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Brain vascular lesions: a clinicopathologic, immunohistochemistry, and ultrastructural approach☆,☆☆

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ABSTRACT

Brain vascular malformations are relatively common lesions that cause serious neurologic disability or death in a significant proportion of individuals bearing them. The purpose of this study was to analyze the clinicopathologic and immunohistochemistry these lesions, looking for common antibodies expressed such as CD31, CD34, CD15, factor VIII, nestin, vimentin, vascular endothelial grow factor (VEGF), vascular endothelial grow factor receptor-2 (VEGF-R2), glial fibrillar acidic protien (GFAP), and fibroblastic grow factor β (β-FGF) and ultrastructure in endothelial cells as well as in vessel walls. Fifty cases of vascular lesions were included in this study: 29 (58%) of them were arteriovenous malformations and 21 (52%) were brain cavernomas. Twenty-six (52%) patients were women and 24 (48%) men. The age range was from 13 to 68 years (mean age, 35.86 ± 15.19 years). The size of the lesions ranged between 1 and 8 cm (3 ± 1.65 cm), and parieto-occipital lesions had a bigger size. Evolution time varied from 1 month to 1 year (mean, 7.5 months). There was a significant statistical correlation between age and sex ($P = -035$), rupture of lesion ($P = .015$), brain hemorrhage ($P = .033$), necrosis ($P = .011$), hemosiderin deposit ($P = .042$), VEGF ($P = .015$), and VEGFR ($P = .037$), as well as localization of rupture ($P = .017$), loss of consciousness ($P = .000$), visual deficit ($P = .026$), hyaline vessels ($P = .000$), and CD31 (.009). Interactions between endothelial cells and mural cells (pericytes and vascular smooth muscle cells) in blood vessel walls have recently come into focus as central processes in the regulation of vascular formation, stabilization, remodeling, and function in brain vascular lesions. However, the molecular mechanisms that underlie the formation and growth of brain arteriovenous malformations are still poorly understood.

1. Introduction

Brain vascular malformations are relatively common lesions that cause serious neurologic disability or death in a significant proportion of individuals bearing them. The most common brain vascular malformations are arteriovenous malformations (AVMs) and cerebral cavernomas (CCs) with detection rates of approximately 1.1 and 0.6 per 100 000 adults per year, respectively [1]. These lesions can occur either sporadically or in the context of genetic syndromes (the primary one associated with cerebral AVM is hereditary hemorrhagic telangiectasia) [1-3]. Neurologic symptoms are associated with brain vascular malformations, which can include hemorrhagic stroke due to lesion rupture, epilepsy, and focal neurologic deficits [4].

Cerebral cavernomas of the brain are congenital lesions clinically divided into hereditary and sporadic forms. Multiple lesions are usually observed in the familial form [5,6]. The prevalence in the general population is 0.5% to 0.7%, without any significant difference between sexes [6]. The proportion of lesions that are in the supratentorial compartment is 79.4%, and that in the posterior fossa is 20.6%, most of them located in the brainstem, especially in pons. Spinal cord cavernomas represent 5% and multiple cavernomas represent 12.6% of the reviewed cases [5].

Cerebral cavernomas are characterized by abnormal sinusoid-like capillaries adjacent to one another with little or no interposed cerebral parenchyma or muscular tissue [5-7]. Brain AVMs are relatively infrequent but are important sources of spontaneous intracranial hemorrhage and may cause life-threatening bleedings in 2% to 6% of cases annually, which would result in high neurologic morbidity in young adults [3,4]. The basic morphology of a mature AVM is a vascular mass called the *nidus*, which is a complex tangle of abnormal, dilated vessels that are not clearly arterial or venous, with close gliosis

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that directly shunts blood between the arterial and venous circulations without a true capillary bed [1-4]. Although the cause of AVM formation is unknown, lines of evidence indicate that brain AVM may occur because of pressure changes in vessels.

The aim of this study was an approach to angiogenic factors and ultrastructural analysis in brain vascular malformations: AVMs and brain cavernomas.

2. Materials and methods

The clinical protocol was approved by the human ethics committee of the National Institute of Neurology and Neurosurgery, Mexico City, and performed according to the recommendations of the Declaration of Helsinki. The AVM and CC nidus were resected from patients undergoing cerebrovascular surgery with conventional indications, using standard microsurgical techniques and normal control of tissues. Tissues were fixed in formalin and embedded in paraffin and then sectioned (4 μm thick). All samples were stained with hematoxylin and eosin, Masson trichrome, elastin van Gieson. Tissue was also fixed in 2% glutaraldehyde for ultrastructure.

2.1. Histopathology

All samples were processed for immunohistologic analysis on paraffin-embedded sections according to the manufacturer's protocol. Antigen retrieval was achieved by immersing the slides in 10 mmol/L citrate buffer (pH 6.0) and boiling for 10 minutes in a pressure cooker. The primary antibodies used were as follows: CD31, CD34, CD15, nestin, factor VIII, VEGF, VEGF-R2, β-FGF, IFIα, vimentin, laminin, GFAP, and α-smooth muscle cell actin (Santa Cruz Biotechnology, Santa Cruz, California; dilution, 1:100), followed by goat antirabbit immunoglobulin G as a secondary antibody (BioSB, Santa Barbara, California). The negative control as an irrelevant goat (Santa Cruz Biotechnology) or mouse immunoglobulin G was used when appropriate. Immunohistochemical staining was performed by Envision technique (BioSB Technology).

