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A POLARIZED LIGHT MICROSCOPY STUDY IN A CASE OF MORPHEA

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ABSTRACT

We report a case of plaque type of scleroderma with specific clinical features and conventional histopathology, with sclerosis and hipocellularity of fibroblasts and preservation of elastic tissue. We describe polarized light microscopy findings, on conventional stained slides and on picro sirius red stained slides. We appreciate that picro sirius red stain allows a better characterization of collagen fibres composition in papillary and reticular dermis, that is severely disturbed in morphea, with an inverse distribution of collagen fibres type I and III comparative with normal dermis.

Keywords: morphea, polarized microscopy, picro sirius red, collagen

Introduction

Morphea, or localized scleroderma, is a fibrosing disease with unknown ethiology, limited on skin, subcutaneous tissue and underlying bone. Clinically it can have more forms of expression and the plaque one is the most frequent (1) and can be generalized or unique. By histological point of view it can be very frustrating, because of the paucity of cells in the conventional methode. Because of the paucity of cells in some evolutive forms of morphea, immunohistochemistry is of no use, so we tried to find some other possibilities of study, i.e. polarized microscopy. We will describe the aspect of the lesion in conventional methode and in polarized microscopy, the last one applied on a conventional slide and after stainig with picro sirius red. We could find a lot of literature data about scleroderma, we found a few data about polarized microscopy in scleroderma on conventional stained slide but we did not find any data about polarized microscopy of picro sirius red stained slides of scleroderma, so we think this is the first description of this type.

Matherial and methods

We recieved an incisional biopsy from the buttock of a man, 63 years old, with clinic

diagnosis of localized scleroderma plaque type. The biopsy was placed in neutral buffer formaline 10%, with minute cold ischemic time, as the protocol of our department and collaborators specifys. The specimen was paraffine embeded, then processsed with automate methods (Diapath line), then sectioned at 5 microns. Microscopical slides were obtained, that were automate stained with Haematoxyline-Eosine, Van Gieson for elastic tisuue (Merck kit) and manually stained with Picro Sirius Red stain kit ab150681.

The slides were examined with microscope Leica DM750, attached to capture camera Leica ICC50HD. The microscope was equiped with Leica polarized light kit for liniar polarization microscopy. The images were then processed with LAS V4.6 soft for Leica cameras.

The methode of stainings was provided by Diapath for Haematoxyline-eosine and Van Gieson for elastic tissue and by its provider for picro sirius red.

Picro sirius red kit protocol of staining summary:

- deparaffinizing sections and hydrate in distilled water
- covering sections in picro-sirius red solution and incubating for 60 min
- washing slide with acetic acid solution
- washing slide with absolute alcohol
- dehydrating, clearing and mounting slide.

The results of PSR kit stain, provided by the manufacturer are: for ligh microscopy collagen fibres red colour, all the other structures yellow, for polarized microscopy type I collagen fibres yellow-orrange birefringence, for type III collagen fibres green birefringence.

Results

Conventional slide, examined in light microscopy, revealed a skin biopsy with an atrophic orthokeratotic epidermis, with hipogranulosis, a band of pink lax homogenuous collagen in superficial dermis, a reticular medium and profound dermis with sclerosis, with broad and red collagen bundles, and with no vizualization of hypodermis, the paucity of hair folicules, the absence of sweat glands and a very mild limpho-monocitary perivascular infiltrate (figure 1). VGET stain showed preservation of elastic fibres in dermis (figure 3). Picro Sirius stained slide showed a lot of red collagen in all dermis (figure 5).

