF map was created from the end-tidal build-up and tissue build-up of xenon measured during this time. The CBF values for each region were computed as the average values of both sides. After the baseline CBF study, each patient received 1 g of acetazolamide intravenously and a second CBF study was conducted 20 minutes later.

Collection of CSF and PlGF, VEGF, Albumin Measurement

CSF was collected from the cerebral ventricles and centrifuged within 15 minutes of collection. The first 2 ml CSF from ventricular drain tube was discarded then following 4ml free of blood was collected. During ventricular tapping, The CSF was kept at –70°C until analysis by a technician who was blinded to the patients' condition. In control group, the CSF was collected during ventricular tapping (for achievement of brain slackness during cranial surgery) before surgery on the pathology. The level of PlGF and VEGF in the CSF was assayed by a standardized sandwich enzyme-linked immunosorbent assay method (R&D Systems, Minneapolis, MN) in duplicate according to the manufacturer's protocol. The albumin level was measured by nephelometry (Immage,Beckman Coulter,Krefeld, Germany) using standard

methods.

Data Analysis

Comparisons of PlGF or VEGF between INPH or control groups were performed by Mann-Whitney rank sum test. The relationships between PlGF and age, CSF albumin, improvement grade or cerebral blood flow were analyzed by linear logistic regression or Mann-Whitney rank sum test with the SPSS software. $P < .05$ was considered statistically significant.

Results

Patient characteristics

Forty-one patients entered our study group. Nine patients, who's CSF were collected from the ventricle for normal data had underlying disease being benign small intracranial tumors (6), small metastatic tumor (1), or unruptured aneurysms (2). The other 32 idiopathic normal pressure hydrocephalus patients were at their age ranged from 36 to 83 years with median age being 69 years. There were 18 male and 14 females. Their symptoms range from 2 month to 7 years, with median being 16 months. Gait impairment was the major problem in 21 patients, memory impairment in 9 patients, urinary symptoms in 1 patients and dizziness in 1 patient. After ventriculo-peritoneal shunt, 25 patients improved (78%), while 18 patients (56%) improved more than 2 points according to modified Larsons score. No shunt complication (shunt infection, obstruction or intracerebral hemorrhage) was found in the postoperative period. Demographic data were shown in table 1.

CSF PlGF and VEGF were elevated in patients with INPH

Normal people had their ventricular CSF VEGF at 23 to 113 with the median being 40, INPH patients' VEGF were significantly higher, CSF VEGF were 23 to 2079 with the median being 148. Normal people had their ventricular CSF PlGF at 13 to 66 with the

median being 25 (Fig.1 (b)); INPH patients' PlGF were higher, at 11 to 221 with the median being 73 (Fig.1 (a)). Both VEGF and PlGF were significantly higher in patients with INPH than normal individuals ($p<0.05$).

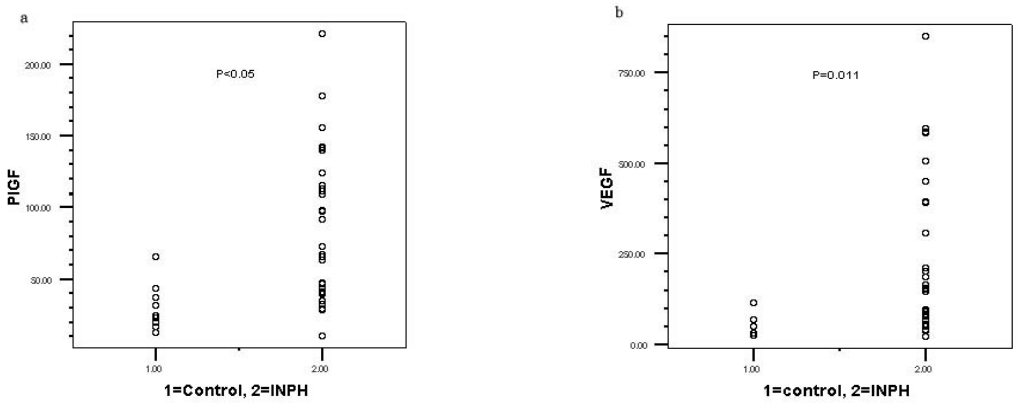

Fig.1 Both VEGF and PlGF were significantly higher in patients with INPH than normal individuals ($p<0.05$).
(a) INPH patients' PlGF were higher, at 11 to 221 with the median being 73.
(b) CSF VEGF were 23 to 2079 with the median being 148.

PlGF level were related with age, cerebral blood flow, yet not with CSF albumin level

In INPH patients, a linear relation was found between VEGF or PlGF (fig. 2). Considering possible causes for PlGF elevation, PlGF was related with age and inversely related to the periventricular cerebral blood flow. Yet it was not related with the CSF albumin level. VEGF, however, didn't show relationship with either the CSF albumin or periventricular cerebral blood flow.

