Markers of Endothelial Disorder after Subarachnoid Hemorrhage Sequential Changes and Impact of Open and Endovascular Surgery

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Abstract

Objective: The goal of this study was to investigate the markers of the endothelial cell disorder after subarachnoid hemorrhage (SAH) and the impact of open and endovascular surgery to the vasculature after SAH. **Methods**: 50 patients were enrolled in this prospective study. 25 patients underwent open surgery and Guglielmi detachable coil embolization, respectively. Serial blood samples were collected on post SAH days 0, 1, 7, and 14. von Willebrand factor (vWF), plasminogen activator inhibitor-1 (PAI-1), E-selectin levels were determined as markers of endothelial cell perturbation. The levels of 6-keto prostaglandin F1 alpha (6-ktPG) were measured as a marker of endothelial cell function. **Results**: The symptomatic vasospasm was observed in nine patients (six in open surgery and three in endovascular surgery). In both treatment strategies, the serum levels of vWF were elevated from day 0 to day 14. Serum levels of PAI-1 and E-selectin were higher in open surgery than endovascular surgery in day 7 and 14 significantly ($p<0.05$). The serum levels 6-ktPG were higher in endovascular surgery than open surgery in day 4 and 7 significantly ($p<0.05$). **Conclusion**: Elevation of parameters on endothelial perurbation and coagulopathy were recognized in both procedures. The inhibition of fibrinolysis by PAI-1, the expression of adhesion molecule, and endothelial dysfunction were higher in open surgery than endovascular surgery. This preliminary result suggests that endothelial disorder associated with open surgical procedure may be predominant than endovascular surgery. running title: Endothelial disorder after SAH.

Keywords: endothelial disorder, subarachnoid hemorrhage, endovascular surgery, surgery, open surgery, vasospasm.

Endovascular surgery has emerged as an alternative therapeutic modality of ruptured cerebral aneurysm. It was reported that the outcome in terms of survival free of disability at one year is significantly better with endovascular coiling (19) and it can also reduce the incidence of vasospasm (20,45). The reasons for this knowledge are that brain damage and the manipulation of arteries required during open surgery may result in unfavorable vascular affect after subarachnoid hemorrhage (SAH) (26). However, it is not clear what happens between such therapeutic assault and the vascular response. Traumatic brain injury such as a mild concussion initiates a cascade of acute and chronic injury responses which include disturbances in the cerebrovasculature that may result in the activation of the endothelial development of a dysfunction endothelium (3). In the pathophysiology of cerebral vasospasm following SAH, endothelial disorder and inflammatory mechanisms which may contribute to cerebral ischemia in experimental SAH has been appreciated (1,11,25).

By producing chemoattractants, expressing adhesion molecules in endothelial cells and increasing the permeability of the endothelial monolayer, inflammatory cytokines activate and attract leukocytes to vessel walls that injure the endothelium and contribute to further thrombus formation and endothelial damage (17). Von Willebrand factor (vWF) is a large adhesive glycoprotein which is suitable circulating marker of endothelial cell perturbation because of its sensitivity, its long half-life, and its relative specificity for endothelial cells(5,30,31). The

