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Tortuosity of terminal arterioles in the basal ganglia is increased in *status lacunaris*.

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Key words: Arteriole, basal ganglia, lacunar disease, *status lacunaris*, tortuous blood vessels.

Abstract. The goal of this study was to evaluate the participation of small (diameter between 26 µm and 90 µm) and terminal (diameter between 10 µm and 25 µm) arterioles in the *status lacunaris* of the basal ganglia and to classify tortuous vascular profiles based on morphometry. Paraffin sections, 40 µm thick, of the basal ganglia from autopsied patients over the age of 45, were stained with PAS. A three-dimensional microscope, R400 (edge) was used to evaluate the structure of the blood vessels. Six patterns of the tortuous profiles were identified: simple kink, loop, knot, tangle, coil, and wave, as well as their combinations. Tortuous arterioles in the basal ganglia were present both in control group and *status lacunaris* cases. However, statistical Student's t-test analysis revealed a significant increment in the number of microfields containing tortuous terminal arterioles in the *status lacunaris* group (mean 7.50±4.62) versus the control group (mean 2.92±1.38) (p= 0.001). A risk for *status lacunaris* was associated with the increased frequency of tortuous terminal arterioles (Odd ratio=1.94, 95%-Confidence Interval=1.17-3.22) (p= 0.008) but not small arterioles (Odd ratio=1.64, 95%-Confidence Interval=0.62-4.38) (p= 0.39). Our findings suggest than an increased number of tortuous terminal arterioles is associated with *status lacunaris*. Six characteristic patterns of the tortuous profiles as well as their combinations were identified.

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Las tortuosidades de las arteriolas terminales en los ganglios de la base están incrementadas en el *status lacunaris*.

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Palabras clave: Arteriola, ganglios de la base, enfermedad lacunar, *status lacunaris*, vasos sanguíneos tortuosos.

Resumen. El objetivo de este estudio fue evaluar la participación de las arteriolas pequeñas (diámetro entre las 26 y 90 µm) y terminales (diámetro entre las 10 y 25 µm) en el *status lacunaris* de los ganglios de la base; así también clasificar los perfiles de presentación de los vasos tortuosos por su morfología. El procedimiento de estudio consistió en secciones de parafina, 40 µm de grueso, de los ganglios de la base de pacientes autopsiados con edad mayor de 45 años, coloreados por la técnica de PAS. Un microscopio tridimensional, R400 (edge) se utilizó para evaluar las estructuras de los vasos sanguíneos. Seis patrones de perfiles tortuosos fueron identificados: simple acodadura, asa, nudo, ovillo, resorte y ondulante, así como también sus combinaciones. Las arteriolas tortuosas en los ganglios de la base estuvieron presentes en aquellos casos tanto del grupo control como del grupo *status lacunaris.* Sin embargo, el análisis estadístico con la prueba de la t de Student reveló un incremento significante en el número de microcampos conteniendo arteriolas terminales tortuosas en el grupo con *status lacunaris* (promedio de 7,50±4,62) *versus* el grupo control (promedio de 2,92±1,38) (p= 0,001). El riesgo para *status lacunaris* fue asociado con el incremento en la frequencia de las arteriolas terminales (Odd ratio=1,94; 95%-Intervalo de Confianza=1,17-3,22) (p= 0,008) pero no con pequeñas arteriolas (Odd ratio=1,64; 95%-Intervalo de Confianza=0,62-4,38) (p= 0,39). Nuestros resultados sugieren que un incremento en el número de arteriolas tortuosas terminales está asociado con el *status lacunaris.* Seis patrones característicos de los perfiles tortuosos así como su combinación fueron también identificados.

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INTRODUCTION

Lacuna is a term used to denote small (<20 mm in diameter) cavities, occurring mainly in the deep cerebral hemisphere parenchyma and the brain stem (1). The condition where lacunas are numerous is called *status lacunaris* (2). It is important to define their pathogenesis because they may warrant treatment different from infarcts involving the cerebral cortex (1,3-8). The pathogenesis of lacunar state is still not understood. The current hypothesis of a lacuna holds that the small deep infarcts are in the territory of a single occluded penetrating cerebral artery (1, 7, 9, 10). However in many instances of lacuna an occlusion of the lumen of the parent artery could not be identified (11). A critically low but not completely absent blood flow (8-23 mL/100 g/min) would be sufficient for development of a lacuna (12-17). These reports support the view that mechanisms other than obstruction in the small blood

vessel possibly contribute to lacuna formation (18-21).

