Ultrastructural aspects of pseudoxanthoma elasticum*

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Abstract: We report the ultrastructural findings in a case of a 72-year-old black woman with confluent yellowish papules in the cervical region. She had no comorbidities. Ophthalmological examination, electrocardiogram, and echocardiogram were normal. Hematoxylin-eosin staining of the affected skin showed strong alterations in the mid-dermis with irregular clumps of eosinophilic material and loss of the normal parallel arrangement of collagen bundles. Orcein staining revealed that the elastic fibers lost their normal linear configuration, showing clump fragmentation, sometimes forming square structures. Transmission electron microscopy showed aberrant elastic fibers with an irregular outline and heterogenic inner structures. We also observed small elastic fibers. Collagen fibers showed a normal structure with irregular distribution. Scanning electron microscopy revealed important disorganization of collagen fibers and small stone-like deposits measuring around 5 µm associated with bigger structures ranging from 10-16 µm. Higher magnification revealed that these small stone-like structures were sometimes polyhedral-shaped or squared.

Keywords: Elastic tissue; Microscopy, electron, scanning; Microscopy, electron, transmission

INTRODUCTION

Pseudoxanthoma elasticum (PXE) is a rare, genetic connective tissue disorder with progressive calcification and fragmentation of elastic fibers, which involves the skin, the retina, the cardiovascular system, and the gastrointestinal tract.

Clinical features of PXE consist of small (1-5mm) asymptomatic yellowish papules localized in flexural areas (neck, axillae, elbows, groin, and knees).[1-3] These lesions may coalesce into larger plaques. The loss of resilience can give the skin a wrinkled appearance, which may cause cosmetic and functional problems, especially with extensive involvement.[4]

Ocular manifestations include angioid streaks, choroidal neovascularization, and subretinal hemorrhage, which may lead to the loss of central vision and sometimes cause blindness.[1] Cardiovascular symptoms consist of weak peripheral pulses and intermittent claudication. A small percentage of patients may have angina or hypertension.[1] At the molecular level, around 300 mutations were described in the ABCC6 gene, which codifies a transporter protein.[2] The real mechanism on how these mutations lead to elastic fibers alteration is not fully understood.

We report the histological and ultrastructural findings in a case of a 72-year-old black woman who noticed skin alterations in the cervical region in the third decade of life. Family history revealed no similar cases. On physical examination, we observed a slight thickening of the cervical region with confluent yellowish papules (Figure 1). Other skin folds were not involved. She had no comorbidities. Ophthalmological examination, electrocardiogram, and echocardiogram were normal. A biopsy of the affected area was performed and processed for light microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM).

RESULTS

Hematoxylin-eosin staining showed strong alterations in the mid-dermis, whereas these changes were less intense in the papillary and deep dermis, showing a normal aspect of collagen bundles (Figure 2). Higher magnification of the mid-dermis revealed the presence of eosinophilic irregular clumps and loss of the normal parallel arrangement of collagen bundles (Figure 2). Lower magnification of the samples stained with orcein confirmed the pres-

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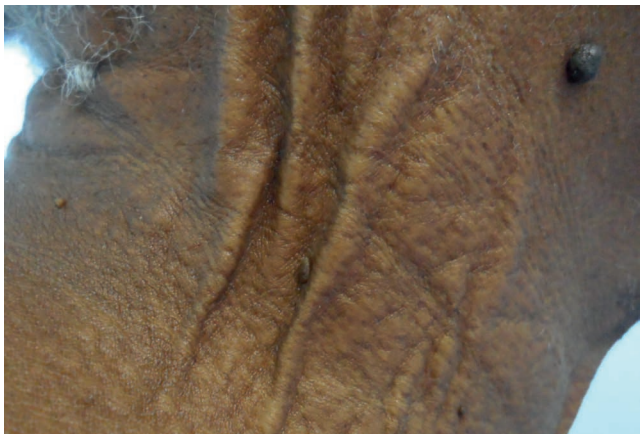

