

ORIGINAL PAPER

Morphologic and histochemical changes in the skin of patients with scleroderma

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Abstract

Background. Systemic sclerosis or scleroderma is a rare collagen disease, characterized by insufficient angiogenesis. Few data are available about the morphologic and histochemical peculiarities of the skin in these patients with this condition. The purpose of the present work was to evaluate the histochemical aspects of sclerodermic skin, obtained through biopsy of the typical lesions from the forearm skin.

Patients and methods. The study was conducted on 31 patients, from which skin biopsies were obtained, after informed consent. The specimens were fixed in buffer formalin, embedded in paraffin and processed for staining with HE, Masson, orcein, Gordon-Sweet silver staining, and alcian blue-safranin, in order to identify elastic fibers, reticular fibers, glycosaminoglycans and mast cells. **Results** are partially similar to other studies: the constant depletion of elastic fibers in the papillary dermis and disorders of the network in the reticular dermis, such as their absence in the skin blood vessels walls. The reticular fibers were absent in the papillary dermis, the reaction in the reticular dermis structure was variable from a case to another. The staining with Alcian blue-Safranin proved that there is a gathering of glycosaminoglycans in the superficial papillary dermis, the heterogeneity of collagen fibers and the decrease of mast cells in the dermis.

Keywords: scleroderma, skin biopsy, elastic fibers, reticular fibers, mast cells.

Background

Scleroderma is a generalized connective tissue disorder with unknown cause [1]. It is characterized by cutaneous sclerosis attributable to increase in collagen synthesis, glycosaminoglycans and other connective tissue substances by dermal fibroblasts [2].

Most of the studies have focused on the excessive angiogenesis, which is present in some conditions such as malign tumors, diabetic retinopathy, endometriosis and others. Insufficient angiogenesis was few investigated, except for the cardiac ischemic disease. Although it is well known that scleroderma is characterized by an insufficient angiogenesis, the basic mechanisms are poorly understood, and the studies published until now have shown few data. Because of these reasons, we investigated the histochemical peculiarities of the sclerodermic skin that could provide new insights about deficient angiogenesis.

Patients and methods

Patients

The study involved 31 patients with macroscopically cutaneous lesions, characteristic for scleroderma. Because the cases are very rare (17–18 cases/million) the patients were selected from The Emergency Hospital Baia-Mare (Rheumatology Department), The Municipal Hospital Timisoara (Clinic of Dermatology), and the Municipal Hospital Arad

(Clinic of Dermatology). There were included only the patients who accepted a cutaneous biopsy.

The material was represented by cutaneous biopsies from the level of typical lesions (forearm). The biopsies were drawn under local anesthesia, having small dimensions of 1.5×1×1 cm; they were washed with buffer saline and immediately put in the fixing solution (buffer formalin).

Methods

After the primary processing of specimens, we applied conventional staining methods with Hematoxylin-Eosin and Masson's trichrome, and histochemical methods in order to identify elastic fibers, reticular fibers and glycosaminoglycans.

The elastic fibers, a constant component of the dermis, were identified with the orcein staining, Unna-Taenzer variant, a simple reproducible method, which assures the contrast between the elastic fibers and other types of connective fibers. The final product of reaction was stained in deep brown, with a light reddish shade, restricted only to the level of the elastic fibers, on the pale background of the section. The reticular fibers were identified with silver staining Gordon-Sweet method.

Glycosaminoglycans were identified with the blue Alcian blue-Safranin method at pH 1.42. Using the method one can identify only the high forms of sulfated glycosaminoglycans, those that could be involved in the angiogenesis process.

This part of the study had two objectives: to show the matrix component and to identify the type and number of dermic mast cells. The method has shown in the skin two types of cells: alcianophilic mast cells (stained in blue) that contain chondroitin sulfates and safraninophilic (redbrick red), which predominantly contain heparin.

Results

In the scleroderma cases, we have noticed a marked depletion of elastic fibers on large areas from the papillary dermis, with or without the persistence of the normal network in the reticular dermis (Figure 1a). In more than half of the cases, we noticed the massive disorganization and fragmented elastic fibers in the reticular dermis, with an important reduction in their number (Figure 1b). In a single case, we have seen a particular aspect, characterized by the absence of elastic fibers from the entire papillary dermis, and in the reticular dermis, and the fibers were arranged into two distinctive layers (Figure 1c). The small and medium sized vessels from the dermis did not have elastic fibers in the structure of the wall.