The pathology of the intracerebral distal arterioles is less well understood than that of the larger cerebral arteries (1, 22, 23) and capillaries (24-27). Morphological changes are described in perforating arteries with a diameter between 90 and 500 microns (µm) but too little in arterioles smaller that 90 µm (1, 3, 28-31). Observations about arterioles, diameters between small and terminal (entrance of capillaries) point to special contribution of these vessels in the pathogenesis of lacunar infarct. In experimental animal models, the density of micro vessels and their tortuous profiles are increased in chronic hypoxic conditions (32-34) as also noted in the brain of aging people (35) and observed in several reports of lacuna (13, 31, 36). The present investigation quantifies the number of tortuous blood vessels (diameter 10-90 µm) in patients who suffered multiple lacunas, and compares it with matched controls. Furthermore, we describe the morphological features of the tortuous arterioles.

MATERIALS AND METHODS

Basal ganglia from 21 autopsy cases of patients over 45 years of age were obtained from the Department of Pathology and Human Anatomy at Loma Linda University Medical Center, Loma Linda, California, USA. Paraffin sections, 40 µm thick, were placed on glass slides and stained with Periodic Acid-Schiff leucofuchsin [PAS] (37). Thirteen cases without lesions in the basal ganglia were selected as a control group. Diagnostic criteria of multiple small (< 20 mm) infarcts in the basal ganglia were fulfilled in eight cases which were selected for the *status lacunaris* group (2, 9, 38). A high definition, real-time, three-dimensional microscope R400 (edge [TM] Scientific Instruments) was used to examine the 40 µm-sections. The diameters of the blood vessels were measured using a filar micrometer eyepiece (American Optical Co. [TM] Scientific Instrument Division, Buffalo, NY, USA). The microscopic fields were chosen according to random and systematic sampling methods by using a transparent, regular, line-lattice test superimposed on the slide (39). Corresponding to distal intracerebral segments from perforating arteries (28), an arteriolar diameter of 25 µm was chosen as a reasonable caliber to separate two arteriolar categories: small (diameter between 26 µm and 90 µm) and terminal (diameter between 10 µm and 25 µm) (40). As previously reported by others arterioles considered tortuous in this study met the following criteria: 1) they had at least one abrupt change in direction that was not artifactual; 2) they clearly deviated from a "natural" course; and 3) the change of direction was 90° or more within a distance approximately equivalent to three times the diameter of the vessel (35). A microfield containing at least one tortuous arteriole was considered positive. Tortuous profiles were classified according to varying structural patterns.

Statistical analysis

The differences in parametric variables, i.e., number of microfields studied, and number of microfields with tortuous arterioles, were calculated using the Student's t-test. The proportions of non-parametric variables, i.e., gender, history of hypertension, history of atherosclerosis, history of ischemic heart disease, and history of diabetes mellitus were compared by Chi-square test, Yates corrected. The association between frequency of tortuous arterioles as a risk factor for *status lacunaris* was suggestive if an odd ratio calculation was above 1.5 (OR>1.5) with a 95% confidence interval (95%-CI) without the value one. Differences were considered statistically significant at $p<0.05$.

RESULTS

The general characteristics of both groups did not show statistical differences (p>0.05) (Table I). Tortuous arterioles were present in both control and *status lacunaris* groups. Six structural patterns (morphological profiles) of the tortuous vascular segments were observed: simple kink, loop, knot, tangle, coil, and wave (Fig. 1). Additionally, combinations of the above structural patterns were observed. In some blood vessels, multisegmented tortuosity was found (Fig. 2).

