ORIGINAL ARTICLE

PARTICULARITIES OF S100 EXPRESSION IN PATIENTS WITH VENOUS PATHOLOGY

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ABSTRACT

Purpose: The aim of this study was to evaluate S100 expression in patients with associated traumatic and metabolic venous disorders. **Material and methods:** The present study included a number of 17 cases of venous fragments collected from patients admitted to the Republican Clinical Hospital, Chisinau, between 2017 and 2019. The morphological and immunohistochemical stainings were performed. **Results:** In patients with associated diabetes mellitus, a small number of S100 positive nerve structures were found in the adventitia- values of 3.33 and 4.33. Some particular aspects were noted to these patient: the existence of isolated, S100 positive cells, with dendritic morphology, in the sub-endothelial layer and outer media, around the zones with inflammatory infiltrate. For the cases without associated metabolic pathology, the predominance of the S100 positive structures was found in the adventitia, with the mean values varying between 4 and 13.33. In the areas with endothelial discontinuities the presence of cells with dendritic morphology and S100 positive nervous fillets in the sub-endothelial layer and a concentration of smooth muscle cells around S100 positive structures were found. **Conclusions:** The present study showed some particularities of S100 expression in patients with associated metabolic disease and in the cases which presented the endothelial discontinuities.
Key words: S100, venous pathology, diabetes mellitus

INTRODUCTION

The S100 protein family name comes from their 100% solubility in saturated ammonium sulfate solution at neutral pH. This protein family was first identified in 1965 by B.W. Moore (1).

Under normal conditions, S100 immunoexpression was noticed in the neuronal and neuroectodermal components of different tissues. Pituitary secretory cells, white adipocytes, Langerhans cells of the skin, express at the cytoplasmic level the S100 protein. In pathological conditions, S100 immunoexpression was noted in melanomas, schwannomas, squamous and basal cell carcinomas, mammary neoplasms, meningiomas and astrocytomas.

In the case of normal veins, S100 expression was found in the peripheral nerves of the adventitia and no S100 positive dendritic cells were reported.

In the case of venous pathology, the presence of cells with dendritic morphology, S100 positive, as a minority cell population, in the intima and in the media of the varicose and thrombophlebitis veins was demonstrated. The localization of these cells with dendritic morphology, S100 positive, was predominant between smooth muscle cells and around neovascularization areas where they co-existed with T lymphocytes. The authors (2) assumed the involvement of dendritic cells in venous inflammatory mechanisms by their interaction with T lymphocytes.

It was demonstrated that mature and immature dendritic cells express S100 (3). Vital et al., demonstrated that in patients with chronic venous disease, S100 positive nerve fibers and CD45 positive cells were present, the density of which was variable from one fiber to another. Non-myelinated C fibers were predominantly located on the outer part of the media, with a small extension in the inner part of adventitia. Isolated inflammatory cells were noticed in the media. The authors demonstrated the existence of non-myelinated C and inflammatory cells in the varicose vein wall. They concluded that non-myelinated C-fibers are present and that further studies are needed to establish their favorable role (4).

Based on these considerations, the aim of this study was to evaluate S100 expression in patients with associated traumatic and metabolic venous disorders.

MATERIAL AND METHODS

The present study included a number of 17 cases of venous fragments collected from patients admitted to the Republican Clinical Hospital, Chisinau, between 2017 and 2019. 8 cases were taken by saphenectomy. 9 cases originated from patients whose lower limbs were amputated. Patient informed consent and the approval of the Research Ethics Committee of USMF N. Testemitanu, Chisinau were obtained. The histopathological diagnosis was established on morphological staining slides. Immunohistochemical technique was performed with the Leica Bond-Max Autostainer (Leica Biosystems, Newcastle Upon Tyne, UK). The first step of immunohistochemical technique involved the use of Bond Enzyme 1, Leica Biosystem Newcastle Ltd, Newcastle Upon Tyne, UK for 10 minutes. 3% hydrogen peroxide applied for 5 minutes was used to block endogenous peroxidase activity. This steps were followed by incubation with the primary antibody S100 (polyclonal, ready to use, Leica Biosystem Newcastle Ltd, Newcastle Upon Tyne, UK) for 30

