



Patients with Invasive Tumors and *eNOS* Gene Polymorphisms with Subarachnoid Hemorrhage Tend to Have Poorer Prognosis

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Abstract

Context Endothelial nitric oxide synthase (*eNOS*) gene polymorphisms are found to predict predisposition to aneurysmal rupture and development of vasospasm in a patient of subarachnoid hemorrhage (SAH). *eNOS* gene polymorphisms are also found to predict invasiveness of malignant cells. Studies are not available in literature to describe the effect of *eNOS* gene polymorphisms and correlation between aneurysm and carcinoma. This study aims to investigate whether positive cancer history influences clinical outcome following SAH and *eNOS* gene polymorphisms.

Materials and Methods The *eNOS* gene polymorphisms were analyzed in seven consecutive patients (mean age, 52.28 ± 20 years) with a diagnosis of invasive systemic tumors from 2011 to 2017. The *eNOS* 4a/4b *eNOS* -786T > *eNOS*894G > T polymorphisms of the *eNOS* gene were determined by polymerase chain reaction and restriction fragment length polymorphism.

Results Seven patients of aneurysmal SAH in association with malignancies were studied for *eNOS* polymorphisms expression and outcome. Three patients had carcinoma cervix: one patient of carcinoma breast and one each of transitional cell carcinoma of urinary bladder, spindle cell carcinoma of left kidney, and untreated patient of atypical pituitary (adenoma). A genotype study of *eNOS* gene polymorphisms in these patients shows common polymorphisms are involved in the determination of disease progression in malignancies and aneurysmal SAH.

Conclusion Patients who expressed 4ab, *eNOS* -786T > TT/CC/TC, *eNOS*894G > T GG/GT polymorphisms did better than patients who expressed only 4bb, though both were associated with poor prognosis.

Keywords

- ▶ invasive malignancy/tumors
- ▶ *eNOS* gene polymorphisms
- ▶ intracranial aneurysms

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Introduction

Intracranial pseudoaneurysms are commonly described in association with choriocarcinoma and cardiac myxoma.^{1,2} The cause of the subarachnoid hemorrhage (SAH) may be berry aneurysms or pseudoaneurysms. Five cases of pseudoaneurysms have been reported in association with pleomorphic adenocarcinoma of lung.² We report seven patients with systemic malignancy presenting with SAH. Endothelial nitric oxide synthase (eNOS) gene polymorphisms are known to be associated with development of vasospasm and increased chances of rupture of aneurysm. eNOS gene is also thought to determine predisposition to metastasis in malignancies. We postulate that common polymorphisms are involved in the determination of disease progression both in SAH and malignancy.

Materials and Methods

Study Subjects

All patients presenting with SAH and with a diagnosis of systemic tumors attending National Institute of Mental Health and Neuro Sciences (NIMHANS) outpatient department or emergency services of NIMHANS institute, Bengaluru, Karnataka, India, were studied. All study subjects belonged to communities of Karnataka and surrounding states. Peripheral blood sample from seven patients were collected between March 2011 and February 2017. Blood sample for DNA analysis and plasma samples were collected after written consent from the patient and/or their legal guardians. The study was approved by ethical committee of Indian Council of Medical Research, Govt. of India, and NIMHANS institute.

Sample Collection

Blood was collected from the subjects in tubes containing ethylenediaminetetraacetic acid (EDTA). Genomic DNA was isolated from EDTA blood according to conventional phenol chloroform method. DNA was quantified using Nano Drop 1000 (Thermo Fisher Scientific, Delaware, United States).

Genotyping

eNOS 4a/4b Polymorphism Screening

Genomic DNA was amplified using polymerase chain reaction (PCR) (primers 5'-AGG CCC TAT GGT AGT GCC TTT -3' (sense) and 5'-TCT CTT AGT GCT GTG GTC AC-3' (antisense) with Taq DNA polymerase (FIREPol [highly processive, thermostable Taq] DNA Polymerase, Solis BioDyne) and supplied buffer under the following conditions: 95°C for 5 minutes, followed by 35 cycles at 94°C for 30 seconds, 59°C for 45 seconds, and 72°C for 45 seconds, followed by a 10-minute 72°C final extension. PCR products were electrophoretically separated on a 2% agarose gel. The 4/4 homozygous 4a allele (containing 4 of 27bp repeats) gave rise to PCR product of 393 bp, the 5/5 homozygous 4b allele (containing 5 of 27bp repeats) gave rise to PCR product of 420 bp, whereas the 4/5 heterozygous 4ab allele gave rise to 420 and 393bps bands.