Tortuous terminal arterioles were more numerous than tortuous small arterioles in the studied microfields in both control and *status lacunaris* groups (Table II). There were no differences in the numbers of tortuous small arterioles in each of the six categories in control and *status lacunaris* groups (Fig. 3). There were a significantly greater number of tortuous arterioles with a diameter between 10 µm and 25 µm (terminal arterioles) [p=0.001] in the *status lacunaris* group (mean=7.50±4.62) as compared with control group (mean=2.92 ± 1.38) (Fig. 3).

Estimate of associated risk for *status lacunaris*, expressed as an odd ratio, was significant with terminal arterioles (OR=1.94; 95%-CI=1.17<OR<3.22) [p=0.008] but not with small arterioles (OR=1.64; 95%-CI=0.62<OR<4.38) [p=0.39] (Table III).

DISCUSSION

A review of literature suggests that *status lacunaris* is a sequela of obstruction of 90 µm – 500 µm blood vessels. However, studies of lacuna, including serial sections, do not document the vascular obstruction in many cases (1, 7, 9, 10, 36). Some reports challenge the obstructive hypothesis of *status lacunaris* (11, 13, 15). Tiny ischemic damages can also result from a loss of autoregulation in distal ramifications of perforating arteries associated with variations in blood pressure (18, 36). On the base of this knowledge, a new approach to the pathogenesis of *status lacunaris* is warranted.

TABLE I
CLINICAL DATA AND GENERAL CHARACTERISTIC OF THE GROUPS

Characteristics		Groups		Significance[a]
		Control	*Status Lacunaris*	
Age (years-old)	mean ± SD	70 ±10	80 ± 11	0.10
Gender:				0.83
Male	number (%)	9 (69%)	6 (75%)	
Female	number (%)	4 (31%)	2 (25%)	
History of:				
Hypertension	number (%)	9 (69%)	8 (100%)	0.13
Atherosclerosis§	number (%)	9 (69%)	5 (63%)	0.87
Ischemic heart disease	number (%)	10 (77%)	8 (100%)	0.40
Diabetes *Mellitus*	number (%)	1 (8%)	2 (25%)	0.64
Number of fields studied	mean±SD	21 ± 6	21 ± 6	0.50

[a]Differences were not statistically significant (p> 0.05). SD, standard deviation.%, percentage. §Include any combination of CNS atherosclerosis, systemic atherosclerosis or both.

