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ARS Medica Tomitana - 2021; 1(27): pag. 27 - 30
doi: 10.2478/arsm-2021-0006

POLARIZED MICROSCOPY STUDY OF 10 CASES OF LICHEN SCLEROSUS USING PICRO-SIRIUS RED STAIN

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ABSTRACT

Lichen sclerosus (LS) is a chronic mucocutaneous sclerosis most commonly affecting the genital area, although extragenital localizations have also been described1 , with unknown pathogenesis.

Our study aims to analyze the morphopathological aspects of LS through the prism of polarized microscopy and with the help of histochemical information provided by special Van Gieson stain for elastic tissue and Picro-Sirius Red special stain.

Picro-Sirius Red staining allows observation of the type of collagen synthesized in pathological conditions such as LS, as well as the appearance of mature hyalinization through loss of elastic tissue, and also the disappearing of piloerectile muscles.

Keywords: Lichen sclerosus; collagen; polarized microscopy; Picro-Sirius Red

Introduction

Lichen sclerosus (LS) is a chronic mucocutaneous sclerosis most commonly affecting the genital area, although extragenital localizations have also been described (1) , with unknown pathogenesis. It mainly affects females with an F:M ratio of 6:1 to 10:1 (1). Apart from the unpleasant symptoms (itching), LS can cause important disturbances in the couple relationship, being a cause of dyspareunia (2), as well as a real aesthetic handicap. Also, not infrequently, it poses clinical problems of differential diagnosis with squamous cell carcinoma (3), as well as clinical management in order to prevent evolution to squamous cell carcinoma.

Clinically, LS is manifested by erythematous patches/plaques with edematous whitish centre, possibly with other additional signs such as erosions, spots, bleeding, superficial hyperkeratosis; may or may not be accompanied by clinical symptoms such as itching or dyspareunia

Histopathological - in early stages an intense band-like, lympho-histiocytic inflammatory infiltrate in the papillary dermis, similar to lichen planus, with vacuolar dystrophy of basal keratinocytes and apoptotic bodies. Subsequently, the inflammatory infiltrate is pushed down and replaced by superficial homogeneous sclerosis

and oedema. Typically the epidermis is atrophic, with hyperkeratosis and basal vacuolation, but rarely it may be hypertrophic. Superficial homogenous sclerosis consists of collagen types I and III and degenerated elastin (1).

Our study aims to analyze the morphopathological aspects of LS through the prism of polarized microscopy and histochemical information.

Material and Method

In this study 10 cases of lichen sclerosus were analyzed, biopsied in the Dermatology Clinic of St. Apostle Andrew Emergency Clinical County Hospital of Constanta, histopathologically processed in the Pathological Anatomy Service of the same hospital. From the total of 10 cases, 9 cases had vulvar localization in women (90%), and 1 case had posterior thorax localization in men (10%). The age of the patients ranged from 32 to 76 years.

Biopsy samples were immediately immersed in fixative (NBF 10%) according to the internal protocol. Sample processing was done automatically with the Diapath Donatella SN 19807 Histoprocessor; microscopic sections were made at 5 microns. Special staining was performed manually following the manufacturer's protocol.

For van Gieson staining for elastic tissue (VGTE) we used Merck kit 115974, with the following results: nuclei - black; elastic fibres - black; collagen - red; muscle fibres - yellow.

For Picro-Sirius Red (PSR) staining we used ab kit 150681, with the following results: collagen - red in conventional light microscopy, and collagen type I - red-orange; collagen type III yellow-green in polarized microscopy. We also noted the internal negativ control of epithelial structures, that do not polarize.

The eligibility criteria for inclusion in the study were clinical suspicion correlated with the presence of specific histopathological lesions, confirmed by special histochemical stains, namely VGTE and PSR.

The changes taken into analysis were: collagen lesions - modes of manifestation, elastic tissue lesions.

In the case of dermal collagen lesions, the following were noted: diffuse papillary dermal sclerosis, peri-anexial sclerosis, collagen „rings" and „balls", hyalinization.

In the case of elastic tissue lesions, the following were noted: absence, reduction, normal presence of elastic tissue in the papillary dermis.

The polarized microscopy study of the cases noted the type of birefringence present in the papillary, adventitial, reticular dermis, according to the 2 groupes of 4 colours (red-orange - corresponding to collagen type I, yellow-green - corresponding to collagen type III).

Results

Diffuse dense sclerosis of the upper dermis was observed in all cases of LS.

Focal hyalinization was considered possible in the usual staining in 5 cases, but in only 2 of these cases it was accompanied by the absence of collagen birefringence in the PSR staining, and in these 2 cases the elastic tissue was absent in the hyalinized area.

The dermal reaction in PSR staining in conventional light microscopy was diffuse red in all cases, dense red in the upper dermis and variable lax red in the lower dermis, the yellow reaction of the hairy erectile muscles not being visible.

The birefringence of dermal collagen in PSR staining was predominantly transdermal red-orange in 9 cases. In only one case we observed green-yellow birefringence in adventitial collagen.

