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Histopathological Studies in Lipodermatosclerosis Associated with Venous Hypertension. Pathophysiological Considerations

Estudios histopatológicos en la lipodermatoesclerosis asociada a hipertensión venosa: consideraciones fisiopatológicas

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Summary
Lipodermatosclerosis (LDS) or sclerosing panniculitis is an induration of the dermis, hypodermis and sometimes the superficial fascia. It is a chronic inflammatory process usually related to venous pathology, with neo-vascularization, adipose tissue necrosis and fibrosis. Several hypotheses have been proposed, but the exact mechanism is still unknown. In this paper we studied the skin biopsies of 6 patients with chronic LDS. We performed histochemical techniques, immunolabelling of methalloproteinases 1, 2 and 3; CD68, alpha smooth muscle actin and plastic semithin sections. Our main findings were: venous vessels were altered, with narrow lumens intimal fibrosis and hyperplasia, some arterial vessels also showed fragmentation and reduplication of internal elastic layer with subintimal fibrosis. There was a remarkable disorganization of reticular and hypodermal layer due to a intense fibrosis. Typical area of pseudomembranous fatty necrosis and pseudocyst were observed. There were patchy mixed inflammatory infiltrates in all skin layers involved. Methalloproteinases 1, 2 and 3 were positive in epidermis, endothelial cells, in some dermal and hypodermal cells, outlining pseudocysts and in pseudomembranous fat necrosis. In plastic semithin sections the presence of lipids droplets permeating between collagen fibers even in superficial dermis were evident.

(Vignale R, Panuncio A, Saralegui P, Di Matteo C, Civila E. Histopathological Studies in Lipodermatosclerosis Associated with Venous Hypertension. Pathophysiological Considerations. Med Cutan Iber Lat Am 2004; 32(3): 111-116)

Key words: lipodermatosclerosis, sclerosing panniculitis, venous hypertension, metalloproteinases, adipocytokines.

Resumen
La lipodermatoeslerosis (LDS) o paniculitis esclerosante es una induración del dermis, hipodermis y en ocasiones de la fascia superficial. Es un proceso inflamatorio crónico relacionado a una patología venosa, con neovascularización, necrosis grasa y fibrosis. Se postulan varias hipótesis patogénicas, pero el mecanismo exacto se desconoce. En este trabajo estudiamos 6 biopsias cutáneas de 6 pacientes portadores de lipodermatosclerosis crónica. Realizamos estudios inmunohistoquímicos para metaloproteinasas 1, 2 y 3; CD68, actina alfa de musculo liso y cortes semifinos de material incluído en plástico. Nuestros principales hallazgos fueron: los vasos venosos estaban alterados, con luces estrechadas con hiperplasia y fibrosis intimal, algunos vasos arteriales también mostraron fragmentación y reduplicación de la capa elástica interna con fibrosis subintimal. Se encontró una marcada desorganización de la dermis reticular e hipodermis debido a la intensa fibrosis. Se observó asimismo, necrosis grasa pseudomembranosa y pseudoquistes con un infiltrado inflamatorio mixto de disposición parcheada. Las metaloproteinasas 1, 2 y 3 fueron positivas en la epidermis, células endoteliales, algunas células dérmicas e hipodérmicas, alrededor de los pseudoquistes y de necrosis grasa pseudomembranosa. En cortes semifinos de plástico, se observó la presencia de gotas lipídicas entre las fibras colágeno, inclusive en la dermis superficial.

Palabras clave: *lipodermatoesclerosis, paniculitis esclerosante, hipertensión venosa, metaloproteinasas, adipocitocinas.*

Lipodermatosclerosis (LDS) or sclerosing panniculitis[1] is an extensive induration of the skin that generally appears in the legs associated to ulcers, most commonly related to venous hypertension. It is a chronic inflammatory process with increased hyalinized fibrous tissue, that involves and replaces the papillary and reticular dermis, as well as the hypodermis[1-5]. Several pathophysiological hypotheses have been mentioned, but the exact mechanism remains unknown. A venous microangiopathy secondary to venous hypertension has been observed, together with a chronic inflammatory cellular infiltrate. These processes trigger the activation of many cytokines and proteolytic enzymes, specially methallo-