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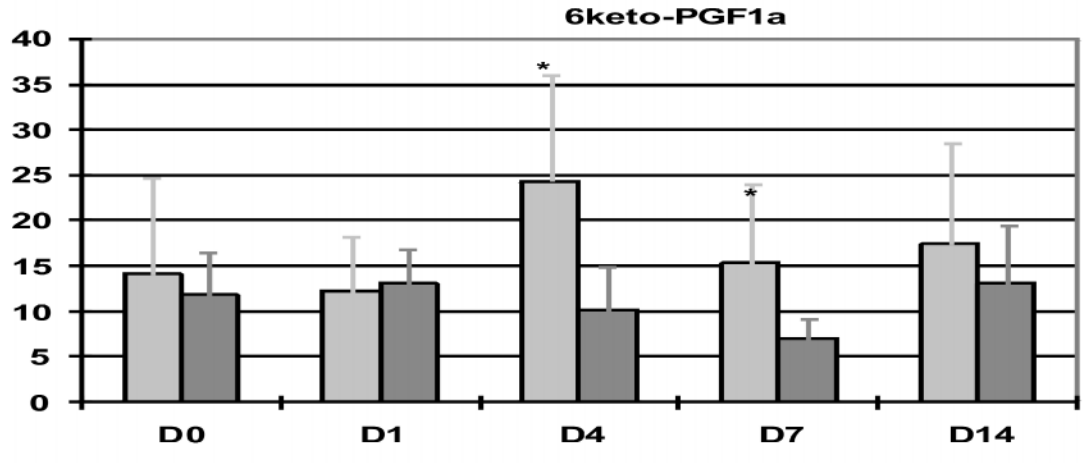
endothelial production of tissue plasminogen activator (t-PA) is increased during ischemia and thrombin stimulation (5). Plasminogen activator inhibitor-1 (PAI-1) is expressed in endothelial cells due to the action of thrombin and cytokines (16, 21). Fibrinolytic activity is regulated by the balance between t-PA and PAI-1 (8). The selectins are transmembrane glycoproteins expressed on activated vascular endothelium (P and E-selectin), activated platelets (P), activated leucocytes (L), and are involved in rolling and activation of leukocytes (22). The detections of E-selectin in serum and cerebrospinal fluid after SAH were reported (32). Vasoprotective function of endothelial cells is associated with biosynthesis and release of nitric oxide (NO), prostacyclin(PGI2), prostagrandin E2(PGE2), and carbon monoxide(CO). These endothelial mediators calm down activated platelets and leukocytes, prevent the occurrence of thrombotic events, promote thrombolysis, maitain tissue perfusion and protect vascular wall against acute damage and against chronic remodeling (10). The expressions of vWF, PAI-1, and selectin in endothelial cells are excellent circulating markers of endothelial cell perturbation and 6-keto prostaglandin F1 alpha (6-ktPG) which is the metabolic product of PGI2 are suitable to investigate the endothelial function.

To evaluate the differences of therapeutic assault to the vasculartur, we prospectively compared the markers of endothelial perturbation which generates an imbalance in coagulofibrinolysis, the expression of adhesion molecules, and the endothelial dysfunction between open surgery and endovascular surgery.

Patients and Methods

Patients

50 patients with SAH were enrolled in this study. All patients were treated within three days of SAH onset (25 underwent direct open surgery and Guglielmi detachable coil embolization, respectively). The patient selection was as the following: neurosurgeons judged that the patient was available in both treatment procedures based on the angio-architectural aspects. The treatment protocol after the operation was the same in both groups. The patients who had a rebleeding and/or had to be operated on again were excluded from the selection criteria. Clinical features from the 50 patients are summarized in Table.1. 8 men and 17 women, aged between 25 to 88 years (mean, 60.4 yr) were treated by endovascular surgery, and seven men and 18 women aged between 30 to 71 years (mean, 57.7 yr), underwent open surgery. None of them were administered with anti-spasmogenic drugs such as calcium channel blockers, papaverine, hydroxyfasdil or thromboxane A2 blockers. All patients were neurologically examined every day after admission. Delayed ischemic neurological deficit (DIND) was determined as a gradual development of focal neurological signs and / or deterioration in the level of consciousness. The occurrence of cerebral vasospasm was confirmed by conventional cerebral angiography in all patients. Nine patients developed DIND (six after direct open surgery and three after the endovascular procedure). The outcomes of them were one good recovery



(GR), one moderate disability (MD), and one severe disability (SD) in endovascular surgery, five GR and one SD in open surgery, respectively.