eNOS -786T > C Polymorphism Screening

Genomic DNA was amplified using PCR (primers 5'-GTG TAC CCC ACC TGC ATT CT-3' (sense) and 5'-CCC AGC AAG GAT GTA GTG AC-3' (antisense) with Taq DNA Polymerase (FIREPol DNA Polymerase, Solis BioDyne) and supplied buffer under the following conditions: 95°C for 5 minutes, followed by 35 cycles at 94°C for 30 seconds, 63°C for 45 seconds, and 72°C for 45 seconds, followed by a 10-minute 72°C final extension. PCR products of 308bps were then digested with NaeI at 37°C for 16 hours and were electrophoretically separated on a 2% agarose gel. The -786 C allele is characterized by diagnostic NaeI restriction fragments of 227 and 81bps. Thus, the gel pattern for individuals with homozygous mutant genotype (-786CC) showed 227 and 81bps bands, whereas -786 TT homozygous wild type did not undergo digestion by NaeI and showed PCR band of 308bps. Individuals with -786 TC heterozygous genotype showed both intact PCR band of 308bps and two cleaved bands of 227 and 81bps.

eNOS 894G > T Polymorphism Screening

Extracted DNA was amplified using PCR (sense primer 5'-AAG GCA GGA GAC AGT GGA TGG A-3'; anti-sense primer 5'-CCC AGT CAA TCC CTT TGG TGC TCA-3') with Taq DNA Polymerase (FIREPol DNA Polymerase, Solis BioDyne) and supplied buffer under the following conditions: 95°C for 5 minutes, followed by 35 cycles at 94°C for 30 seconds, 67.5°C for 45 seconds, and 72°C for 45 seconds, followed by a 10-minute 72°C final extension. PCR products of 248bps were then digested with Dpn II at 37°C for 16 hours and were electrophoretically separated on a 2% agarose gel. The 894T allele is characterized by diagnostic Dpn II restriction fragments of 163 and 85 bps. Thus, the gel pattern for individuals with homozygous mutant genotype (894 TT) showed 163 and 85bps bands, whereas 894 GG homozygous wild type did not undergo digestion by Dpn II and showed PCR band of 248 bps. Individuals with 894 GT heterozygous genotype showed both intact PCR band of 248bps and two cleaved bands of 163 and 85bps.

Results

Seven cases of SAH with common influence of eNOS gene polymorphisms in determining their clinical course are discussed in **Table 1**.

Case 1

A 70-year-old lady presented with severe headache. She is a known case of stage 2b cervical carcinoma received who had chemoradiotherapy and intracavitary brachytherapy; she completed adjuvant therapy 3 months prior to presentation with SAH. On examination, she was conscious, obeying, with World Federation of Neurological Surgeons (WFNS) grade 1. Computed tomography (CT) shows diffuse SAH digital subtraction angiography (DSA)—(1) anterior communicating artery (Acom) aneurysm, (2) left frontopolar artery aneurysm, and (3) left middle cerebral artery (MCA) bifurcation aneurysm. She underwent clipping of aneurysms. Immediate postoperative Glasgow coma score (GCS) was 15(E4M6V5)

