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Electron microscopy analysis of skin biopsies in CADASIL disease

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Abstract

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is an inherited vascular disorder, non-amyloid and non-atherosclerotic, affecting predominantly the central nervous system. We examined samples of skin biopsies from six patients (men, 43–52-year-old), admitted for treatment in the Neurology Clinic regarding the presence of partial motor impairment on upper and lower right limbs, facial asymmetry and phrasing impairment (three of the patients); These three patients had family history remarkable for early-onset strokes: mother and two brothers deceased by early strokes (40–50-year-old). Skin biopsy samples were fixed in glutaraldehyde and post-fixed in osmium tetroxyde. After dehydration, tissue samples were embedded in Epon. Ultrathin sections were mounted on copper grids and stained with uranyl acetate and lead citrate as usual and examined with a transmission electron microscope Phillips CM100. In all cases ultrastructural study showed granular osmiophilic material (GOM) in extracellular locations, between degenerating smooth muscle cells in dermal arteries or in their indentations. Deposits of GOM varied in size and electron density. Degeneration and loss of smooth muscle cells (SMCs) leads to abnormal enlargement of the space between these cells Ultrastructural analysis in three cases showed chromatin condensation and peripheral aggregation of nuclear material suggesting cells entry to apoptosis. These aspects and the marked destruction of the vascular wall were correlated with MRI findings and the severity of clinical manifestations at these patients. Our study showed that findings of GOM deposits, degeneration and loss of SMCs (probably by apoptosis), cell adhesion elements disturbance are characteristic for CADASIL disease and sufficient for diagnose of certainty. Moreover, electron microscopy analysis of skin biopsies is a useful tool for a differential diagnosis and can be considered as first choice method.

***Keywords*:** CADASIL, electron microscopy, Notch, granular osmiophilic material.

Introduction

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is an inherited vascular disorder, non-amyloid and non-atherosclerotic, affecting predominantly the central nervous system [1]. Although the symptoms are almost exclusively neurological, pathological changes are not limited to the cerebral arteries. The same pathological findings can be seen in medium-sized and small arteries of almost all organs [2].

Clinically, CADASIL manifestations begin early in middle age (from 27 to 65-year-old) with migraine (with or without aura), lacunar strokes or transient ischemic attacks in the absence of risk factors such as hypertension and dyslipidemia [3, 4]. About 40% of patients develop mood disorders, memory disturbances and progressive stepwise cognitive decline and subcortical dementia in the course of the disease and another 20% have psychiatric disturbances [3, 5].

Magnetic resonance imaging (MRI) of the brain shows varying degrees of diffuse hyper intensity in the periventricular and subcortical white matter, along with lacunars infarctions in the thalamus, basal ganglia and brain stem with normal-appearing cerebral cortex and cerebellum [3, 6].

CADASIL disease is caused by mutations of Notch3 gene located in chromosome 19q12, which encodes for a large Notch3 transmembrane receptor protein, with an important signaling function during development: influence cell differentiation, proliferation and apoptotic events at all stage of development. Gene expression for Notch3 is highly restricted to the vascular smooth muscle cells, being important in maintenance the phenotypic stability of these cells and for arterial maturation after birth [7]. In adults, Notch3 may promote cell survival by inhibiting apoptosis, is involved in wound healing of vascular injuries etc. [7]. The mutations lead to either a gain or loss of a cysteine residue in the extracellular N-terminal part of the molecule, causing a conformational and functional alteration [8].

The diagnosis of CADASIL must therefore be established by clinical and neuroimaging data, ultrastructural analysis and/or immunohistochemical analysis of skin blood vessels and/or by genetic testing (some-

times difficult due to the large size and various mutations of the Notch3 and more expensive than electron microscopy studies, which provide pathognomonic finding in CADASIL and is considered the method of first choice) [9].

Our study displays the morphologic, ultrastructural analysis of the skin biopsy samples from patients admitted in the Neurology Clinic of „Prof. Dr. Nicolae Oblu" Hospital, Iassy, who showed clinical and radiological features suggestive for CADASIL disease.

Material and Methods

We examined samples of skin biopsies from six patients (men, 43–52-year-old), admitted for treatment in the Neurology Clinic regarding the presence of partial motor impairment on upper and lower right limbs, facial asymmetry and phrasing impairment (three of the patients); memory disturbances, frequent migraine attack with aura, mood disturbances (agitation, emotional lability) and phrasing impairment for the other patients. These three patients had family history remarkable for early-onset strokes: mother and two brothers deceased by early strokes (40–50-year-old).

Skin biopsy samples were fixed in glutaraldehyde and post-fixed in osmium tetroxyde. After dehydration, tissue samples were embedded in Epon. Ultrathin sections were mounted on copper grids and stained with uranyl acetate and lead citrate as usual and examined with a transmission electron microscope Phillips CM100.

Results

In all cases ultrastructural study showed granular osmiophilic material (GOM) in extracellular locations, between degenerating smooth muscle cells in dermal arteries or in their indentations. Deposits of GOM varied in size and electron density.

Smooth muscle cells (SMCs) of examined dermal small arteries was irregular in shape, with electrolucent vacuoles in their altered cytoplasm, indentations on the cell surface and separated from their neighboring cells. In three of six cases their cytoplasm showed also large, irregularly, dense bodies and disorganization of cytoskeletal elements.

Degeneration and loss of SMCs leads to abnormal enlargement of the space between these cells (Figure 1, A and B).

Endothelial cells also showed modifications, irregular shape, sometimes with clear vacuoles in their cytoplasm. We observed also lumen stenosis, detachment of the cells and disorganization of the normal architecture with increase of electrolucent areas located between the endothelium and the smooth muscle cells (Figure 2, A–C).