In four cases, we have noticed a depletion of elastic fibers on large areas in the papillary dermis and in that reticular one (Figure 2a). In two cases the elastic fibers were absent from those two components of the dermis (Figure 2b), excepting for the adjacent regions of the hair follicle, where they were concentrated as a continuous layer (Figure 2c). The complete (Figure 3a) or partial fragmentation (Figure 3b) was noticed in 25% of the cases.

In cases with scleroderma we have observed aspects that significantly differ from the normal skin, concerning the presence and distribution of the reticular fibers. In all cases, we were not able to identify the basal membrane and we noticed the absence of reticular fibers in the papillary dermis (Figure 4a). In addition, the basal membrane of the small vessels from the papillary dermis was absent (Figure 4b). In comparison with this, the blood vessels from the deep part of the dermis had many reticular fibers in the media and adventitia layer (Figure 4c).

At the level of dermis, the number of the reticular fibers varied from one case to another. In some cases, we noticed fibers with a similar architecture as the normal skin (Figure 5a), in other cases, they were numerically reduced, short, without forming networks (Figure 5b); in the third group, they were absent. In this last condition, the absence of the reticular fibers was associated with two models of distribution for the collagen fibers: spaced, with ground substance well-represented (Figure 5c) and conglomerate, forming an amorphous, homogeneous substance (Figure 5d). Thanks to the results obtained until now, we can presume that the grade of depletion in reticular fibers depends of the severity and age of the lesions.

In only one case, we have noticed the complete collagen transformation of the papillary and reticular, superficial dermis, the entire area being occupied by a smooth, fibrillar and granular material, with tinctorial

composition similar to the collagen type I. In this area, we did not notice the presence of the reticular fibers (Figure 6).

In scleroderma cases, using alcian blue–safranin, we have constantly noticed pronounced reaction for Safranin in the areas with transformed horn-like cells, with hyperkeratosis of the epidermis (Figure 7a). In most of the cases, in the superficial papillary dermis, next to the basal layer of the epidermis, we have seen a massive accumulation of glycosaminoglycans, intensely positive with Alcian blue and amorphous structure (Figure 7b).

In the reticular dermis, the intensity of the reaction at the level of the collagen fibers was variable. In some cases, the final reaction product was heterogeneously distributed, only some collagen fibers being moderately stained (Figure 8a). On occasion, we have noticed, intense Alcian blue reaction, in the cases where we had seen depletion of reticular fibers (Figure 8b). In two cases, we have noticed the existence of some areas with variable extension, with degeneration through fragmentation and homogenize of the collagen fibers (Figure 8c). The more severe the degenerative lesion was, the more intense the alcianofilia was found.

We remarked just a small number of mast cells, in the papillary dermis, as well as in the reticular. In the control, the density of the mast cells in the papillary dermis was of 0.82/0.72 mm², in the cases with scleroderma the density was less than 0.2 on the same measured unit. We mention the absence especially of the alcian blue positive mast cells from the papillary dermis. There are only rare mast cells in the reticular deep dermis, in the loose connective tissue around the sweat glands (Figure 8d).