Table 1 Case details with eNOS polymorphisms

Patient details	Previous history	SAH clinical details	SAH radiological details	Treatment	Postoperative	eNOS polymorphisms	Follow-up and outcome
1) 70 y/f	Stage 2b cervical carcinoma with right ovarian cyst had chemoradiotherapy and intracavitary brachytherapy; completed adjuvant therapy 3 months prior to presentation with SAH	Severe headache 3 days prior GCS was 15 (E ₄ M ₆ V ₅) WFNS grade 1	CT—diffuse SAH DSA— 1) Acom aneurysm 2) Left frontopolar artery aneurysm 3) Left MCA bifurcation aneurysm	Clipping	GCS was 15 (E ₄ M ₆ V ₅) right lower limb paresis (power) [MRC]-3/5	4bb type in 4a/b, TT type in T786C and GG type in G894T	Right lower limb paresis (power) [MRC]-3/5] persistent at 1 month of follow-up
2) 50 y/f	10 months prior diagnosed with stage 3 carcinoma cervix and treated with chemoradiotherapy and intracavitary radiotherapy Completed adjuvant therapy 5 months prior to SAH	Headache and altered sensorium for the last 2 days with one episode of GTCS Papilledema was present: WFNS grade-4 GCS was 14 (E ₃ M ₆ V ₄)	CT brain-left gyrus rectus hematoma with diffuse SAH and intraventricular hemorrhage involving bilateral lateral, 3rd and 4th ventricle. DSA—single saccular Acom aneurysm	Clipping	GCS was 4 (E ₁ M ₃ V _{ET}). Postoperative CT showed increase in size of left frontal hematoma. Re-exploration and evacuation of contusion was done. Neurological condition deteriorated to GCS 3 (E ₁ M ₁ V _{ET})	4bb type in 4a/b, TC type in T786C and GT type in G894T	Poor GCS of 3 at 1 week follow-up
3) 45 y/f	Insignificant	Severe headache, 3 days prior, weakness of right upper and lower limb with irrelevant talking and became unresponsive 3 days post ictus. GCS was 8 (E ₂ M ₅ V ₁) with right upper motor neuron facial palsy and right sided power ([MRC]-2/5). WFNS grade was 5. General examination showed a lump in the breast of size 4 × 5 cm, irregular in shape, hard in consistency and fixed to chest wall	CT brain—diffuse SAH Chest X-ray—coin shaped lesion in right lung field CT thorax—multiple calcified lesions in breast with metastatic lesions in right lung upper lobe, suggestive of carcinoma breast DSA—Left Acom artery saccular aneurysm. Left anterior cerebral artery was in spasm with good cross-circulation	Due to the poor general condition did not consent for either clipping or coiling	—	4bb type in 4a/b, CC type in T786C and GG type in G894T	Poor GCS at 1 month of follow-up
4) 72 y/f	Diagnosed case of transitional cell carcinoma of urinary bladder receiving intravesical chemotherapy, date of diagnosis not available	Headache and multiple episodes of vomiting for the last 3 days GCS 15 E ₄ M ₆ V ₅ (WFNS grade 1).	DSA—small saccular left MCA bifurcation aneurysm Small saccular aneurysm arising from cervical segment of the LICA	Coiling	GCS of E ₄ M ₆ V ₅ with no postoperative deficits	4ab type in 4a/b, TT type in T786C and GG type in G894T	Died one and half year post-aneurysmal surgery

(Continued)

Table 1 (Continued)

Patient details	Previous history	SAH clinical details	SAH radiological details	Treatment	Postoperative	eNOS polymorphisms	Follow-up and outcome
5) 42 y/f	Known case of spindle cell carcinoma of left kidney operated 3 years back and post RT/CT	Altered sensorium for the last 1 day with left sided weakness and multiple episodes of GTCS. Positive neck rigidity. GCS was E ₂ M ₂ V _{aphasic} (WFNS grade 5) with relative left sided paucity moving against gravity	CT—SAH in right sylvian and ambient cistern with no hydrocephalus and midline shift. DSA—right MCA bifurcation saccular aneurysm measuring 4 × 3.8 × 2.8 mm		E4M6V5 with no residual paucity	4bb type in 4a/b, TT type in T786C and GT type in G894T polymorphisms	Died on 14/8/2014 after 7 months of aneurysm surgery
6) 32 y/f	Menstrual cycles were irregular occurring once in 3 months with scant flow. History of polyuria and polydipsia	Headache and blurring of vision for the last 1 month. Fundus showed papilledema	MRI brain (P + C)—sellar-suprasellar lesion T1 hypo, T2 hetero solid-cystic with cystic component extending anterior in right basifrontal region. Solid portion in sellar-suprasellar region was enhancing on contrast. Features suggestive of papillary type of craniopharyngioma Pituitary macroadenoma MRA—Acom aneurysm. T3–53 decreased, T4–4.3 decreased, TSH–2.72 N, FSH–5.31, and LH–0.72 decreased, GH–0.13 N. Cortisol –5.29 Field-Rt eye 6/24 with temporal hemianopia and left eye 6/60 with tubular vision. Histopathology Report was suggestive of pituitary adenoma sellar-suprasellar (features suggest atypical adenoma)	Subtotal decompression of sellar-suprasellar lesion and clipping of the A-comm aneurysm	Uneventful with follow-up serum prolactin of 4.67. Visual acuity did not deteriorate post-surgery	4bb type in 4a/b, TT type in T786C and GG type in G894T polymorphisms	Died 1 year after aneurysm surgery
7) 55 y/f	K/c/o carcinoma cervix IIB taken radiation in 2010 (5 years back)	Severe headache giddiness and fall 2 days back E ₄ M ₆ V ₅ (WFNS grade 1) with no paucity	CT—left sylvian fissure SAH with hydrocephalus. DSA—Left ICA communicating segment aneurysm	Coiling	No postoperative deficits	4ab type in 4a/b, TT type in T786C, and GG type in G894T	No deficits after one and half year of follow-up