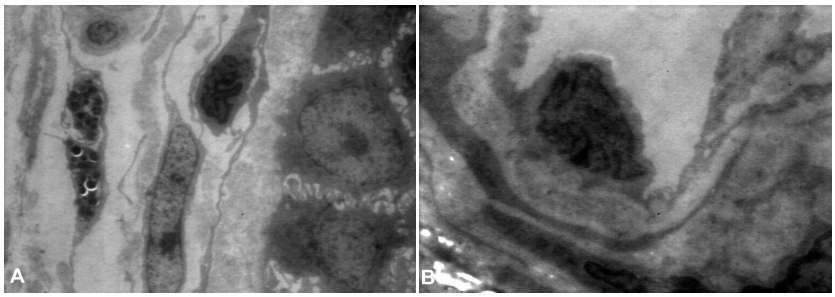

Figure 1 – ***Degeneration and loss of SMCs leads to abnormal enlargement of the space between these cells: (A) 1250×; (B) 2400×.***

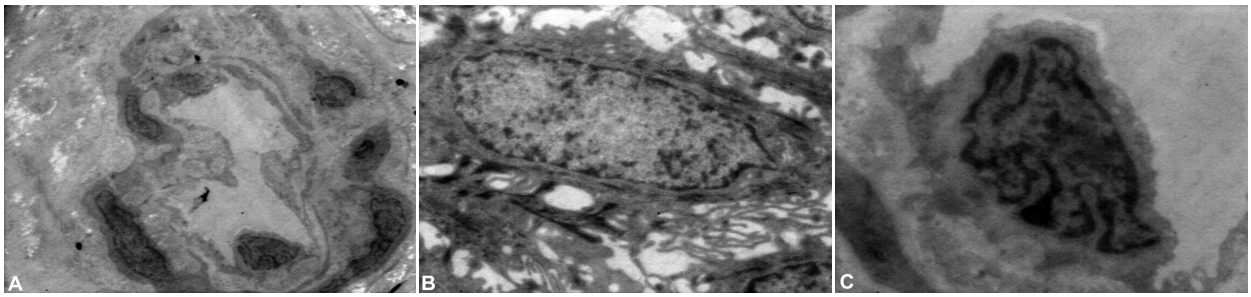

Figure 2 – ***(A–C) Lumen stenosis, detachment of the cells and disorganization of the normal architecture with increase of electrolucent areas located between the endothelium and the smooth muscle cells.***

Ultrastructural analysis in three cases showed chromatin condensation and peripheral aggregation of nuclear material suggesting cells entry to apoptosis. These aspects and the marked destruction of the vascular wall were correlated with MRI findings and the severity of clinical manifestations at these patients.

Discussion

The diagnosis for CADASIL disease was suspected in these patients due to clinical features, recurrent ischemic episodes beginning earlier than classic atherosclerotic strokes, the absence of high blood pressure and the family history. Biochemical and cardiovascular investigations helped to rule out other causes of ischemia.

All patients exhibited MRI abnormalities and these findings agreed with recent studies and demonstrate that cranial MRI is a sensitive tool for screening patients for CADASIL. However, these changes on MRI, often characteristic, are uncertain and non-specific [10].

Our study shows that skin biopsy can be used as confirmation diagnosis for CADASIL disease while genetic tests are more expensive and demanding. Even if irrelevant symptoms and minimal MRI changes are present, the electron microscopic examination shows at least GOM presence. Ruchoux MM *et al.* [9] were the first who describe the presence of GOM within the vessel walls of skin biopsies in a patient affected by this

disease. Up to now, all skin biopsies in subjects carrying the CADASIL haplotype (100%) have been characterized by the presence of GOM [11]. These GOM are present in the SMCs basal lamina of the skin vessels or between them and appears to be unique and pathognomonic for CADASIL [11].

Until recently, the biochemical structure of GOM deposits was unknown. This material usually stains with PAS, does not contain amyloid, elastin, chromatin, calcium or iron [12]. Some immunohistochemical studies suggested that GOM issues from SMCs or are debris of degenerated muscle cells or basal lamina [13] while other recent studies show that their molecular structure contains mainly Notch3 extracellular domains.

Enlargement of subendothelial spaces and the presence of electrolucent areas located between endothelium and the smooth muscle cells involve changes in the structure and function of the gap junctions and consequently on the intercellular communication mechanisms; this influence the homeostasis of the vascular wall and the vasoactive response [14].

Disturbances in normal distribution of cytoskeleton elements in examined samples may also indicate the supposed progressive involvement of cell adhesion at vascular wall level (mainly in focal contacts) and the link to CADASIL pathogenesis.

The ultrastructural aspects, indicatives of cellular apoptosis prove that Notch3 dependent signaling mechanisms are essential for smooth muscle cells. These observations are consistent with recent data illustrated in literature [7], which states that Notch3 receptor activation induces an anti-apoptotic mechanism in SMCs.

A defective Notch3 receptor may be responsible for apoptosis and degeneration of SMCs.

Degeneration of SMCs in CADASIL disease could lead to decrease secretion of vascular endothelial growth factor (VEGF), a potent permeability factor [15]. These two aspects lead to a decrease in permeability and loss of vessel wall tonicity.

Conclusions

Our study showed that findings of GOM deposits, degeneration and loss of SMCs (probably by apoptosis), cell adhesion elements disturbance are characteristic for CADASIL disease and sufficient for diagnose of certainty. Moreover, electron microscopy analysis of skin biopsies is a useful tool for a differential diagnosis and can be considered as first choice method. The study confirms, as well, the role of vascular dysfunction in the pathogenesis of CADASIL disease the correlation between clinical symptoms and ultrastructural changes. Therefore, we submit that analysis of skin vessel morphology may serve as a paradigm for the study of other forms of subcortical vascular dementia.

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