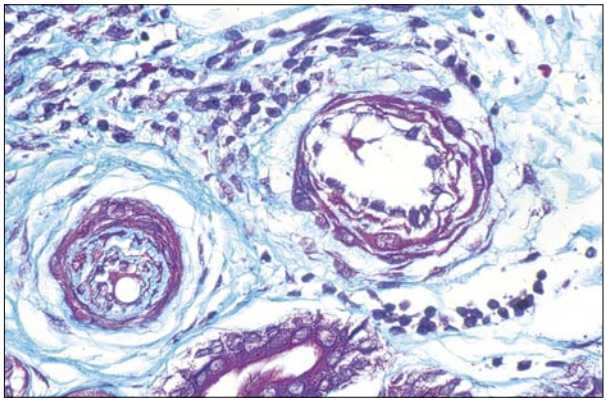

Figure 1. *Blood vessel with marked alterations in endothelial cell, media (right and below) and intimal hypertrophy with severe obliteration of the lumen (left and above).*

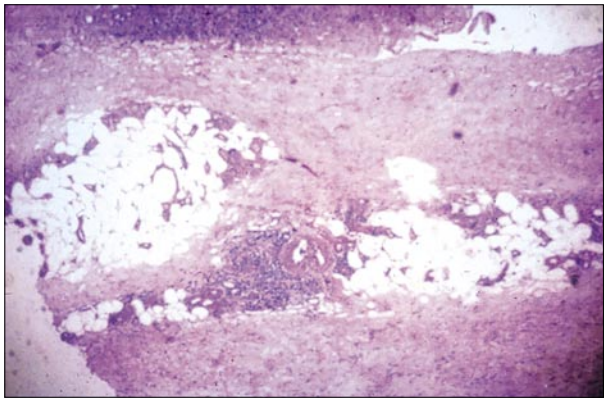

Figure 2. *Adipose lobule surrounded by fibrosis. Thickening of the connective septae, peri-vascular inflammatory infiltrate. Blood vessels with enlarged walls are located equidistantly.*

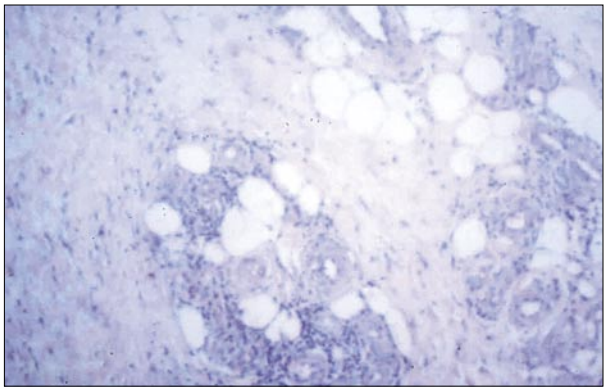

Figure 3. *Left: Thicker and closer vessels and more developed loss of fatty tissue. Right: Most of the fat of the adipose lobule is lost and vessels are grouped in clusters.*

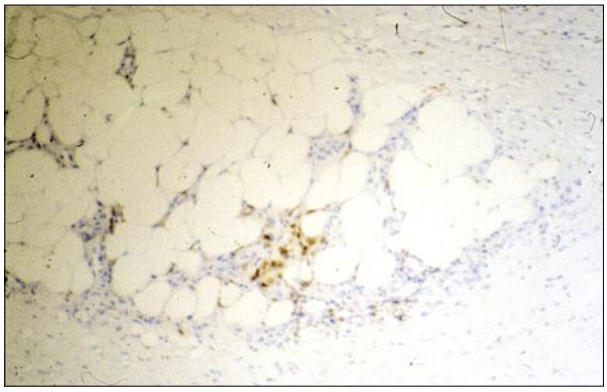

Figure 4. *CD68 immuno-labelling in the cells invading the adipose lobule.*

proteinases (MMPs), and also determine an increased fibroblastic activity[3, 5-8]. In this paper we studied 6 patients with long-standing LDS, all of whom presented chronic leg ulcers in the past, while one presented an active ulcer in the inner aspect of the right leg. Skin biopsies of these patients were stained with histochemical techniques and evaluated with immunohistochemistry for methalloproteinases 1, 2 and 3, alpha smooth muscle actin (ASMA), and CD68. Plastic semithin sections were performed in one case. We propose a hypothesis to account for the pathophysiological mechanism involved in this condition.