Figure 1: Cervical involvement with yellowish papules

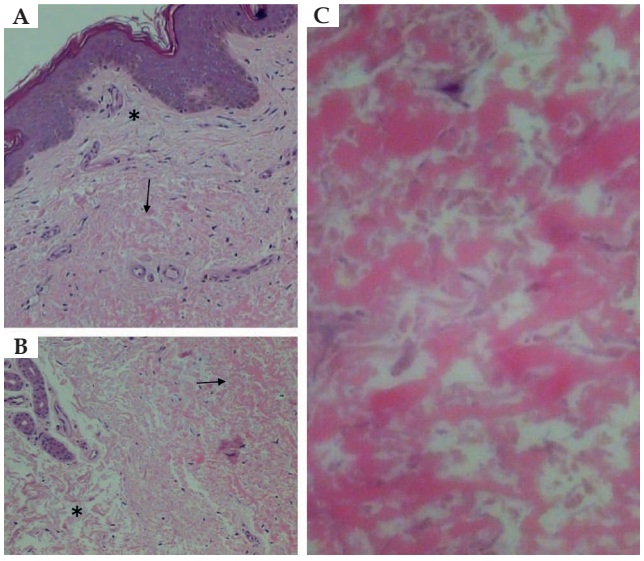

Figure 2: Light microscopy with HE staining. **A** and **B -** low magnification (X100) showing eosinophilic degeneration of the mid-dermis (arrows). Note the papillary and deep dermis showing less intense changes (asterisks). **C -** detail of the mid-dermis showing intense eosinophilic changes (X400)

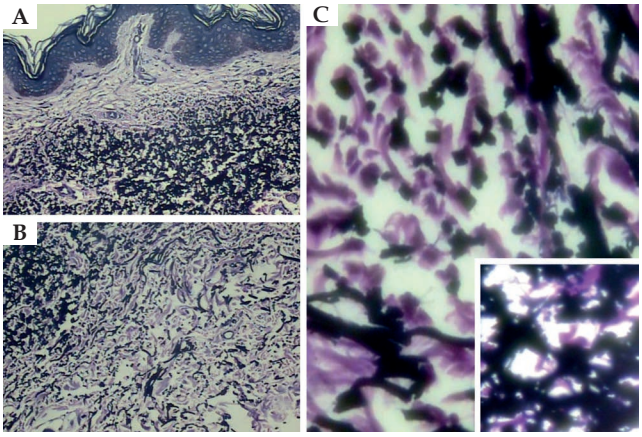

Figure 3: Light microscopy with orcein staining. **A** - and **B -** low magnification (X100) showing degeneration of the mid-dermis, confirming that the papillary and deep dermis show less intense changes. **C -** detail of the mid-dermis showing elastic tissue changes with clumping and square structures (inset with detail of the clumping) (X400).

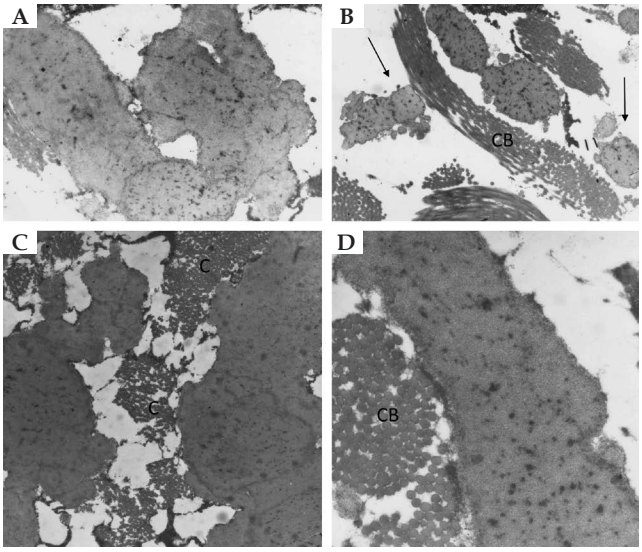

Figure 4: Transmission electron microscopy. **A -** irregular and clumped elastic fiber (X7,000). **B -** area with smaller elastic fibers with a square outline (arrows) and a collagen bundle (CB) (X7,000). **C -** large irregular elastic fibers near to lose collagen fibers (C) (X7,000). **D -** detail of an elastic fiber showing irregular distribution of black dots in its core and a collagen bundle in cross section demonstrating normal collagen fibers (CB) (X20,000)

ence of more intense alternations in the mid-dermis, with lighter involvement of the papillary and deep dermis (Figure 3). Higher magnification also revealed that the elastic fibers lost their normal linear configuration, showing clump fragmentation, sometimes forming square structures and some areas of intense accumulation of clumped material (Figure 3). Von Kossa staining showed no calcium deposits.

TEM examination showed aberrant elastic fibers with irregular outline and heterogenic structures. We also observed smaller elastic fibers, sometimes with a square outline (Figure 4). At higher magnifications, elastic fibers showed an irregular distribution of black dots normally seen in their cores. Collagen fibers revealed a normal structure, sometimes forming bundles, showing a possible disorganization secondary to the elastic defect (Figure 4). We observed no electron-dense calcium deposits.