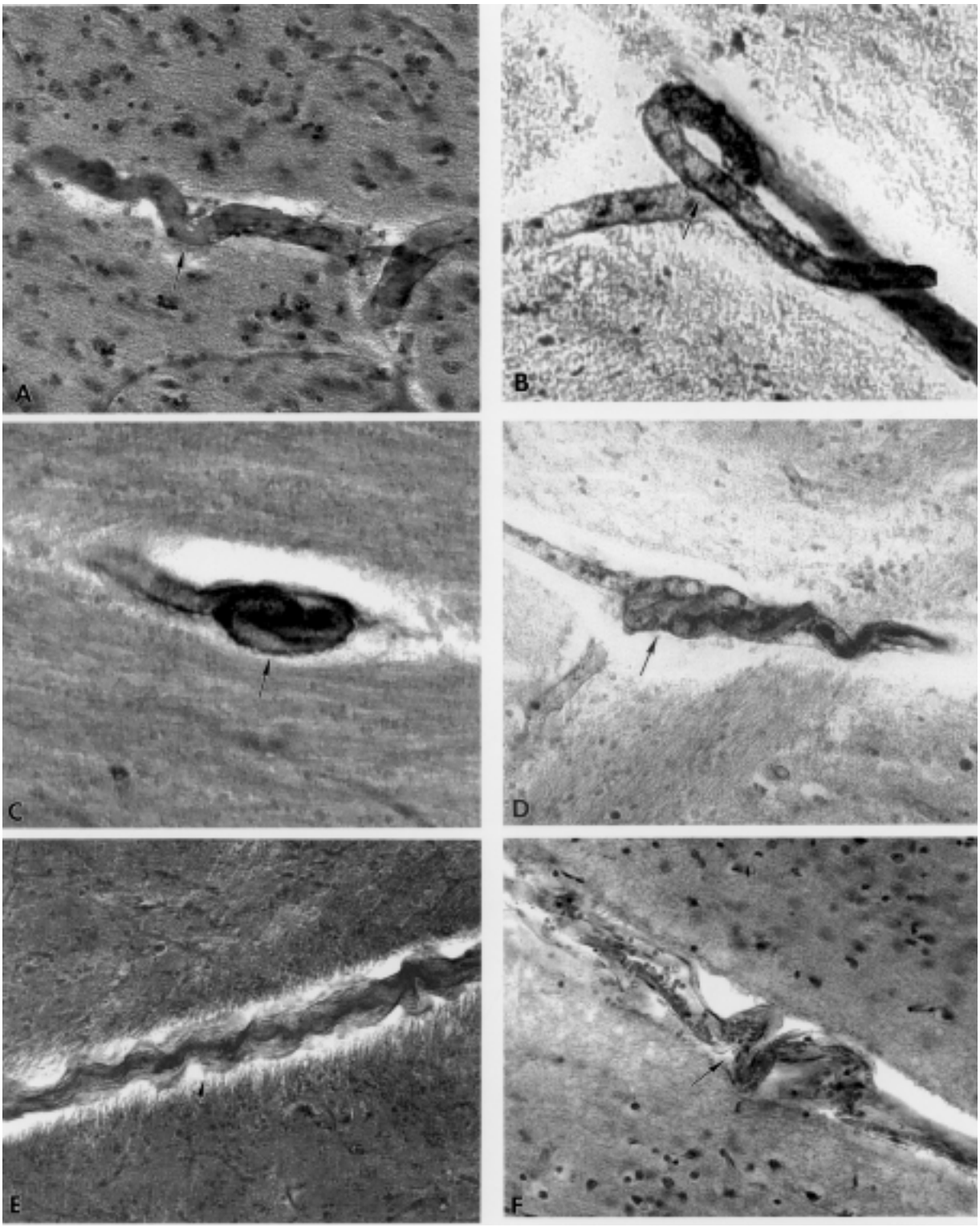

Fig. 1. Types of tortuous arterioles in basal ganglia: (A) single kink; (B) loop; (C) knot; (D) tangle; (E) coil; and (F) wave. Diameters of the blood vessels are between 10 and 90 µm.

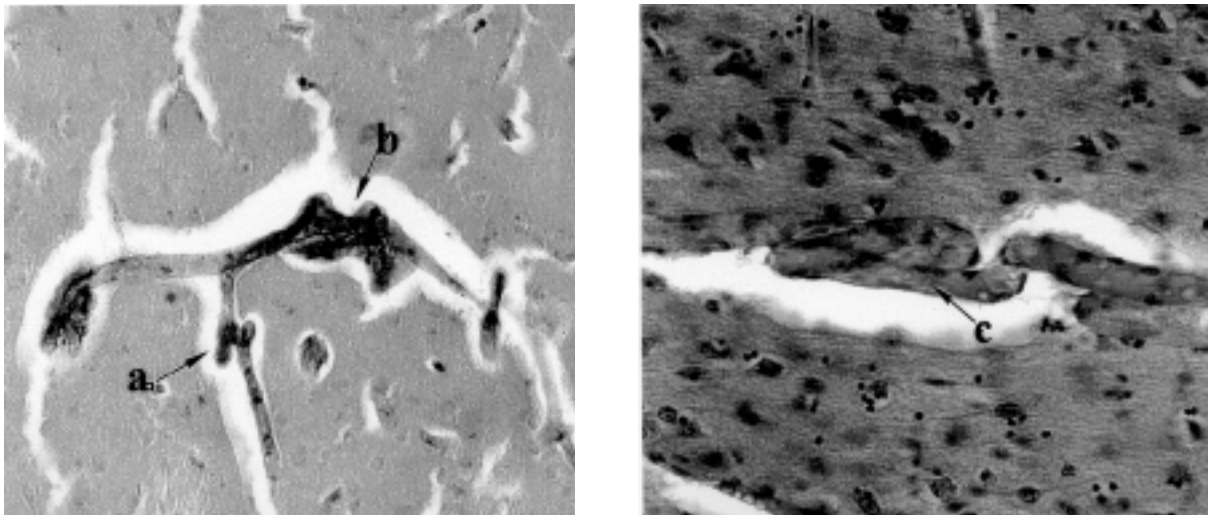

Fig. 2. Multiple tortuous profiles in arterioles of the basal ganglia: (a) coil; (b) tangle, and (c) complex tortuosities. Diameter of the blood vessels are between 10 – 90 µm.