Material and methods

After informed consent we obtained punch biopsies involving the epidermis, dermis and hypodermis from 5 patients in the more indurated area of chronic LDS, far from the scar tissue of a previous ulcer; another patient was sampled at the edge

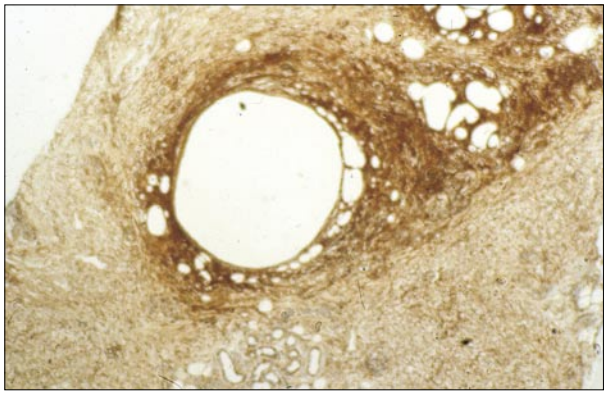

Figure 5. *Immuno-labelling for MMP1 shows marked stain outlining the pseudo-cyst.*

of an active ulcer. Ages ranged from 48 to 57 years. Four patients were female, and 2 were male. Three women and one man were obese. All of them presented symptoms of venous pathology. None of them had a history of erysipelas

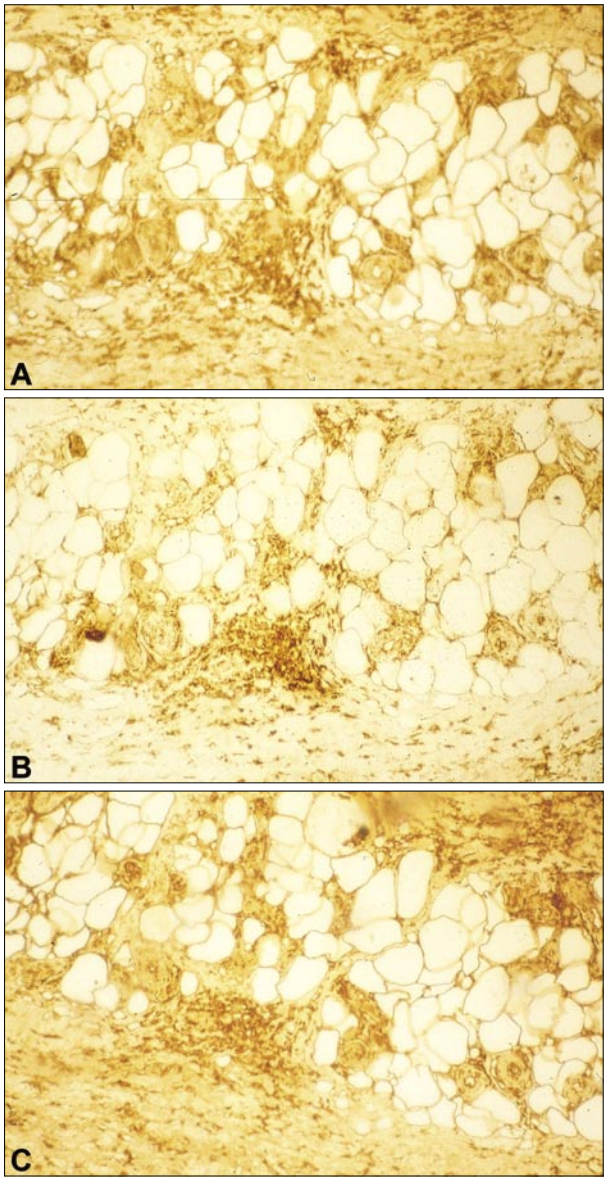

Figure 6. *a), b), c) Immuno-labelling in adipose lobule and cells of the fibrotic tissues. (a: MMP1; b: MM2; c: MMP3).*

or any other skin disorders, diabetes, arterial hypertension or other systemic diseases. The Echo Color-Doppler study revealed alterations in the venous circulation without apparent arterial pathology. All laboratory tests were normal. The skin biopsies were fixed in 10% formalin embedded in paraffin. Sections were stained with Haemalum Eosin, Periodic Acid Schiff, Masson Trichrome, and Orcein. Formalin-fixed, paraffin-embedded tissue sections were evaluated immunohistochemically using the Envision Plus kit with diaminobencidine (DAB) as a chromogen. The antibodies used were: mouse