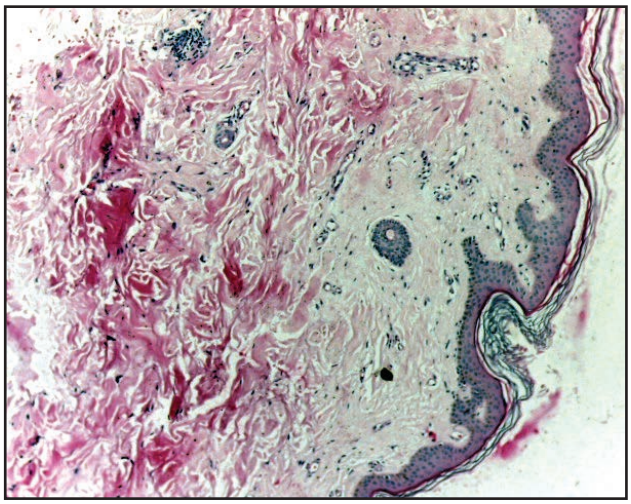

Figure 1 HE, 10X

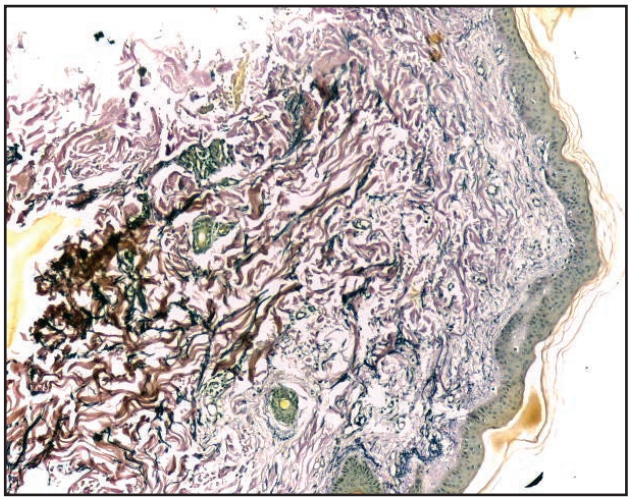

Figure 3 VGET, PM, 10x

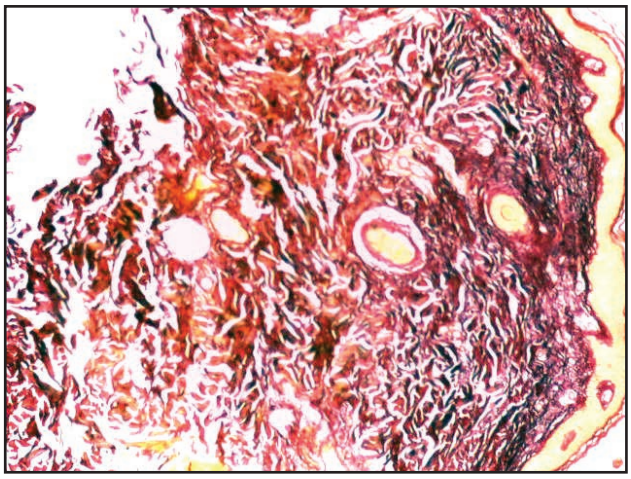

Figure 5 PSR, 10x

Polarized light examination of HE and VGET slides showed intense birefringence of collagen in medium and profound dermis, in the area of sclerosis, with broad collagen bundles and variable reduced birefringence in the area of homogenuous pink collagen from superior dermis (figure 2, 4). The birefringence was a litle bit enhanced in VGET (figure 4) than in HE stain (figure 2).

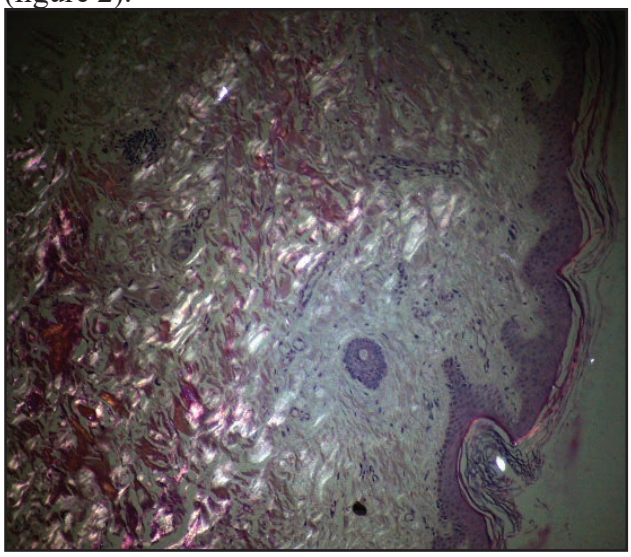

Figure 2 PM, HE, 10x

Polarized light examination for PSR stain revealed the yellow-orange birefringence of collagen fibres in the superior dermis, in the area corresponding to lax pink homogenuous collagen, and a mix of green and yellow-orange birefringence of the sclerotic area form medium and profound dermis. We noticed the absence of birefringence in epidermis and all the epithelial structures, as internal negative control, and the green weak birefringence of hair shafts as internal positive control (figure 6).