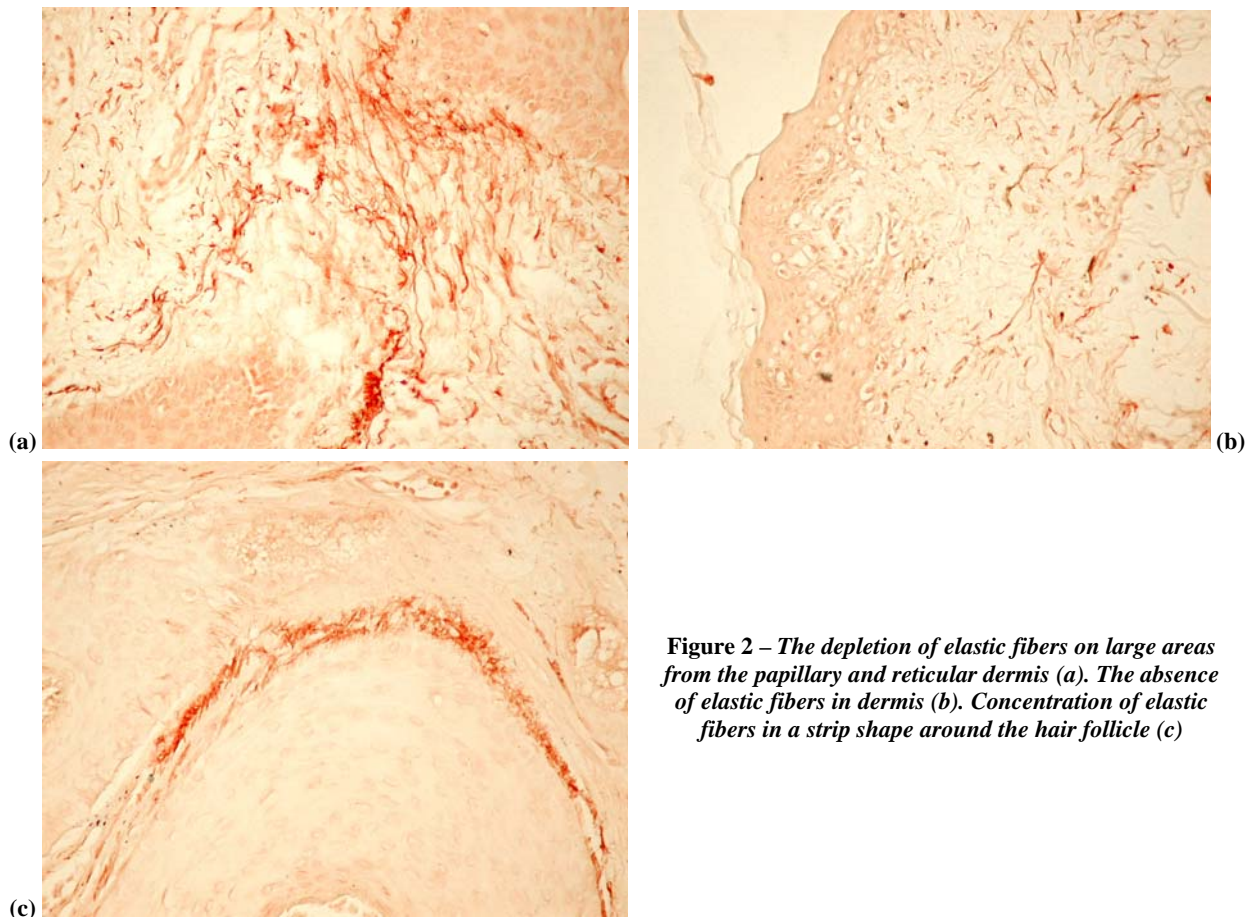
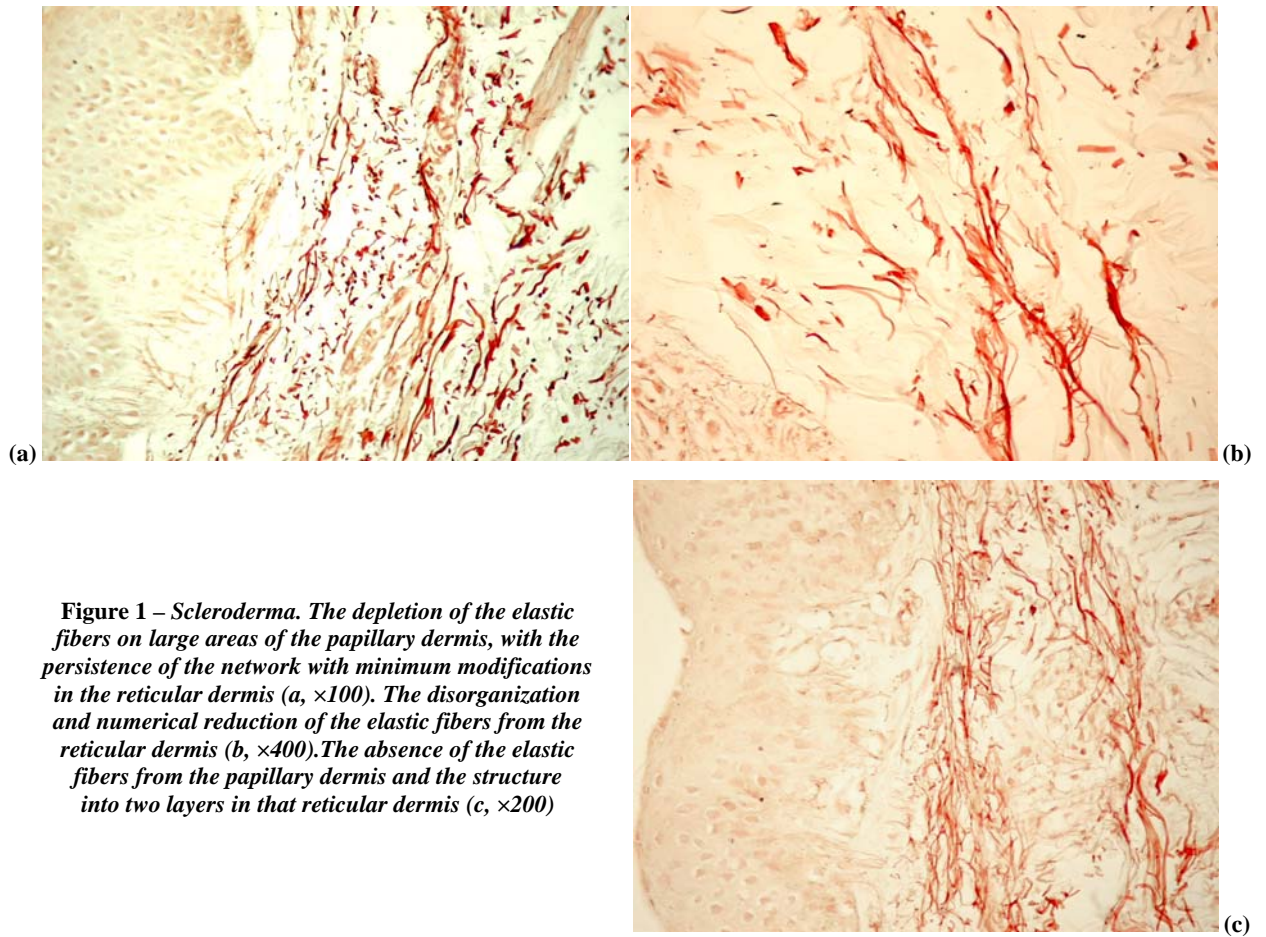
Discussions