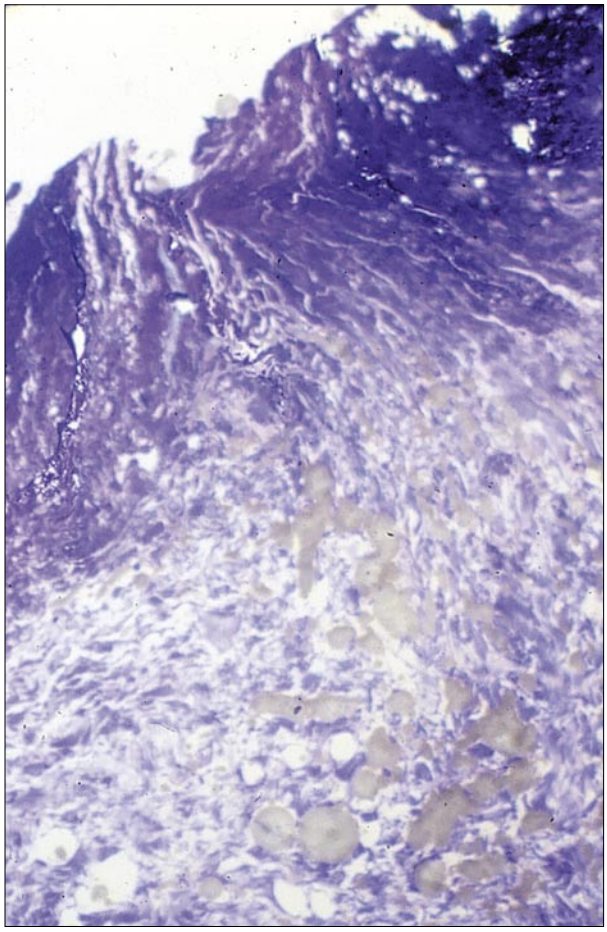

Figure 7. *Ulcerous bed with small fat droplets (10x).*

monoclonal antibodies to MMP1 (interstitial collagenase, clone 148-1A3), MMP2 (gelatinase A, clone A-4001), MMP3 (stromelysin, clone 148-1A3)), Alpha Smooth Muscle Actin (ASMA) and CD68 (Macrophage). The immunostain kit and all the antibodies were from Dako, USA. Appropriate positive and negative controls were also performed. The Shi retrieval antigen method[9] was used for MMP1 and 3, and tripsin for MMP2. Routine embedding in Durcupan was performed and plastic semithin section were stained with toluidin and fucsin.

Results

Epidermal changes ranged from acanthosis of the epidermis and interpapillary crests to hypotrophic and ulcerated epidermis in one case. A patchy inflammatory infiltrate with lymphocytes and polymorphonuclear cells were observed in the edematous papillary and reticular dermis, and hypodermis. Throughout the reticular dermis we found extensive fibrosis reaching and invading the hypodermis, which presented a

significantly increased number of venous vessels. All cases showed marked venous alterations. The veins presented narrow lumens, and some exhibited endothelial cells disorders, intimal fibrosis and hyperplasia (Figure 1). A few lymphocytes were occasionally found in the vessel walls, involving the adventitious layer and generally extending into the perivascular tissue. Some arterial vessels also showed fragmentation and reduplication of the internal elastic layer, as well as subintimal fibrosis, and slight myointimal hyperplasia. In two cases we observed dilated lymph vessels.

The hypodermis was globally disorganized. There were groups of preserved adipose cells encircled by marked fibrosis. The more preserved adipose tissue showed thickened small vessels in an equidistant pattern of distribution (Figure 2). Other adipose lobules with marked fibrosis presented thicker venous vessels, and the distance between them was decreased (Figure 3, left). As the fat cells disappear, venous vessels form clusters with a glomerular-like appearance, surrounded by a dense fibrous tissue (Figure 3 right). Typical areas of pseudomembranous fat necrosis and pseudocysts were also observed. Lymphoid cells and CD68 positive cells were observed within the adipose lobules (Figure 4). Immunolabelling for MMPs 1, 2 and 3 was positive in the epidermis and endothelial cells. There was a positive staining for MMPs in dermal and hypodermal cells, outlining the pseudocysts (Figure 5), and in the pseudomembranous fat necrosis. In addition, the cells within the adipose tissue were also positive (Figures 6 a, b and c). ASMA was positive in the thickened vessels, and in isolated cells in the hypodermis which were interpreted as myofibroblasts.

Plastic semi-thin sections showed lipid droplets permeating between the collagen fibers, and spreading from the hypodermis to the superficial dermis, as is shown in the ulcerous bed (Figure 7).