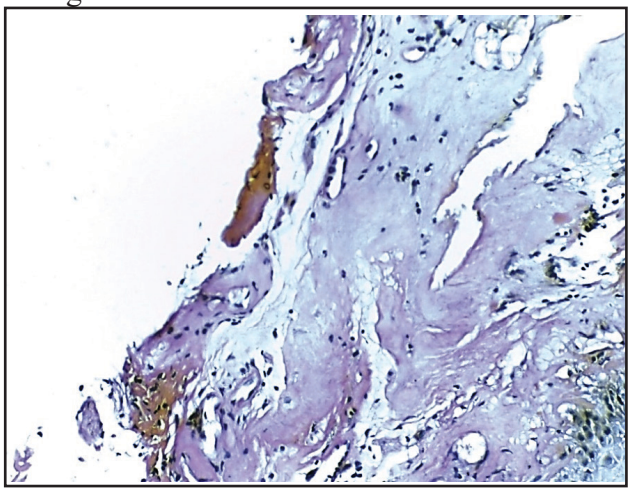

Fig. 1 - Lichen sclerosus; homogenized dermal collagen tissue in upper dermis, possible hyalinization ; HE, 200x

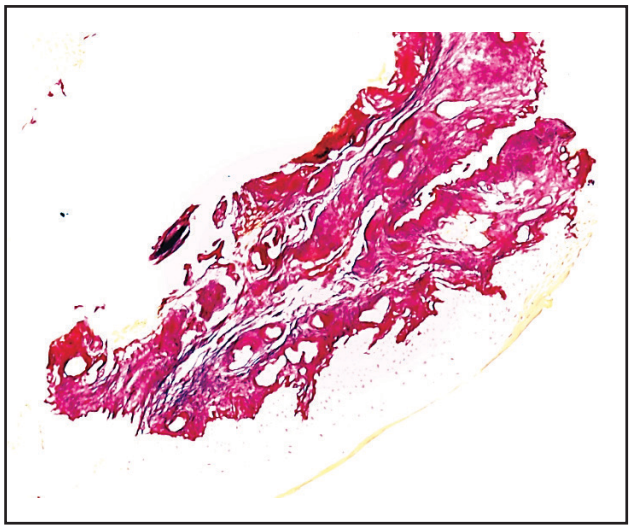

Fig. 2 - Lichen sclerosus; diffuse deep red reaction of dermal collagen; PSR, 100x

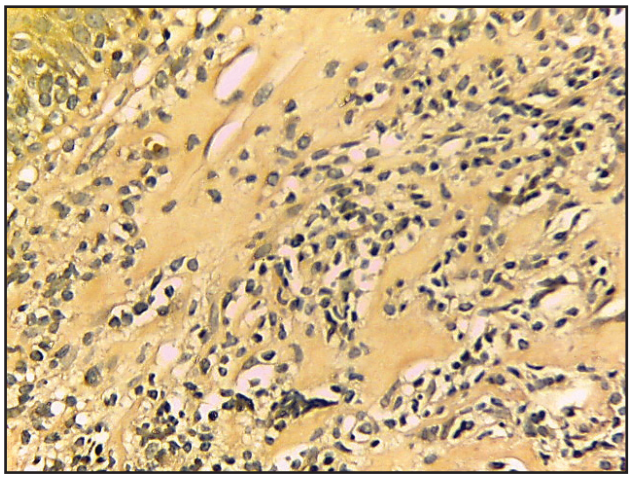

Fig. 3 - Lichen sclerosus; elastic tissue absent, dermal collagen homogenized; VGTE, 400x

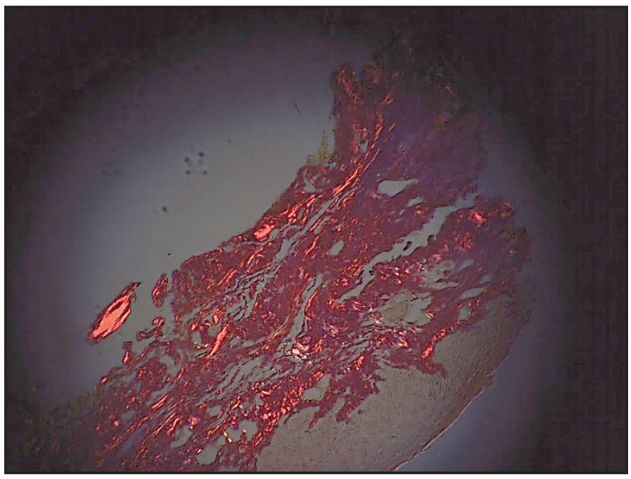

Fig. 4 - Lichen sclerosus; collagen birefringence is focal absent and confirms presence of hyalinization; PSR/MP, 100x

Discussions

Our study confirms the replacement of immature, thin type III collagen, normally present in papillary and adventitial dermis, with mature, thick type I collagen in LS as a functional deficiency of the dermal extracellular matrix.

The absence of visualization of the hairy erectile muscles is for the first time reported in this study, and we think it may be real, by destructive mechanism, or it may be just an optical effect, as the birefringence of the structures is based strictly on physical-chemical criteria (4,5)and four images of each section were digitized for an angle of the polarizer with the section varying from 0-67.5 degrees in steps of 22.5 degrees. The amounts of transmitted light for each setup of the polarizer were combined in order to extract the values of the azimuth angle (modulo 90 degrees . Complementary studies must be done on this issue.

The presence of adventitial type III collagen in only one of the cases analyzed is thought to correlate with the younger age of the lesion.

Hyalinization of dermal collagen correlates with the absence of elastic tissue and is objectified by the absence of birefringence of the hyalinized area in PSR staining.

To the best of our knowledge, this study is the first to analyse lichen sclerosus under polarised microscopy on PSR-stained sections.

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