Data collection

Blood samples were collected on post SAH days 0, 1, 7 and 14 for the markers of endothelial perturbation, and days 0, 1, 4, 7, 14 for 6-ktPG after receiving informed consent from each patient. Plasma isolated by centrifugation at 500g for 10 minutes was stored at -30 degrees C. We assayed serum concentrations of endothelial marker and a marker of endothelial function using commercially available kits. Serum levels of vWF were measured using STALIA test vWF (DIAGNOSTICA STAGO, Asnier-sur-seine, France). The total PAI-1 levels were measured using Latex photometric immunoassay-tPAI kits (Yuka Medias Co., Tokyo, Japan). Serum levels of E-selectin were measured using Parameter Human soluble E-selectin Immunoassay kits (R and D Systems Inc., Minneapolis, USA). 6-ktPG were measured using 6-keto prostaglandin F1 alpha (^{125}I) radioimmunoassay kit (PerkinElmer ™life Science, Boston,USA).

Data are presented as means±standard deviation and were analyzed using the chi-square test and Student's t test. A p value less than 0.05 was considered significant.

Results

Levels of markers on endothelial disorder

In both open and endovascular procedure, levels of serum vWF elevated over normal range (50%-145%) from day 0 to 14th after the onset of SAH (Fig.1). The differences between open surgery and endovascular surgery were not significant, however, there was a tendency towards an increase in its expression in patients

Fig. 1: Sequential changes of von Willebrand factor (vWF) after open and endovascular surgery. Normal range of serum vWF is between 50% and 145%. The serum levels of vWF increased in both open and endovascular surgeries in all days. The significant differences between open surgery and endovascular surgery were not found.

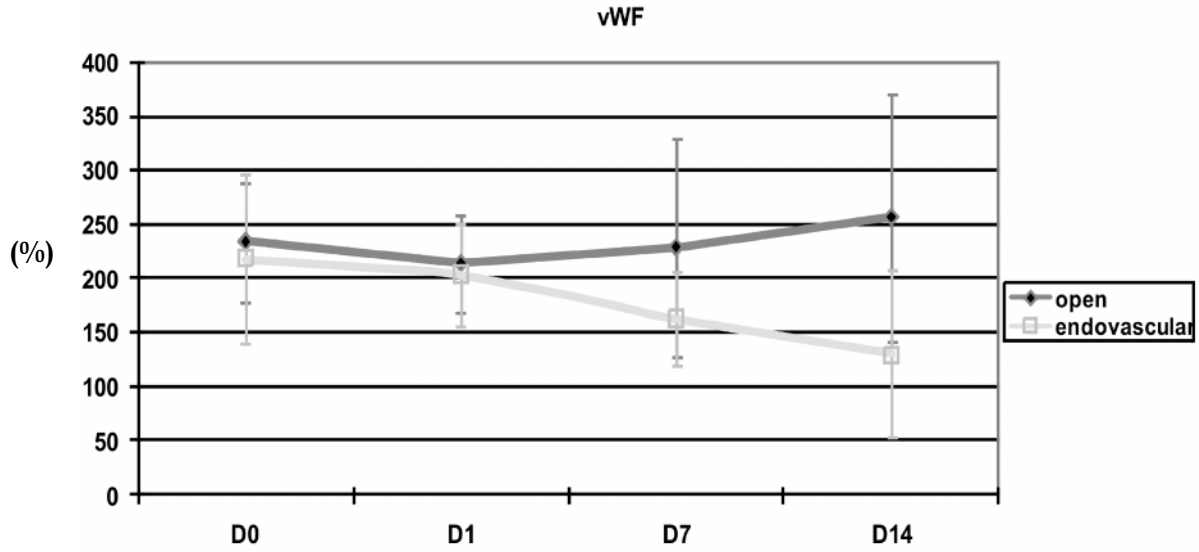


Fig. 2: Sequential changes of serum plasminogen inhibitor-1 (PAI-1) after open and endovascular surgery. Normal range of plasma PAI-1 is below 50ng/ml. Serum levels of PAI-1 after open surgery were over 50ng/ml between 0 to 14 days whereas those after endovascular surgery were within the normal range. Difference was significant day 7 (65.2±49.1ng/ml vs. 32.3±12.5ng/ml, p=0.0125)and day 14 (63.67±27.4ng/ml vs. 39.3±13.3ng/ml, p=0.002).