The choice for the histochemical methods needed to identify the elastic, reticular fibers and the glycosaminoglycans were generated by the direct implications that these elements had in normal and pathological angiogenesis. Therefore, the elastic and reticular fibers are normal components of the vascular wall, and are essential in order to maintain the local dermic homeostasis. On the other hand, the glycosaminoglycans are involved in the aggregation of the collagen fibers and are components of the mast cells' granules.

Mast cells produce a variety of cytokines and hematopoietic growth factors that may have paracrine and autocrine functions, which are important in the development of the inflammatory cell. Increased numbers of mast cells are also found in many fibrotic conditions, including scleroderma [3].

Mast cells participate in the process of inflammation, angiogenesis, matrix degradation and tissue remodeling [4].

Mast cells in scleroderma have been discussed for past decades without any definite conclusion. It was shown that in scleroderma skin, in the edematous stage, in both papillary and reticular dermis, mast cells density was increased as compared with normal skin [5, 6].



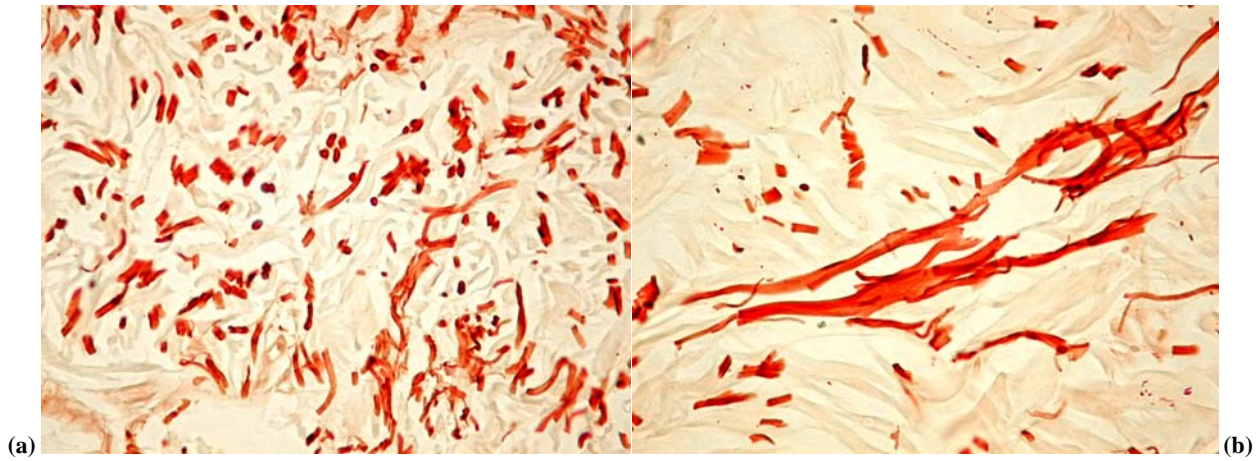


Figure 3 – The complete (a) or partial fragmentation (b) of the elastic fibers in the dermis