Positive immnunoexpression for each one of the different antibodies used was evaluated in endothelial cells, muscular layer, vasa vasorum, internal lamina, and astrocytes. Because it is difficult to distinguish between arteries, veins, and layers of the vascular wall in AVM, we quantified the content of the entire vascular wall of both arteries and veins rather than in specific layers of arteries. Endothelial labeling index was calculated for CD43, CD41, nestin, CD15, VEGF, VEGF-R2, and vimentin. VEGF, VEGF-R2, β-FGF, vimentin, and α-actin and were evaluated for the intensity of immunoreaction of the endothelial cells and muscular layer. GFAP was evaluated in astrocytes: reactive vs normal tissue. Electron microscopy was used to compare features of AVM vs CC. Special attention was directed to the components of the vascular wall, endothelial cell morphology, intercellular tight junctions, and the subendothelial layer.

2.2. Statistical analysis

Labeling index had quantitative results that were expressed as the mean $\pm$ SEM. The statistical significance between means was evaluated. The statistical differences between different groups were assessed by 2-way analysis of variance. Statistical analyses were done using SPSS v.20 software for Windows (SPSS, Chicago, Illinois). Values of $P < .05$ were considered statistically significant. Kaplan-Meier survival curves were also performed.

3. Results

Patient demographics and lesion characteristics are summarized in Table 1. We analyzed the tissue specimens from 50 patients with cerebral vascular lesions: 29 were AVMs (58%) and 21 (42%) were CCs.

Table 1
Clinical and demographic characteristic of patients

	AVM	CC	*P* value
	29 (%)	21 (%)	
Age	27.72 $\pm$ 10.87	44.33 $\pm$ 11.56	
Women	13 (45)	13 (62)	.183
Men	16 (55)	8 (38)	
Localization of the lesion			.082
Frontal	13 (45)	8 (38)	
Temporal	7 (24)	5 (24)	
Parietal	5 (17)	5 (24)	
Occipital	1 (3)	1 (5)	
Cerebellum	3 (10)	9 (43)	
Bulbar	0	2 (10)	
Embolization	4 (14)	1 (5)	.291
Cephalea	20 (69)	13 (62)	.047
Seizures	15 (52)	8 (38)	.253
Rupture	11 (38)	4 (20)	.130
Fistula	2 (7)	0	.219
Hemorrhage	15 (52)	5 (24)	.044
Alertness disturbances	9 (31)	11 (52)	.110
Motor disturbances	9 (31)	11 (52	.110
Visuales disturbances	9 (31)	11 (52)	.110
Sensitive disturbance	7 (24)	7 (33)	.475
Personality disorders	9 (31)	11 (52)	.110
Surgical complications			.385
Hemorrhage	1 (3)	0	
Hematoma	0	1 (5)	
Fistula	1 (3)	0	
Neurological disorders	13 (45)	13 (62)	
None	14 (48)	7 (33)	
Size of the lesion	3.55 $\pm$ 1.80 mm	2.50 $\pm$ .865 mm	.001
Follow-up	Median 8 mo	Median 6 mo	.485

Twenty-six (52%) patients were women and 24 (48%) were men. Patients ages ranged between 13 and 68 years (mean, 35.86 $\pm$ 15.19 years); lesion size varied between 1 and 8 cm with bigger size in parieto-occipital lesions (3 $\pm$ 1.65 cm); and evolution time went from 1 to 365 months (mean, 7.5 months).

The histologic findings are summarized in Table 2. Arteriovenous malformations show vessels at different stages of differentiation with thick vessels in veins and venulization of arteries (Fig. 1A). Vessels also show diffuse thickening and irregularities in their walls (Fig. 1B). Rolled vessels affect directly adjacent cerebral tissue (Fig. 1C). The surrounding astrocytes are reactive, and the neuropilo is edematous and fragmented (Fig. 1D).

However, cavernomas show numerous blood vessels with a variable vessel wall (Fig. 1E) splendidly built (Fig 1F) with different grades of hemorrhage with hemosiderophages (Fig. 1G).The muscular layer shows calcification or hyaline changes. Blood vessel walls also show endothelial cells that break apart and proliferate, and reactive astrocytes with hemosiderophages were observed (Fig. 1H). Immunohistochemistry results are summarized in Table 3. CD34 was positive in endothelial cells of AVM (Fig. 2A) and negative in CC (Fig. 2B).

Table 2
Histologic findings

	AVM (n = 29), n (%)	CC (n = 21), n (%)	*P*
Flat endothelial cells	18	7 (33)	.002
Endothelial cell proliferation	7 (24)	18 (86)	.002
Muscular layer	18 (62)	7 (33)	.002
Hemorrhage	4 (14)	11 (52)	.004
Hemosiderophages	9 (31)	15 (71)	.005
Dystrophic calcifications	9 (31)	15 (71)	.005
Reactive gliosis	4 (14)	11 (52)	.004
Hyalinized vessels	12 (41)	13 (62)	.284
Necrosis	15 (52)	8 (38)	.135
Inflammation	9 (31)	15 (71)	.005
Thrombosis	6 (21)	4 (20)	.589