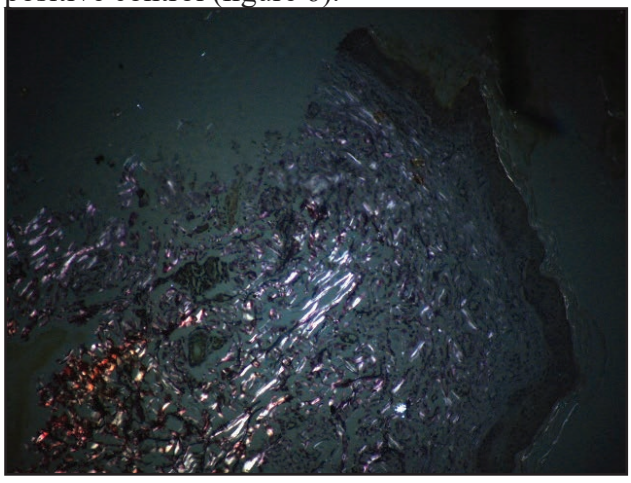

Figure 4 PM, VGET, PM, 10x

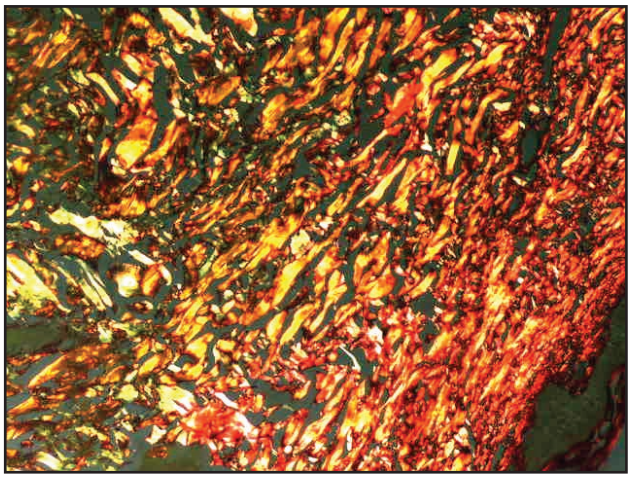

Figure 6 PM, PSR, 10x

Abreviations: HE Haematoxyline-eosine, VGET van gieson for elastic tissue, PSR picro sirius red, PM polarized microscopy

Discussions

Morphea or localized scleroderma is a fibrosing disease with unknown etiology, limitated to skin, subcutaneous tissue and underlying bone. Unlike the sistemic scleroderma, it does not associate sistemic disease, sclerodactilia, Raynaud fenomenon and capillaries changes of nail folds (1). The plaque type is by far most frequent, i.e. 2/3 from the cases (1). It affects especially women. It appears as erythematous oval variable circumscribed patches or plaques, that become sclerotics, no hair bearing, anhydrotic, that migrate centrifugal and become white and cicatricial (2). The skin becomes hard and as thick as the sclerosis depth is. In active lesions there is a patognomonic lilac ring at the perifery of the lesions. The old lesions become tan.

Histopathology of morphea is poor, and its description as „the red desert of the dermis" fits very well with the pattern of sclerosis with a reduced number of fibroblasts. Sclerosis compresses and destroys skin anexes and extends in hypodermis in a pseudopodal manner (2). The adipose tissue distruction is clinically expressed by skin depressing (3). A variable lymphocitic inflamatory infiltrate maybe found in the dermis, superficial and profound, with rare plasmocytes and even more rare eosinofils (4). Hypodermal

vessels have thick walls and small lumens. In the mature faze of the disease the cellular density is very small and the preservation of elastic tissue is an important clue for differentiation from lichen sclerosus. Radiodermitis is another important differential, but the history of previous radiation, the absence of plasmocytes and the presence of „rings" of collagen are the clues for differentiation (5).

Polarized microscopy, used especially for diagnosis of non-scarring alopecia, because it was noticed that the birefringence of collagen is absent in the remainings fibrous tract after hair follicule distruction (6), Collagen birefringence depends on collagen fibres features, as density, orientation, type of deposit (7). Amira Elbendary and Dirk M. Elston and all. reports in a study a diffuze and strong collagen birefringence of collagen in morphea, with enhancing in hyalinized areas, unlike lichen sclerosus, which, in their study showed absence or reducing of birefringence in superior dermis (8).

In our case of morphea, we noticed a variable collagen birefringence band-like in superior dermis, with preservation of elastic tissue, when examining HE and VGET stained slides, and a strong white and pink birefringence of the collagen from medium and profound dermis.

Collagen birefringence was stronger for VGET stained slides that for HE stained slides.

In the case of PSR stained slides we noticed an overall strong birefringence of the collagen, with predominance of type I collagen in the superior dermis, and a mixture of type I and III collagen in the mid and profound dermis, with predominance of type III collagen.

Conclusions

Collagen birefringence was stronger and wider in PSR stained sections than in VGET and HE, and was stronger in VGET than in HE stained slides.

PSR had let us notice for our case of morfea a modified distribution of collagen fibres comparative with normal proportions, ie a predominance of type I fibres in papillary dermis, and a predominance of type III collagen fibres in reticular dermis, instead of predominance of type III in papillary dermis and type I in reticular dermis, as in the normal dermis.

Acknowledgements

Nothing to declare

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