Discussion

All cases studied presented modifications in the epidermis, a global disruption of the dermis and hypodermis, an increased number of pathologic venous vessels, patchy chronic inflammatory cells, extensive fibrosis with loss of fat tissue, pseudomembranous fat necrosis and also pseudocysts. These findings are in agreement with those reported by other authors in LDS due to venous hypertension[1-8]. Multiple pathogenic mechanisms had been involved in this entity. It seems that LDS results from a cascade of events. Venous hypertension produces the activation of endothelial cells with release of numerous cytokines and many proinflammatory substances that attract many inflammatory cells. A chronic inflammatory reaction is established and causes necrosis of adipose cells[10-18].

We observed in plastic-embedded material, fat droplets of different sizes, specially in the ulcerous bed (Figure 7). This histological finding is difficult to asses in paraffin embedded material. We think that adipose cell debris and free lipid droplets in the extra-cellular matrix, contribute to maintain the chronic inflammatory process. Experimental studies in mice with ligated hind limb veins support this presumption. When subcutaneous lipase is injected in mice limb, an acute necrotizing panniculitis occurs, with subsequent fibrous replacement of the subcutaneous tissue[19, 20]. Simultaneously to adipose tissue necrosis, activated macrophages stimulates fibroblasts which cause the synthesis of pathologic hyaline collagen.

We found an increased methalloproteinase immunolabelling, mainly in the ulcerated areas and altered adipocytes (Figures 6 a, b and c), in the periphery of pseudocysts (Figure 5), inflammatory cells (Figures 6 a, b and c), and in the pseudomembranous fat necrosis. These results agree with those of other authors, but they mainly used biochemical methods to demonstrate MMPs activity[21-22]. It is intriguing that fibrous tissue is augmented in spite of the high level of the methaloproteinases[23-31]. It was described the existence of a substantial increase of altered cross-links in the hialinized collagen[32-33]. In order to explain this fact, we suggest that these anomalous cross-links could hide to MMPs specific collagen-binding sites, thus the enzymes are unable to find them.

We observed adipose lobules in different stages of involution in a patchy and non-homogeneous way from the surface to the deepest hypodermis (Figures 2 and 3). We think that it is possible to propose a probable sequence in the degenerative process of adipose tissue. The more preserved adipose lobules had few mononuclear cells as well as less MMPs stain (Figures 6 a, b and c). When the adipocytes begin to disappear, blood vessels come closer and also presented a thicker muscular layer (Figure 3, left), and finally form clusters of glomerular-like vessels among the hyaline fibrous tissue (Figure 3, right).

Adipose tissue is an active organ that secretes multiple substances such as growth factors and adipocytokines[34-37]. The reduction or absence of these substances in LDS probably contributes to the misbalance in tissue homeostasis. There is no adipocyte regeneration in the hypodermis of patients with LDS. In active lesions the adipocytes are gradually replaced by fibrous tissue. Why this occurs is still an enigma. In vitro studies of preadipocytes demonstrated that the extra-cellular matrix and proteinases play a highly regulated role in the differentiation of preadipocytes into adipocytes[34-37]. Adipocyte differentiation occurs when fibronectin, and collagens I and III fall and collagen IV increases in the culture media[37]. High matrix levels of collagen I

and III present in LDS, allows us to guess that preadipocytes are forced to differentiate in fibroblasts.

It was difficult to us to differentiate arteriolar from venous vessels due to the remarkable alterations that they exhibited. Following the criteria described by Sanchez Yus[38] we found that most vessels corresponded to altered small veins. These vessels frequently presented mixed histological features corresponding to arterial and venous vessels. The main change observed in venous vessels was the absence of the normal elastic tissue network in the medial layer. These modifications could be explained as a consequence of venous hypertension. We wonder if there is in LDS a real venous vessel proliferation or rather, myointimal hyperplasia of preexistent small vessels, as is shown in the sequential changes above mentioned in adipose lobules (Figures 2 and 3).

We conclude that LDS is initiated by an alteration in the walls of small venous vessels due to venous hypertension. This, in turn, triggers the activation of many inflammatory cells. Activated macrophages secrete numerous enzymes, cytokines and growth factors, leading to the destruction of adipose tissue. Consequently, free fat appears in the extracellular matrix. These lipids contribute to stimulate macrophages, which also activate fibroblasts, thus maintaining a chronic inflammatory process. The increase in MMPs 1, 2 and 3 is not effective in diminishing fibrosis, probably due to the architectural and molecular alterations of collagen fibers. These matrix changes forced preadipocytes to differentiate into fibroblasts, and more collagen fibers are produced. A deeper knowledge of these pathophysiological events, will allow us to improve the treatment of this condition.

Acknowledgements

We would like to thanks to Mariángel Parnisaro, Andrés Silveira and Mariana García for their excellent technical assistance.

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