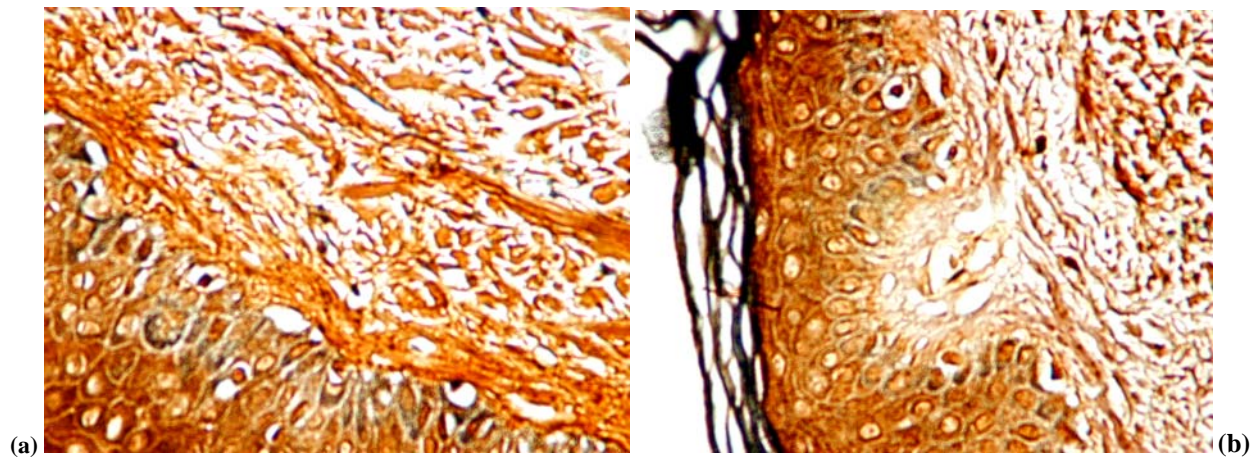


Figure 4 (above and left) – The absence of the basal membrane of the layered epithelium and of the reticular fibers from the papillary dermis (a). The absence of the basal membrane of the endothelium in the small vessels of the papillary dermis (b). Blood vessel from the dermis with many reticular fibers (c). Silver staining Gordon–Sweet, ×400

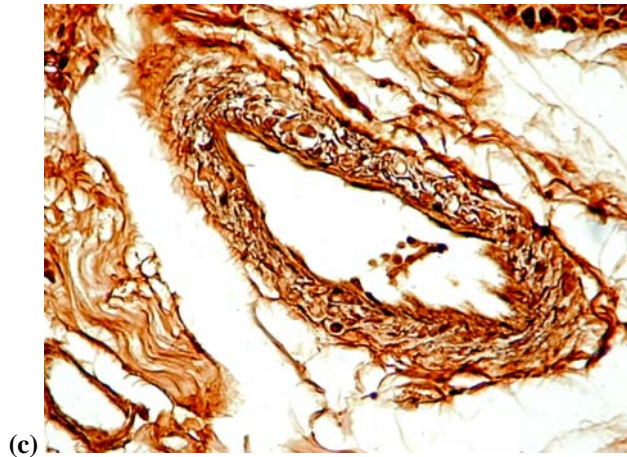
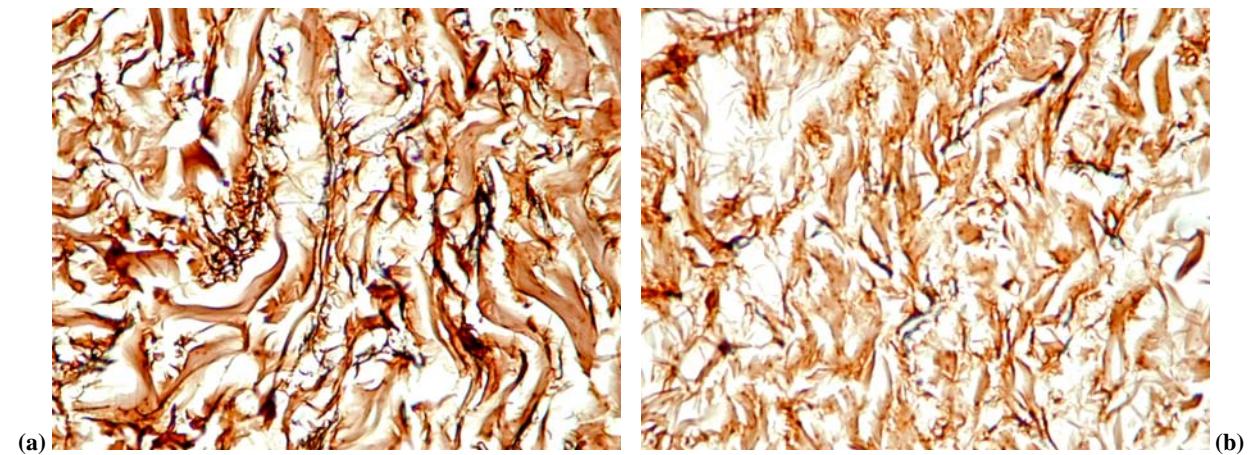