TABLE II
NUMBER OF MICROFIELDS WITH TORTUOUS ARTERIOLES PAIRED BY CATEGORIZATIONS OF ARTERIOLES IN THE SAME GROUP

Variables	Number of Pairs	Mean ± SD NMWTA	Significance*
Control group:			
Small arterioles	13	0.76 ± 0.83	0.20
Terminal arterioles	13	2.92 ± 1.38	
Status lacunaris group:			
Small arterioles	8	1.25 ± 1.58	0.74
Terminal arteioles	8	7.50 ± 4.62	

*Differences were not statistically significant ($p>0.05$), t-test for paired samples. SD, standard deviation. NMWTA, number of microfields with tortuous arterioles.

In our study, we focused on a population of blood vessels ranging in diameter from 10 µm to 90 µm and found that in all cases of *status lacunaris*, tortuosity of these blood vessels was seen. The presence of tortuosity of 10 µm – 90 µm blood vessels of basal ganglia was not confined to *status lacunaris*; such blood vessels were also seen in normal controls. However, when the group of 10 µm – 25 µm blood vessels was analyzed, a statistically significant increment in tortuosity in *status lacunaris* when compared to control was seen (Fig. 3). In all cases of *status lacunaris* patients had hypertension which perhaps is the cause of the blood vessels distortion. Should this be the case, hypertension occurring in patients without *status lacunaris* could also be the cause of tortuosity. It is possible that persistent hypertension can lead to *status lacunaris* by increasing the number of 10 µm – 25 µm tortuous blood vessels. The question remains in dilucidating the role of tortuosity in the development of *status lacunaris.* The possibility exists that the affected blood flow in a distorted blood vessel can lead to hypoxia (41-45).

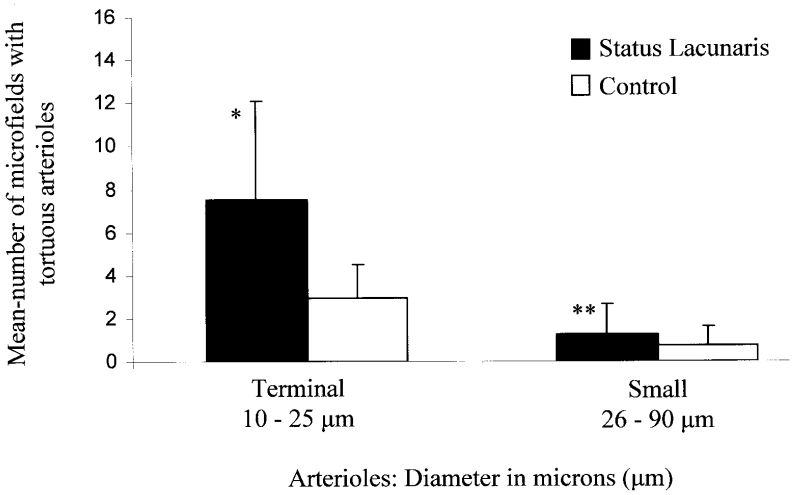

Fig. 3. Mean-number of microfields with tortuous arterioles in control vs status lacunaris groups. (*) p=0.001, (**) p=0.218, t-test for independent samples.

TABLE III
TORTUOUS ARTERIOLES AS A RISK FACTOR IN THE DEVELOPMENT OF *STATUS LACUNARIS*

Categories of Tortuous Arterioles	Odd Ratio	95%-Confidence	Interval Significance
Small (26 µm – 90 µm)	1.64	0.62<OR<4.38	0.390
Terminal (10 µm – 25 µm)	1.94	0.17<OR<3.22	0.008*

* The difference was statistically significant (p<0.05), t-test for independent samples. OR, odd ratio.

Finally, we found six distinct types of arteriolar tortuosity. However, studies on a larger number of cases are needed to establish the real significance of the different types of tortuosities.

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