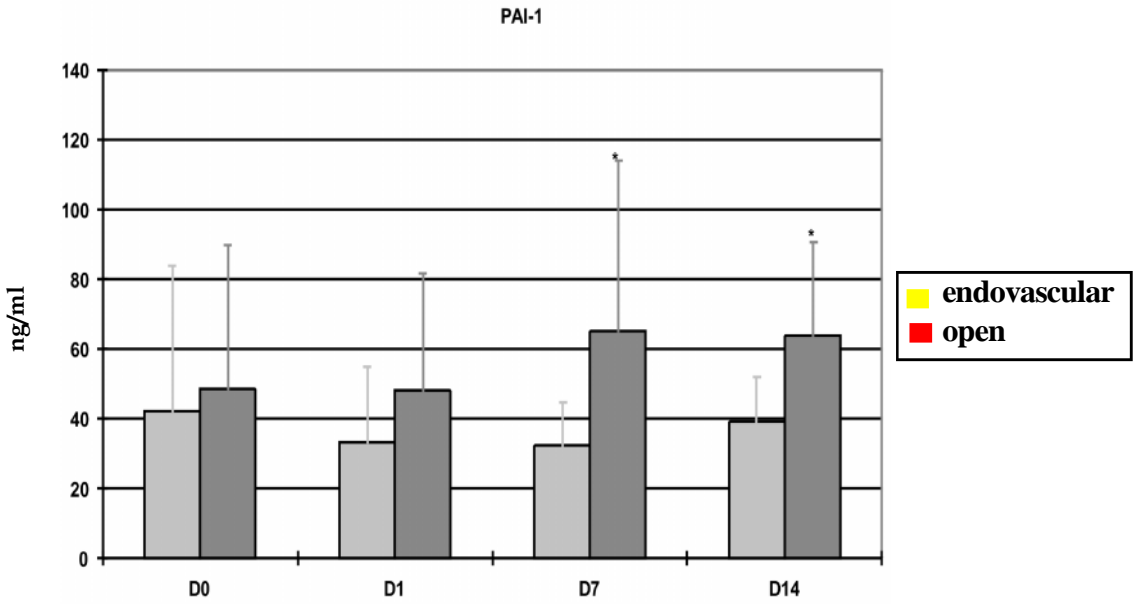


Fig. 3: Sequential change of E-selectin after open and endovascular surgery. Normal range of serum E-selectin is between 29.1ng/ml and 63.4ng/ml. Serum levels of E-selectin in open surgery were higher than endovascular surgery between 0 and 14 days following SAH, and the difference was significant on day 7 (63.6±37.6ng/ml vs. 40.8±24.7ng/ml, p=0.039).

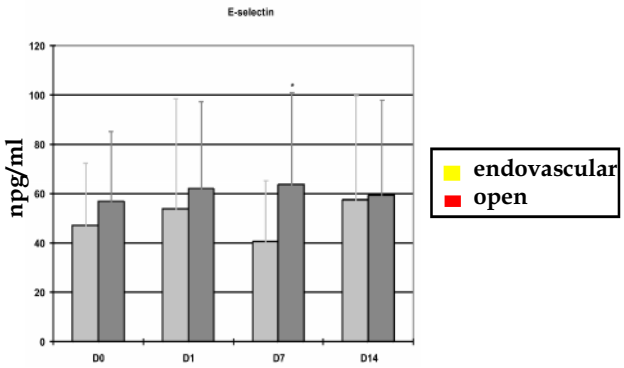
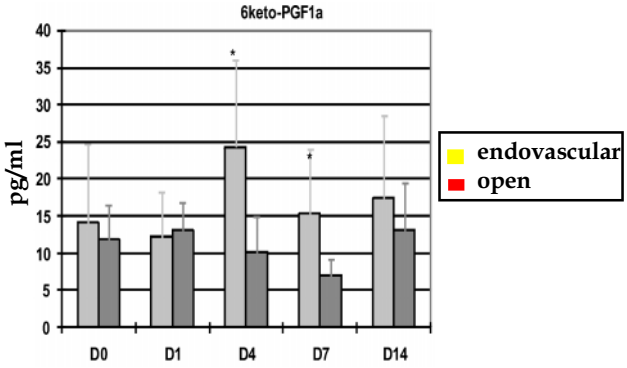