Figure 5 (below) – Reticular dermis, scleroderma. Reticular fibers, morphological and normal distributed (a). Small number of reticular fibers (b). Silver staining Gordon–Sweet, ×400



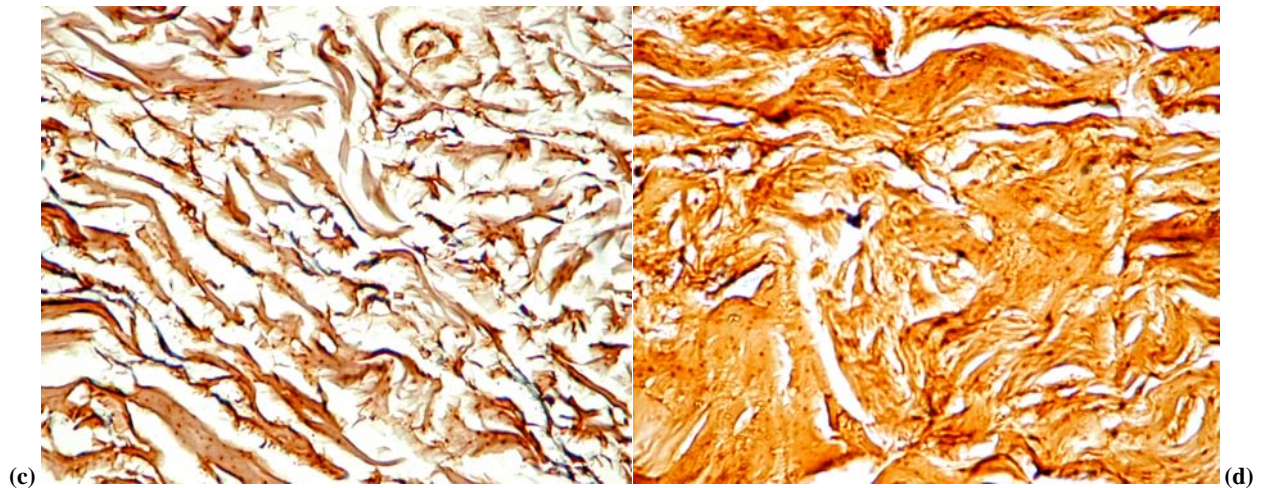


Figure 5 – Reticular dermis, scleroderma. Absent reticular fibers with spaced collagens (c). Conglomerate of collagen fibers, without reticular fibers (d). Silver staining Gordon–Sweet, $\times 400$

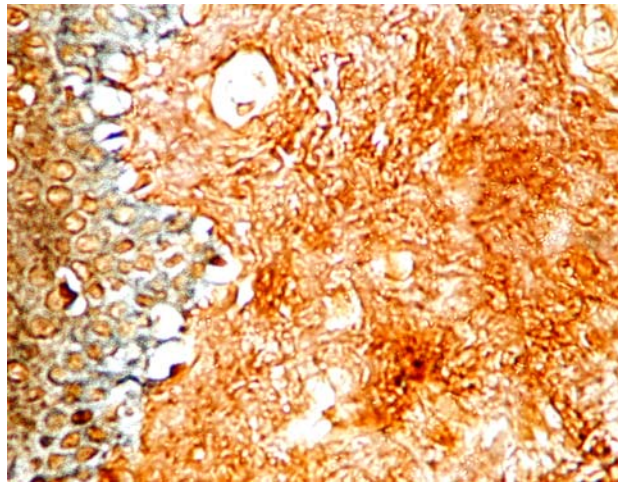


Figure 6 – Amorphous mass of collagen fibers (yellow-brown), without reticular fibers that occupied the papillary and reticular superficial dermis. Silver staining Gordon–Sweet, $\times 400$