Abbreviations: Acom, anterior communicating artery; CT, computed tomography; DSA, digital subtraction angiography; eNOS, endothelial nitric oxide synthase; FSH, follicle-stimulating hormone; GCS, Glasgow coma scale; GH, growth hormone; GTCS, generalized tonic-clonic seizure; ICA, internal carotid artery; LH, luteinizing hormone; MCA, middle cerebral artery; MRI, magnetic resonance imaging; SAH, subarachnoid hemorrhage; TSH, thyroid-stimulating hormone; WFNS, World Federation of Neurological Surgeons.
Abbreviation: eNOS, endothelial nitric oxide synthase.

right lower limb paresis (muscle power assessment [MRC]-3/5). Serum eNOS polymorphism analysis showed 4a/b, TT type, and GG type. Follow-up at 1 month, she was conscious, obeying, with right lower limb paresis (power [MRC]:3/5).

Case 2

A 50-year-old woman presented with sudden onset headache and altered sensorium for 2 days and one episode of generalized tonic-clonic seizures. She was diagnosed with stage 3 carcinoma cervix and received chemoradiotherapy and intracavitary brachytherapy and completed adjuvant therapy 5 months prior to presentation with SAH. On examination, papilledema was present, WFNS grade 4, GCS was 14 (E3M6V4). CT brain showed left gyrus rectus hematoma with diffuse SAH and intraventricular hemorrhage involving bilateral lateral, 3rd and 4th ventricle. DSA showed single saccular Acom aneurysm. She underwent clipping. Postoperatively patient's GCS was 4 (E₁M₃V_(ET)). Neurological condition deteriorated to GCS 3 (E₁M₁V_(ET)). Repeat CT showed recollection with severe cerebral edema and intraventricular hemorrhage and ventriculomegaly. It was decided not to reoperate considering poor cardiac condition and risks of re-exploration. Poor prognosis was explained to relatives. Serum eNOS gene polymorphism analysis showed 4bb type in 4a/b, TC type, in T786C, and GT type in G894T polymorphisms. She had Poor GCS of 3 at 1 week follow-up.

Case 3

A 45-year-old woman presented with severe headache for 3 days with weakness of right upper and lower limb and irrelevant talking. On examination, GCS was 8 (E₂M₅V₁), disoriented, right upper motor neuron facial palsy and right upper limb and lower limb weakness (power [MRC]-2/5), WFNS grade 5. General examination showed a lump in the breast of size 4 × 5 cm, irregular in shape, hard in consistency, and fixed to chest wall. CT showed diffuse SAH. Chest X-ray showed coin-shaped lesion in right lung field (►Fig. 1). CT thorax showed multiple calcified lesions in breast with metastatic lesions in right lung upper lobe. DSA showed saccular aneurysm arising from Acom (►Fig. 1). Left anterior cerebral artery was in spasm with good cross-circulation (►Fig. 1). Serum eNOS gene polymorphism analysis showed 4bb type in 4a/b, CC type in T786C, and GG type in G894T polymorphisms. The possible prognosis of the patient due to the poor general condition and the untreated carcinoma

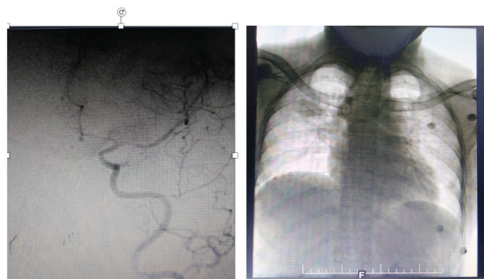


Fig. 1 Case 3 shows a saccular ACOM aneurysm on DSA, whereas X-ray chest shows a coin-shaped lesion in the right upper lung field.

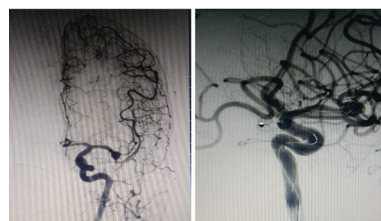


Fig. 2 Case 4—DSA. Shows right MCA aneurysm as shown.

breast and metastasis to lungs was discussed with patient and relatives. They did not consent for either clipping or coiling of the aneurysm. She had poor GCS at 1 month of follow-up.