Fig. 4: Sequential changes of 6-keto prostaglandin F1 alpha (6-ktPG) after open and endovascular surgery. Serum levels of 6-ktPG in open surgery are lower than endovascular surgery between 1 and 14 days following SAH, and the difference was significant on day 4 (10.2±4.7pg/ml vs. 24.3±11.7 pg/ml, p=0.01) and day 7 (7.0±2.1 pg/ml vs. 15.4±8.7 pg/ml, p=0.03).



that underwent open surgery.

Levels of PAI-1 obtained after endovascular surgery were below 50ng/ml between days 0 and 14 after the onset of SAH. These data were within the normal range. However, the serum levels of PAI-1 obtained after direct open surgery were higher than endovascular surgery in all days. Differences between the two procedures were significant on day 7 (65.2±49.1ng/ml vs. 32.3±12.5ng/ml, p=0.0125) and day 14 (63.67±27.4ng/ml vs. 39.0±13.3ng/ml, p=0.002) (Fig.2).

The serum levels of E-selectin in open surgery were higher than endovascular surgery between 0 and 14 days following SAH, and the difference was significant on day 7 (63.6±37.6ng/ml vs. 40.8±24.7ng/ml, p=0.039) (Fig. 3).

Levels of a marker of endothelial function

Serum levels of 6-ktPG in open surgery are lower than endovascular surgery from day 4 to day 14, and significant differences are recognized on day 4(10.2±4.7pg/ml vs. 24.3±11.7pg/ml, p=0.01) and day 7 (7.0±2.1pg/ml vs. 15.4±8.7pg/ml, p=0.03) (Fig.4).

Relationship between markers of endothelial perturbation and DIND

Statistical analyses showed a significant difference between patients with DIND and without DIND in 6-ktPG on 7th day (p=0.03), but did not show a significant difference regarding the markers of endothelial perturbation.

Discussion

Enodothelial perturbation after SAH

The endothelium is an active paracrine organ that produces potent vasoactive, procoagulant, anticoagulant and proinflammatory substances. Endothelial cells have two important roles, namely adaptive and constitutive functions. During acute inflammation, endothelial cells assume adaptive functions. They become chemoattractants, facilitating leukocyte adhesion, activation and migration, and also become prothrombotic and demonstrate vascular permeability. Levels of coagulation factors, inflammatory cytokines and adhesion molecules during vasospasm following SAH are elevated (1, 4, 6, 13, 22, 37). These reports support the notion that the activation of endothelial cells following abnormal stimulation after SAH gives rise to this adaptive function. PAI-1 and vWF are expressed in endothelial cells through the action of thrombin and cytokines (34). The production of E-selectin in endothelial cells is increased by inflammatory cytokines and E-selectin is released into serum as a soluble type (17). Therefore, the elevation of these circulating markers indicates that the endothelial perturbation following activation is predominant. The relationship between the markers of endothelial perturbation and SAH has been recognized (4, 6, 11, 28, 29, 31, 37, 42). The endothelial cell perturbation in brain was found in mild concussive injury (3) and multiple sclerosis (7). Even though the mechanisms of cerebral endothelial perturbation are not clear, many direct and indirect results of injury such as impact on cerebral vessels, hemodynamic stress, hypoxia, cerebral ischemia, or brain edema are posited (46).