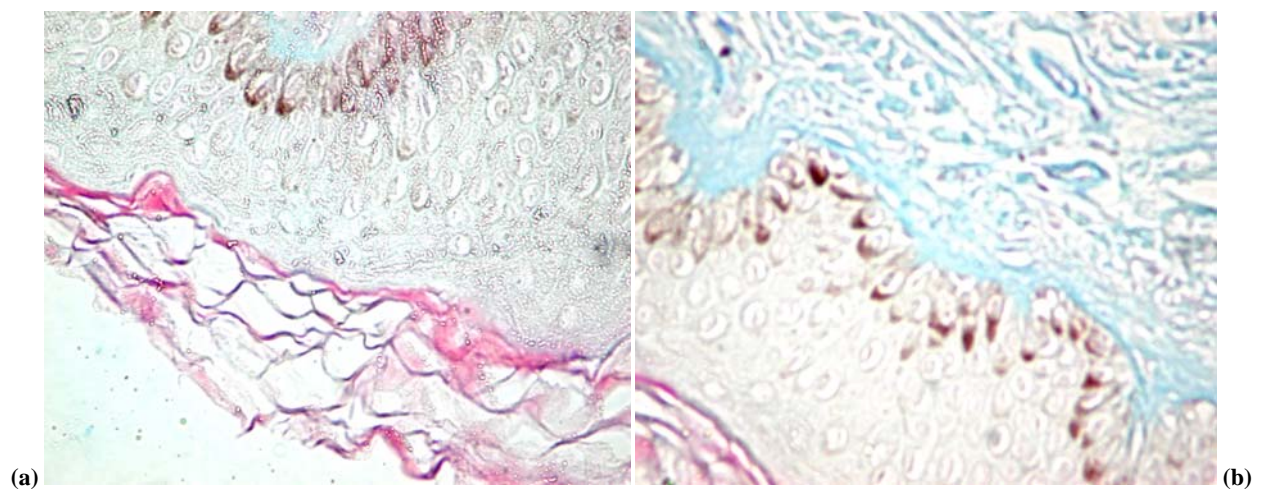


Figure 7 – Scleroderma. Pronounced safraninophilia in the hyperkeratinised areas. (a) Accumulation of alcianophile material in the papillary dermis, at the interface epidermis–dermis. (b) Alcian blue–Safranin, pH 1.42, $\times 400$