Case 4

A 72-year-old female with h/o sudden onset headache and multiple episodes of vomiting for 3 days. Her systemic and CNS examination showed no evident abnormalities, while GCS showed E₄M₆V₅ (WFNS grade 1). She was a diagnosed case of transitional cell carcinoma of urinary bladder receiving intravesical chemotherapy; date of diagnosis not available. DSA showed small saccular MCA bifurcation aneurysm (►Fig. 2). Balloon-assisted coiling of left MCA aneurysm was done (►Fig. 2) Also a small saccular aneurysm in cervical segment of the LICA. Serum eNOS gene polymorphism analysis showed 4ab type in 4a/b, TT type in T786C, and GG type in G894T polymorphisms. Post-coiling, she had no deficits and was discharged with a GCS of E₄M₆V₅. She died 1 and half year later.

Case 5

A 42-year-old female presented with altered sensorium for 1 day and left-sided weakness and multiple episodes of generalized tonic-clonic seizures. General examination showed positive neck rigidity, and GCS was E₂M₅V_{aphasic} (WFNS grade 5) with relative left-sided paucity moving against gravity. She is a known case of spindle cell carcinoma of left kidney operated 3 years back and post-chemoradiotherapy. CT showed SAH in right sylvian and ambient cistern with no hydrocephalus and midline shift. DSA showed right MCA bifurcation saccular aneurysm (►Fig. 3). Serum eNOS gene polymorphism analysis showed 4bb type in 4a/b, TT type in T786C, and GT type in G894T polymorphisms. She underwent clipping of aneurysm. Post-clipping, the GCS

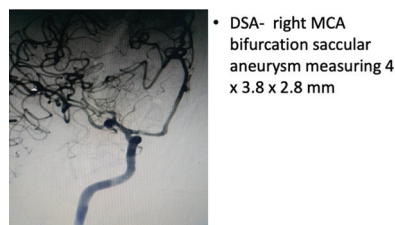


Fig. 3 Case 5 –DSA. Shows right MCA aneurysm as shown.

improved over a period of 5 days during hospital stay to E4M6V5 with no residual paucity. She died 7 months later.

Case 6

A 32-year-old married lady presented with a history of headache and blurring of vision for 1 month. Her menstrual cycles were irregular occurring once in 3 months with scant flow. History of polyuria and polydipsia was present. On examination, the patient was moderately built, nourished, GCS was 15, WFNS 1. Fundus showed papilledema. Magnetic resonance imaging (MRI) of brain plain with Contrast was suggestive of a T1 hypo, T2 hetero solid-cystic sellar-suprasellar lesion T1 hypo, T2 hetero solid-cystic with cystic component extending anterior in right basifrontal region. Solid portion in sellar-suprasellar region was enhancing on contrast. Magnetic resonance angiography showed Acom aneurysm. T3–53 and T4–4.3 were decreased, thyroid-stimulating hormone was 2.72 (normal), follicle stimulating hormone was 5.31 (normal), and luteinizing hormone was 0.72 (decreased), growth hormone –0.13 (normal), whereas cortisol was 5.29 (normal). Visual fields were as follows: right eye 6/24 with temporal hemianopia and left eye 6/60 with tubular vision. Histopathology report was suggestive of pituitary adenoma sellar/suprasellar (features suggest atypical adenoma). Patient underwent subtotal decompression of sellar-suprasellar lesion and clipping of the Acom aneurysm. Postoperative course was uneventful with follow-up serum prolactin of 4.67. Visual acuity did not deteriorate post-surgery. She died 1 year later.

Case 7

A 55-year-old female K/c/o carcinoma cervix IIB taken radiation in 2010 (5 years back) presented with sudden onset severe headache, giddiness, and fall 2 days back. She had a history of brief loss of consciousness and multiple episodes of vomiting. On examination, she was conscious obeying commands with GCS of E₄M₆V₅ (WFNS grade 1). CT showed left sylvian fissure SAH with hydrocephalus. DSA showed left internal carotid artery (ICA) communicating segment aneurysm. She underwent endovascular coiling of left ICA communicating segment aneurysm with no postoperative deficits. Serum *eNOS* showed 4ab type in 4a/b, TT type in T786C and GG type in G894T polymorphisms. She had no deficits at one and half year of follow-up.