Endothelial dysfunction after SAH

The constitutive function of normal endothelial cells prevents vascular permeability, regulates vascular tone by producing PGI2 and NO, and suppresses inflammation, endovascular thrombosis by controlling the production of t-PA and PAI-1, and intimal proliferation for regulation of vascular metabolism. The present study shows the endothelial dysfunction after open surgery is more predominant than after endovascular surgery, because serum 6-ktPG levels were significantly reduced in open surgery. The level of 6-ktPG on 7 day in the patients with DIND was statistically lower than in the patients without DIND. After the acute inflammatory state,endothelial NOS and cyclooxygenase 1 down-regulation causes reduced PGI2 and NO production (43). Sasaki described a mechanism associated with endothelial damage in the major cerebral arteries with regard to the pathogenesis of vasospasm (35). Several reports indicate that the diminished synthesis of PGI2 and NO caused by endothelial dysfunction is associated with cerebral vasospasm after SAH (12, 24, 35, 38, 44, 45). Thromboxane synthetase inhibitor increases plasma levels of 6-ktPG (41). Fasudil is an anti-spasmogenic drug that prevents the development of endothelial injury (36). These reports support the notion that endothelial dysfunction associated with endothelial perturbation plays an important role in cerebral vasospasm following SAH.

Hematological component and SAH

The hematological component is a key factor in the pathophysiology of cerebral vasospasm. In normal brain tissue fibrinolytic activity is low (40), whereas thromboplastic activity is extremely high in comparison with other organs (2). Hirashima et.al reported that the hypercoagulation state is associated with cerebral vasospasm (13). Thrombosis formation in the artery of vasospasm was confirmed in autopsy cases and experimental study (27,29). Ikeda et.al reported the elevation of serum and

CSF levels of PAI-1 in the patients with SAH(14). vWF acts as a bridging adhesive molecule between platelets and components of the extracellular matrix or other platelets, and it may become the cause of pathological thrombus formation leading to arterial occlusion (33). Artery-to-artery embolism, such as high intensity transient and microembolic signals, have been confirmed using transcranial Doppler sonography during the period of DIND (9). Patients who underwent either open or endovascular surgery had serum vWF elevation. It showed the tendency towards procoagulopathy after the occurrence of SAH and it was promoted by the elevation of serum PAI-1 in open surgery.

Cereberal vasospasm and therapeutic assault

Cerebral vasospasm is an important causative factor of morbidity and mortality in patients with SAH. Blood in the subarachnoid space and the degradation product of hemoglobin spreading over the vessels may contribute to the development of symptomatic cerebral vasospasm. Removing blood from the subarachnoid space should be useful as a treatment of vasospasm (18, 23). On the other hand, physiological stress such as brain damage, manipulation of arteries and disturbance of peripheral cerebrospinal fluid circulation during open surgery may contribute to the occurrence of vasospasm after SAH (15, 26). In addition, it has been reported that the incidence of cerebral vasospasm following SAH can be reduced by endovascular surgery (20, 45). But, what happens between therapeutic assault and the vascular response remains unclear. When the endothelium is physically disrupted or functionally damaged, prothrombotic and proinflammary state is characterized by platelet and leukocyte activation and adhesion (expression and upregulation of vWF and E-selectin), promotion of thrombin formation, coagulation and fibrin deposition at the vascular wall (expression of PAI-1), and unprotected state of vascular wall (reduction of PGI2). Our study suggested these inflammatory disorders of endothelial cell following SAH were boosted by open surgery than endovascular surgery. However, whether endothelial disorder is a casual or indirectly related factor in the pathogenesis of cerebral ischemia after SAH is still uncertain. Actually, in the present study we could not recognize significant differences between patients with and without vasospasm in sequential changes of these endothelial perturbations after SAH. Further study is needed to clarify the relationship between the incidence of vasospasm and therapeutic assaults in larger series of patients.

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