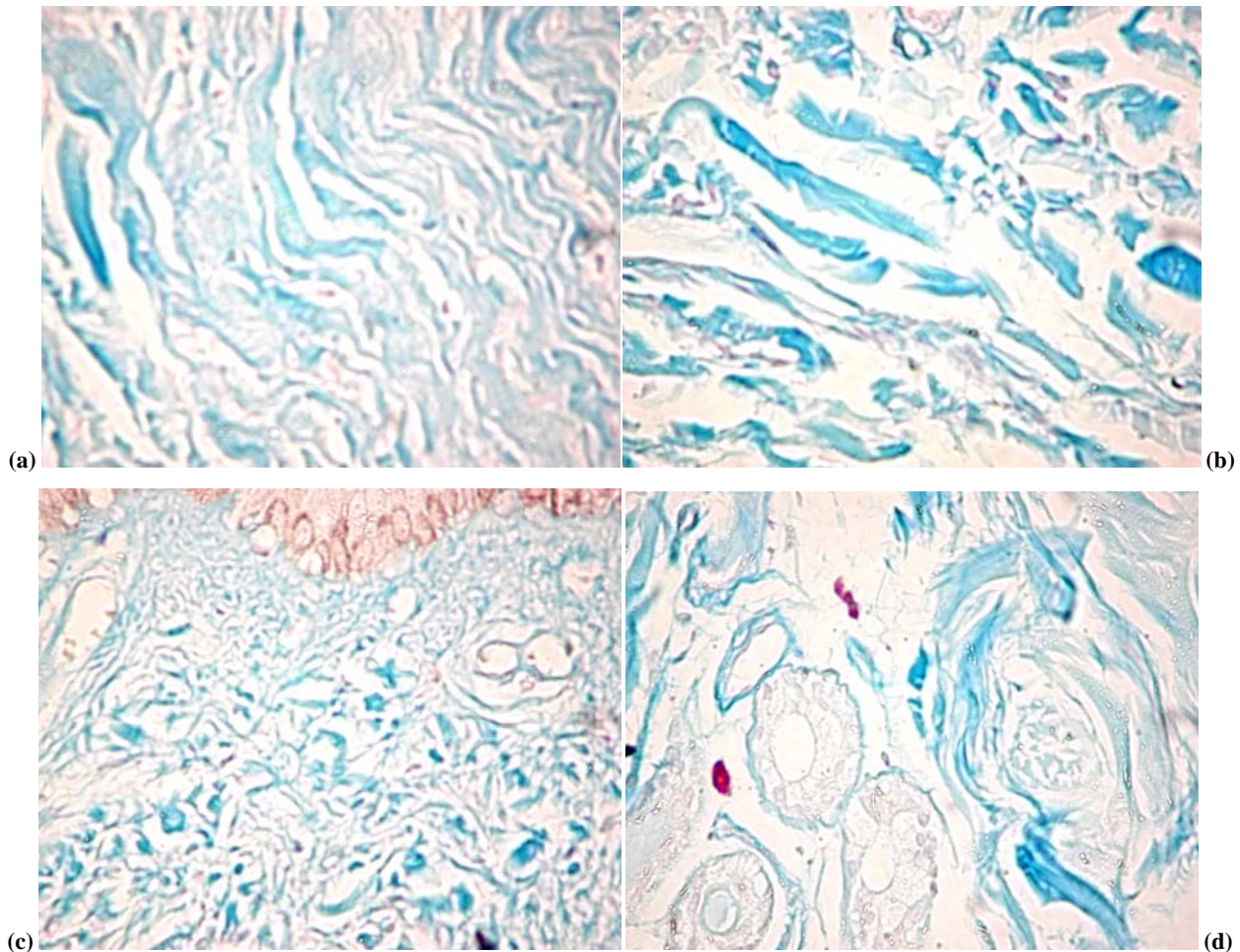


Figure 8 – Scleroderma. The thick collagen fibers from the reticular dermis, with heterogenic affinity for the blue alcian (a). Intense alcianophilia in the collagen fibers (b). Fragmentation and degeneration of the collagen fibers (c). Safranophilic mast cells in the reticular deep dermis next to the sweat glands (d). Alcian Blue–Safranin, pH 1.42, $\times 400$

However, in the sclerotic stage, characterized by homogenization of collagen bundles, mast cells density was significantly decreased [7, 8].

Cutaneous mast cells density is variable among persons and is known to decrease with age. Mast cells have direct effects on fibroblast physiology. In the studied cases, we have noticed the decrease of the mast cells in the papillary dermis as well as in the reticular, in concordance with the data from the literature referring to the advanced stages of scleroderma. The contribution of mast cells and their precise functional role remain to be further investigated in scleroderma.

We have not found in the any published studies aspects referring to the presence and distribution of the reticular fibers in the cutaneous biopsies of the patients with scleroderma. We can presume that the level of depletion in the reticular fibers depends on the severity and age of the lesions. The skin of the patients with scleroderma is characterized by an excessive accumulation of collagen in the extracellular matrix of the fibrotic reticular dermis.

Elastic fibers are disrupted in this disease; however, relatively few studies have shown evidences concerning the changes that occur in elastic fiber in scleroderma. Our results are in accord with those of some studies

that show the disintegration of the elastic fibers in the sclerodermic skin and suggest the possibility that the degradation products from the elastic matrix to act as a signal of fee back for the increase of matrix production [9].

The tight skin (TSK-1) mouse has been proposed as a model for systemic sclerosis based on increased accumulation of collagen and glycosaminoglycans in the skin and by the presence of serum autoantibodies [10]. TSK-1 mice had a highly significant increase in the percentage of elastic fibers (19.6%) In the dermis, as compared to control mice (7.9%) ($p < 0.001$).

☐ Conclusions

The histochemical study, applying the orcein staining, silver staining and alcian blue–safranin methods on biopsies taken from the patients with scleroderma has revealed the following particular aspects.

By orcein staining, we have seen the depletion of elastic fibers from the papillary dermis and frequently the disorganization and fragmentation of the network from the reticular dermis. The elastic fibers were absent from the wall of the blood vessels in the dermis.

The silver staining has shown the constant absence of the reticular fibers from the papillary dermis, including the specific components of the basal membrane (of the covering epithelium and endothelium); in the papillary dermis the reaction varied, there were some cases with normal network with depletion of reticular fibers.

The absence of the vascular basal membranes suggests either the existence of a degenerative process or the persistence of the lymphatic vessels.

We noticed accumulation of glycosaminoglicans in the papillary superficial dermis, heterogeneity and degeneration through division of the collagen fibers and a small number of mast cell in the two components of the dermis.

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