Discussion

Malignancy can present with SAH due to rupture of berry aneurysms or pseudoaneurysms. Embolization of tumor material into large vessels leading to aneurysm formation at the site of the occlusion is thought to be the cause of neoplastic intracranial aneurysms. It is hypothesized that tumor material embolizes to peripheral artery in brain and gets attached to the wall and later invades the wall, resulting in segmental weakening of internal elastic lamina and formation of aneurysmal dilatation. On histopathology, wall of these aneurysms shows presence of malignant cells of primary tumor.^{3,4} Cervical carcinoma and breast carcinoma have hematogenous metastasis and this may predispose to

development of intracranial pseudoaneurysms in these patients and thus to SAH). Pseudoaneurysms from metastasis of malignancies are known to occur in association with cardiac myxomas¹ and choriocarcinoma.² Twenty cases have been described in association with choriocarcinoma and 34 cases have been described in association with cardiac myxoma,⁵ true incidence of aneurysms in choriocarcinoma and cardiac myxoma is not known. There are five cases reported in association with pleomorphic adenocarcinoma lung.² Association of intracranial aneurysm has been reported with hypernephroma,⁶ papillary thyroid carcinoma,⁷ testicular carcinoma,⁸ glioblastoma multiforme,^{3,9} pituitary adenoma,¹⁰ and meningioma.¹¹ It is important to note that none of these studies have confirmed that the aneurysms were because of thromboembolic phenomenon from systemic metastasis. Shibahara et al¹² in a retrospective analysis of 498 SAH patients spanning 6 years reported that compared with SAH patients without cancer history, those with cancer history had poorer Hunt & Hess grade at SAH presentation, and poorer modified Rankin Scale score at discharge, and positive cancer history remained an independent risk factor.¹² From the above data, it appears patients with malignancy may present with early aneurysmal rupture.

Carcinoma cervix may be associated with brain metastasis in 0.5 to 1.2%.¹³ The route of spread from cervical cancer is hematogenous. Brain metastases are frequently seen with poorly differentiated cervical tumors.¹⁶ These metastatic lesions may cause SAH. Duarte et al reported a case of cervical carcinoma presenting with severe hyperprolactinemia and panhypopituitarism due to supraclinoidal aneurysm after 2 years of treatment and achievement of remission from carcinoma,¹⁴ but no SAH was reported. In the current study, two patients presented with aneurysmal SAH within 6 to 8 months period of detection of cervical carcinoma and completion of treatment. Breast carcinoma is the second most common cause of brain metastasis and this metastasis is more common in advanced stages of breast carcinoma.¹⁵ Brain metastasis from carcinoma breast occurs by hematogenous route,¹⁵ where tumor emboli lodge in peripheral vessels and cause secondaries that can cause SAH. In a study by Koppelman et al on incidental findings on brain MRI in long-term survivors of breast cancer, it is found that 2.6% had an aneurysm of 191 subjects.¹⁶ The patient 3 of our study with undiagnosed and therefore untreated carcinoma breast was in morbid condition because of metastasis to lung and SAH.

Some studies have shown^{17–36} correlation between *eNOS*, tumors, and SAH (► **Tables 2** and **3**) The evaluation of *eNOS* genotypes (► **Table 4**), between natural history of aneurysmal SAH and polymorphisms of *eNOS* gene, has shown all the seven patients were found to have 4b polymorphism in 4a/b gene that is the most common polymorphism in the population and two having 4a and 4b both. In G894T gene, five patients had GG polymorphism, which is wild phenotype and two patients had GT, which is heterozygous with one mutant allele. In T786C gene, three patients showed three different genotype: CC (mutant), TT(wild), and TC (heterozygous). In a study by Khalkhali-Ellis and Hendrix,