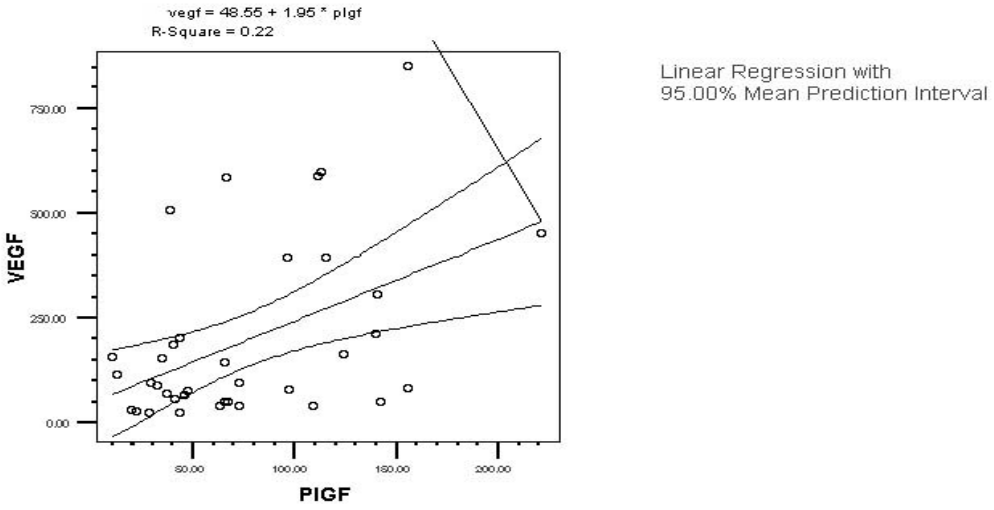

Fig.2 In INPH patients, a linear relation was found between VEGF or PlGF.

Taking into account the age factor, CSF albumin are relating to shunt outcome

CSF albumin arose from the serum. Ventricular albumin elevation was thought to caused by the stagnation of the CSF outflow. Taking into account the age factor, when we assumed Larson's grade improvement 2 scale as good outcome, the CSF albumin level was inversely related to outcome (Fig 3). The patients with good outcome had mean CSF albumin level at 8, yet patient with poor outcome had CSF albumin level at 21. Neither VEGF or PlGF was significantly related with the response to the shunt. Yet a reverse trend was found comparing shunt responsiveness to CSF PlGF level.

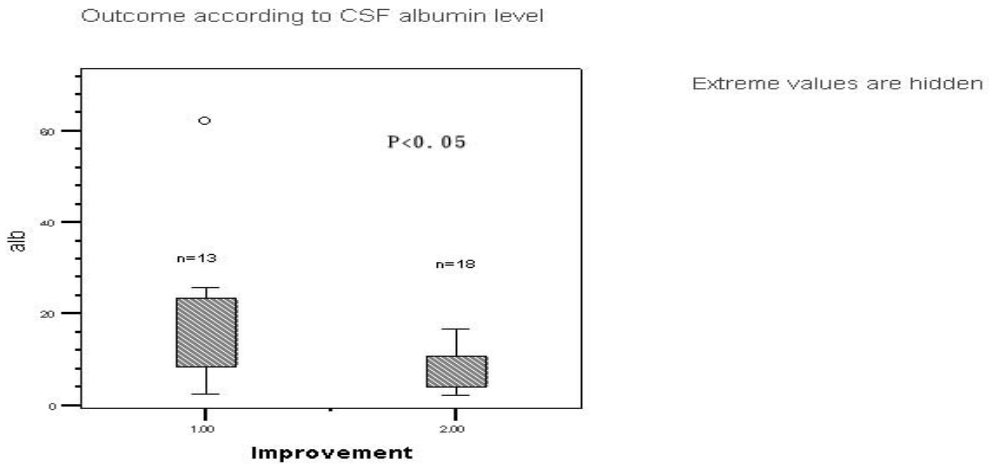

Fig.3 Taking into account the age factor, when we assumed Larson's grade improvement 2 scale as good outcome, the CSF albumin level was inversely related to outcome.

Discussion

INPH is a common disease occurred in patients at their 6th or 7th decade. Treatment for INPH is by shunting with a variety of device including programmable shunt valve, anti-siphon device, flow controlled device etc. The response rate is aboout 59-80% and complication rate is about 18-36%[4, 5]. Our patients received shunt surgery at medium age at 69 years, showed 78% responsive rate with no complication. Though all patients received medium pressure shunt device without flow control or programmable valve, our results were comparable with other reports[4, 5].