SEM examination of the dermis at low magnification revealed important disorganization of collagen fibers and deposits of small structures measuring around 5 μm associated with bigger stone-like structures ranging from 10-16 μm (Figure 5). In some fields, we could visualize normal aggregations of collagen fibers

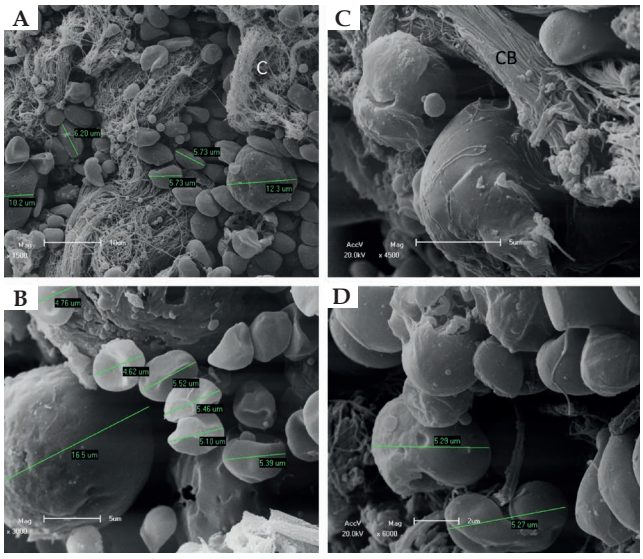

Figure 5: Scanning electron microscopy. **A -** low magnification with irregular distribution of collagen fibers (C) and deposition of small structures with around 5 µm and bigger ones over 10 µm (X1,500). **B -** detail of the stone-like structures seen in the mid-dermis (X3,000). **C -** collagen bundle (CB) with two small stone-like structures (X4,500). **D -** detail of the small structures, some with a square outline (arrow) (X6,000)

forming bundles (Figure 5). Observations at higher magnification revealed that these small stone-like structures were sometimes polyhedral-shaped or squared, similar to TEM and HE findings (Figure 5).

DISCUSSION

PXE is a rare inherited disease, which has been recently associated with mutations in the ABCC6 gene (ATP-binding cassette subfamily C member 6) that encodes a transmembrane ATP binding efflux transporter, normally expressed in the liver and kidney.[5,6] At least one mutation was found in 80% of patients.[6] Many cases occur sporadically as in the present patient.[3]

PXE presents an estimated prevalence ranging from 1:25,000-1:50,000 and females are more commonly affected than males at a ratio of 2:1.[1]

The diagnosis is based on clinical and histopathological aspects and on genetic studies.[5] A skin biopsy is essential for PXE diagnosis because it shows typical histological alterations such as aberrant and fragmented aggregation of elastic fibers in the mid-dermis, which were stained black with orcein in the present case.

This patient showed a mild dermal involvement in a 40-year follow-up. We observed no cardiac and ocular involvement.

Light microscopy confirmed the elastic tissue alteration with fragmentation of elastic fibers. Some areas showed small squared elastic fibers, whereas others revealed intense clumping, which was more conspicuous in the mid-dermis. TEM confirmed the involvement of elastic fibers, which were irregular and clumped, sometimes squared and small. Collagen was secondarily involved, with intense disorganization, but with normal fiber structures. TEM revealed no calcium deposition according to von Kossa staining.

SEM also demonstrated intense disorganization of collagen, with occasional bundle formation. The deposition of small structures around 5 µm and bigger ones ranging from 10-16 µm among the collagen fibers was an impressive finding, which should represent the aberrant elastic fibers seen with TEM and light microscopy. These examinations also revealed smaller and bigger structures in a bidimensional manner.

There is little information about PXE ultrastructure in the literature. Most authors report cases of fragmentation of elastic fibers.[7-9] Calcium deposition – described as electron-dense material – has not been reported in all cases, similar to our findings.[9,10] Although the use of SEM is not usual in conditions with dermal involvement, it can also contribute by describing three-dimensional features of connective tissue changes.[11] As an example, clumping of elastic fibers was demonstrated in a previous report with SEM.[12]

An important differential diagnosis is late-onset focal dermal elastosis. Our patient showed skin changes in the third decade of life and light microscopy showed no increased aggregates of normal-appearing elastic fibers in the reticular dermis, as described in this condition.[13-14]

Our findings showed important deposition of stone-like structures, which should represent the altered elastic fibers seen in TEM. The ultrastructural aspects of this case contributes with additional information about the elastic tissue involvement in PXE. ❑

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