Table 2 eNOS gene association with various cancers and SAH

Cancer type	eNOS polymorphisms	Outcome	References
Invasive breast cancer	Intron 4a/b and -786T > C 894G > T polymorphisms	eNOS -786T > C and 894G > T polymorphisms are associated with the increased risk of breast cancer	18
Bladder cancer	G894T polymorphisms NOS3 GT genotype	The frequency of the 894T allele was significantly higher in patients with bladder cancer (51%). No association was identified for eNOS T-786C and intron 4 VNTR 4a/b polymorphisms between patients with bladder cancer and control groups in Turkish population	29,34
Meta-analysis 1 (cancer)	The CC of T-786C and 4a/4a of 4b/a polymorphism are associated with increased cancer risk	The eNOS G894T polymorphism may not be a major risk factor for most types of cancers. The CC of T-786C polymorphism and 4a/4a of 4b/a polymorphism are associated with cancer risk, especially in Caucasians. There is significant association between T786C polymorphism and breast cancer risk	36
Meta-analysis 2 (cancer)	T-786C, G894T polymorphism	The eNOS T-786C polymorphism is associated with elevated cancer risk; the G894T polymorphism contributes to susceptibility to breast cancer and cancer generally in females; and the 4a/b polymorphism may be associated with prostate cancer risk	35
Meta-analysis 3 (aneurysmal)	eNOS T786C polymorphism	The presence of the T786C polymorphism as a predictor for the development of IA in the cerebrovascular system	28
Cerebral vasospasm, delayed cerebral ischemia (DCI), delayed ischemic neurologic deficit (DIND)	eNOS T786C, VNTR intron 4 a/b, G894T); and haptoglobin (Hp) 1/2 phenotypes	eNOS VNTR and Hp polymorphisms appear to have the strongest associations with DIND and radiographic vasospasm, respectively	30
Aneurysmal	NOS3 27-bp-VNTR b/b genotype	NOS3 27-bp-VNTR b/b genotype independent of other risk factors act in concert with male sex to substantially increase risk of SAH. This effect is not mediated by any single NOS3 haplotype	23
Aneurysmal	eNOS gene T786C and IL-6 gene G572C polymorphism	IL-6 gene G572C polymorphism (OR 7.08, 95% CI 2.85–17.57; $p < 0.0001$) both showed a significant association with ruptured/unruptured IA. The IL-6/G174C polymorphism exerted a significant protective effect against IA	31
Aneurysmal SAH and vasospasm	eNOS single nucleotide (rs1799983, variant allele) polymorphisms	eNOS gene plays a role in the response to SAH, which may be explained by an influence on CV	17
Aneurysmal SAH and vasospasm	eNOS gene T to C substitution in the promoter at position -786 and the a-deletion/b-insertion in intron 4	DNA sequence differences in eNOS gene influence the risk of cerebral vasospasm in subarachnoid hemorrhage	22
Vasospasm post-aneurysmal SAH	eNOS T-786 SNP	Genetic variation influencing NO regulation contributes to the risk of angiographic vasospasm in patients with SAH. The specific role of the promoter SNP (-786T→C) may determine the effect of NO regulated by this pathway, distinct from other known eNOS polymorphisms	32
Aneurysmal	Polymorphism in exon 7 of the endothelial nitric oxide synthase gene G894T	eNOS polymorphisms were not indicative of which Japanese patients with IA would suffer an SAH	21,27
Aneurysmal SAH and vasospasm	(eNOS 27 VNTR) predicts susceptibility to IA rupture, while the eNOS gene promoter T-786C single nucleotide polymorphism (eNOS T-786C SNP)	eNOS T-786C genotype may be a factor influencing the size at which an aneurysm rupture	19,20

Abbreviations: eNOS, endothelial nitric oxide synthase; IA, intracranial aneurysm; IL-6, interleukin-6; SAH, subarachnoid hemorrhage.

Table 3 SAH association with various cancers and outcome

SAH	Cancer and conclusions/Outcome	Studies
(22%) had SAH	42 different cancers ICH in these patients often occurs from unique mechanisms	25
ICH with various cancers	Patients with systemic cancer have higher mortality and less favorable discharge outcomes after ICH than patients without cancer. Cancer subtype may influence outcomes after ICH	24
ICH with various cancers	ICH in these patients often occurs from unique mechanisms	33
Ischemic and hemorrhagic	Lung cancer, pancreatic cancer, colorectal cancer, breast cancer, prostate cancer Incident cancer is associated with an increased short-term risk of stroke. This risk appears highest with lung, pancreatic, and colorectal cancers	26

Abbreviations: ICH, intracranial hemorrhage; SAH, subarachnoid hemorrhage.

Table 4 Tumors with various eNOS expressions

Cancer diagnosis	eNOS 4a/4b	eNOS -786T > C	eNOS-894G > T
1. Carcinoma cervix	4bb	TT	GG
2. Carcinoma cervix	4bb	TC	GT
3. Carcinoma breast	4bb	CC	GG
4. Bladder carcinoma	4ab	TT	GG
5. Renal carcinoma	4bb	TT	GT
6. Atypical pituitary macroadenoma	4bb	TT	GG
7. Carcinoma cervix	4ab	TT	GG