CSF VEGF and PlGF were significantly higher in our patients with INPH than control samples (median 148 vs 40; and 73 vs 25; $p<0.05$). CSF VEGF obtained from spinal tap has previously found to be higher in children hydrocephalus[22]. VEGF was also elevated following cerebral ischema or in meningitis[25, 32]. Meanwhile, cerebral PlGF was found to be elevated following cerebral ischemia[20]. Contrary to the early response of VEGF following tissue ischemia, delayed elevation of PlGF mRNA was detected by microarray analysis of rodent brain following experimental focal ischemia-reperfusion[21]. The control group in our series includes patients with small benign brain tumors or unruptured aneurysms who received brain surgery. All of these 9 patients had normal ventricular size, had no symptoms related to global cerebral dysfunction. Besides, their ventricular CSFs were taken before major cranial

procedures. We therefore assume that these may represent normal ventricular CSF data, instead of the lumbar punture samples. Our finding that CSF PlGF was higher in INPH patient has never been reported yet.

CSF albumin can reflect hydrodynamics of the ventricular outflow. The CSF/serum ratio of albumin, usually used as a marker for blood-CSF barrier function, is afftected by CSF dynamics or flow pattern[29, 30]. Increase in the CSF albumin should come from either blood-CSF barrier breakdown or decreased CSF flow[26, 27]. Different from meningitis or Guillian-Barre syndrome that inflammation and blood brain barrier breakdown were the major pathophysiologic finding, no major blood brain barrier breakdown was detected in patients with INPH or aqueductal stenosis patients. Therefore the major determinant for CSF albumin should be the local flow pattern or global CSF absorption disturbance[28].

The pathophysiology of INPH remained inconclusive. It has be suggested that brain distortion by ventriculomegaly, CSF stagnation with accumulation of toxic metabolites or periventricular cerebral ischemia be the contributing factors[4, 15]. Elevation of CSF VEGF or PlGF may therefore be the consequence of either of these. By correlation study on angiogenesis factors with cerebral blood flow or CSF turnover related protein (CSF albumin), CSF VEGF and PlGF elevation be caused by cerebral ischemia or mearly by accumulation by stagnation can be analyzed. Our

finding that age and frontal periventricular white matter blood flow was significantly related to the CSF PlGF level suggested ischemic condition at the periventricular area is a ongoing process. Because steady state decrease cerebral blood flow with acompanying neuronal damage usually brought down the angiogenesis factor[21, 33, 34]. CSF PlGF not relating to CSF albumin level reflected that CSF flow disturbance is not a major factor for PlGF elevation. CSF PlGF can therefore be used as indicator for cerebral ischemia in patients with INPH.

Though a close and parallel realtionship between VEGF and PlGF was found. VEGF was not significantly related to age, CSF albumin or cerebral blood flow in our series. VEGF, a angiogenesis factor that was rapidly triggered by ischemic threat is also elevated in inflammatory disorders, infection, surgery and even physical exercise[32, 35, 36]. VEGF response to ischemia or stress usually followed a rapid but highly variated course[21]. In the brain VEGF also had other roles like neurotroprotective effect or neurite outgrow promotion[37, 38]. Our data showed that VEGF presented at wide range, 23-2079, this accounts for its limiting statistical analysis power.

Association of VEGF and PlGF, relationship of PlGF to periventricular blood flow detected by Xenon computed tomography despite excluding age and CSF albumin factor, elevation of CSF angiogenesis factor can be regarded as reflection of periventricular ischemia.

Data derived from CSF samples taken from different regions can not be equally judged. Samples are not only influenced by adjacent pathology but also CSF flow disturbance or blood brain barrier changes. It was found that many brain or blood derived proteins has gradients between ventricular samples or CSF taken by lumbar puncture[25]. Even within the cerebrum, cisternal CSF or ventricular CSF samples showed different data following subarachnoid hemorrhage. Since cerebral blood flow decrease or water content increase in periventricular regions at MRI has been reported to be correlated with symptom severity and adjacent axons distortion is the most salient pathological findings, the ventricular CSF data should be best to represent the pathophysiology of the INPH.

Albumin were found to be the factors relating to shunt outcome. When comparison was made between patients who improved for more than 2 Larson's score, albumin was the sole factors that was inversely related to the patients medial term outcome (6 month). This is compatible with Tisell's finding that ventricular CSF albumin was inversely related to patients' shunt outcome[39].This finding implies that CSF flow disturbance may still be the main factor in INPH pathogenesis. Low pressure shunt device has been found to be effective in cognitive recovery even for patients of Alzheimer's disease[40]. Better clearance of CSF galanin, a forebrain inhibitory neuropeptide, was associated with cognitive recovery following shunt surgery[41].

Periventricular ischemia was also an important pathophysiologic finding, yet less predictive of shunt responsiveness.

Conclusion

VEGF and PlGF are elevated in ventricular CSF in INPH patients. They reflected decrease of periventricular cerebral blood flow and possibly ischemia. Though significant, the CSF albumin level is still the major determinant for shunt responsiveness.

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