it was shown that reduced levels of nitric oxide in breast carcinoma cells are associated with increased cell motility and invasion and increased NO levels can decrease this nature by inducing a gene called maspin.³⁷ In another study by Schneider et al, it was found that eNOS -786TT and eNOS -894GG genotypes are associated with greater chances of invasive disease and eNOS-894GG genotype is associated increased chances of having metastatic disease.³⁸ Cheon et al showed that eNOS 4b genotype occurs in significantly higher frequency in lung cancer patients.⁴ NO levels and eNOS gene polymorphisms have been implicated in the prognosis of other malignancies like prostate cancer and colorectal carcinoma.³⁹ eNOS gene polymorphism 4a/b has been thought to predict susceptibility to rupture of intracranial aneurysms, predicting increased chances when 4a allele is expressed in genotype, while 786TC polymorphism is thought to predict post-SAH vasospasm in presence of C allele.^{19,40} In the current study, patient number 2 and 3, who had deteriorating clinical course, have shown C allele in their genotype, one in homozygous state and other in heterozygous state. In the patient with breast carcinoma, the genotypes, 4bb, GG and CC, show the possibility of genetic predisposition to invasive breast carcinoma and poor WFNS grade SAH and development of vasospasm. Reduced levels of NO are postulated to be the cause of vasospasm, which is major cause of mortality in patients with SAH. Though there exists a controversy on whether these genotypes are associated with aneurysm formation, there is

evidence to believe they have effect on clinical manifestations and progress of intracranial aneurysms. There is common association of eNOS gene polymorphisms in clinical course and poor prognosis in both malignancies and aneurysmal SAH.

It is well known that tumor can embolize and get seeded in parts of brain and vessel wall that can lead to formation of pseudoaneurysms. But usually, such thromboembolic phenomenon involves distal circulation and low caliber vessels. All the patients presented here have aneurysms in proximal large caliber vessels and one even at bifurcation of ICA. Most thromboembolic phenomenon due to tumor metastasis should cause Intracerebral hemorrhage as compared with SAH. Thus, it is difficult to predict whether the SAH is due to tumor emboli or berry aneurysmal bleed. One more thing to note here is most of our patients had only one aneurysm, whereas multiple aneurysms are usually seen in thromboembolic tumor metastasis. We agree that there is still no study available to properly predict the time lag between brain parenchymal metastasis versus tumor emboli in cerebral blood vessels, but none of the patients presented here showed any possible brain metastasis in CT/MRI at the time of diagnosis of aneurysms or prior. This reiterates our assumption that the aneurysmal bleed in our patients is most likely due to de novo aneurysmal rupture unrelated to thromboembolic phenomenon. Also, out of seven patients, five had undergone clipping; there was no intraoperative impression of tumor emboli aneurysmal bleeding in these

patients. We assume that the SAH caused in our patients is most likely due to berry aneurysmal rupture than due to pseudoaneurysm. However, further histopathological analysis of these aneurysmal specimens needs to be done to arrive at a conclusion.

Also, after reviewing the last 10 years database from PubMed and NCBI sites, we have formed concise tabular formats relating to cancers, SAH and eNOS gene polymorphisms. As per our analysis, we think a clear association of eNOS with SAH and metastatic cancers is possible. Also, a notable conclusion speculates that most of our observational cases had SAH within few months of diagnosis of cancer following the completion of full course of chemoradiotherapy. It is noteworthy that in spite of complete treatment the occurrence of SAH was seen quite early in the course of disease post-complete treatment. None of these patients were diagnosed with aneurysms while receiving chemotherapeutic full course of treatment. Additionally, none of these had any neurological manifestations before they presented for SAH.

Our study reports conclusively that aneurysms and aneurysmal SAH in invasive systemic cancers have a definite correlation with eNOS gene polymorphisms and more so of 4bb. Polymorphisms associated with 4bb were associated with early occurrence of SAH with relatively poorer prognosis.

Limitations

A larger sample size is needed to confirm the strength of the above association. Also, histopathological analysis post-surgery may add to and help in further strengthening the evidence of this association.

Conclusion

If patients with malignancy present with SAH due to either berry aneurysm or pseudoaneurysm, the clinical course and prognosis may be poor. The cause and effect of eNOS gene polymorphism specially on the higher stage malignancy are not fully understood. The course of the patients with higher stage malignancies may be associated with higher incidence of complications, especially if they present with SAH. Early recognition of these complications and treatment may decrease the morbidity and mortality in these patients. Also, the variable presence of 4ab and 4bb polymorphisms in certain patients can be helpful in predicting the poor outcome in association with SAH. To our knowledge, there is no literature associating presence of malignancies and poor prognosis in SAH associated with eNOS gene polymorphisms. A further study with a larger sample may be required to determine the strength of this association.

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Conflict of Interest

None declared.

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