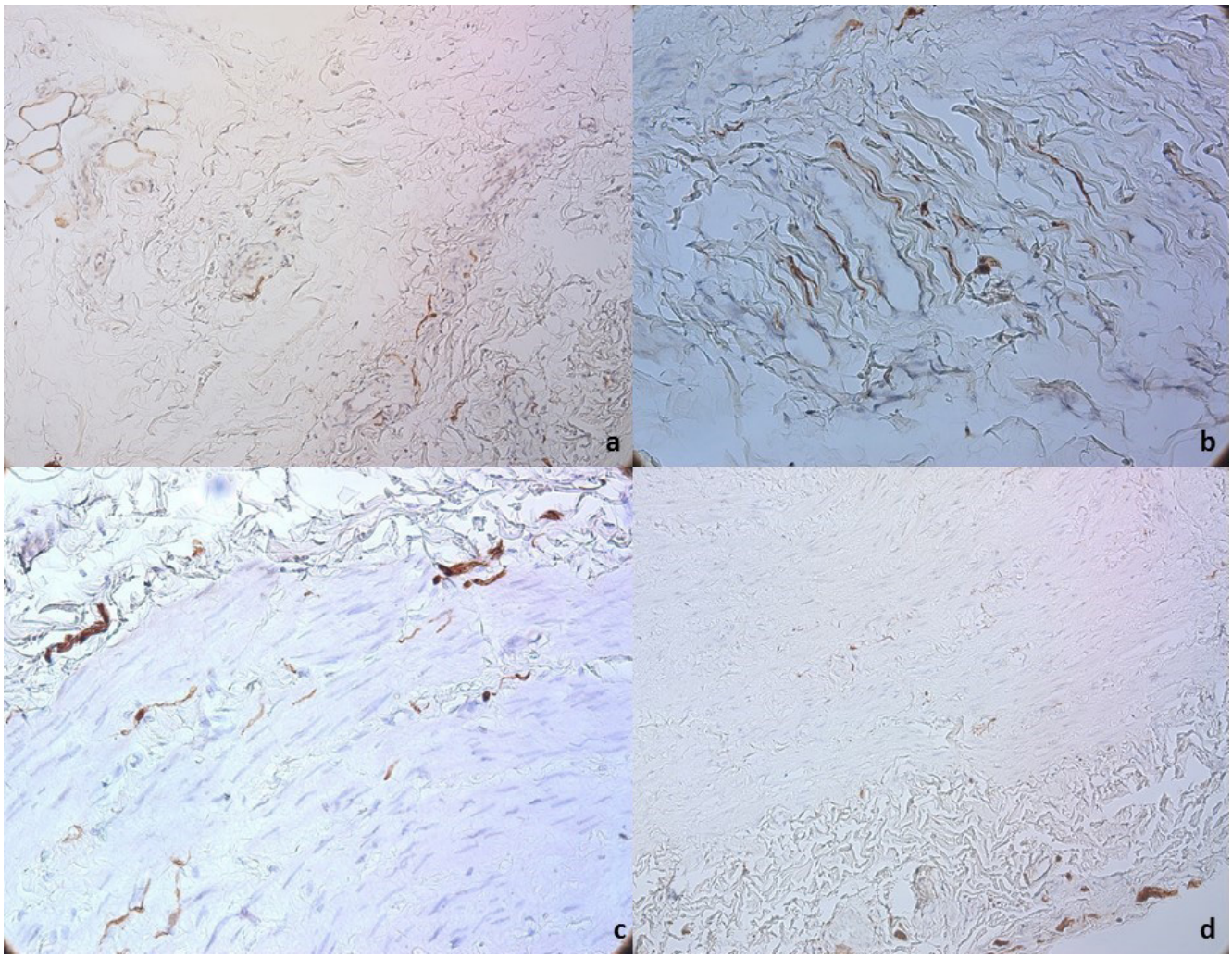

Figure 1.
a)Immunoexpression of S100 in the white adipocytes, ob.X20
b) Nervous fibers with perpendicularly trajectory in the outer media, S100 immunostaining, ob.X20
c) S100 immunoexpression in the adventitia and media of the veins, in the patients without metabolic associate disease, ob.X40
d) Numerous S100 positive nervous fillets in the adventitia and in a reduced number in the outer media of the vein, S100 immunostaining, ob.X10

minutes. Visualization was made with the Bond Polymer Refine Detection System. 3.3 diaminobenzidine (for 10 minutes) was used as chromogen, while for counter-staining hematoxylin was applied for 5 minutes.

RESULTS

Internal positive control of the reaction was assessed at the cellular level, where the cytoplasmic expression of S100 in the white adipocytes and Schwann cells was noticed (Figure 1a).

The following mean values of S100 positive structures in the adventitia were obtained: 13.33, 9.33, 9, 7.33, 6.66, 4.66, 4, and 4.33 and 3.33 respectively (in the associated diabetes cases).

In one case, the nerves with a perpendicular trajectory, in the outer layer of the media were noticed, through the smooth muscle cells, at the changing direction of smooth muscle cells beams. (Figure 1b).

The evaluation of the case without associated metabolic disorders indicated the presence of S100 positive nerve structures predominantly in the outer part of the media and in the connective areas which separating the smooth muscle cells fascicles of the media (Figure 1c).

Four of the evaluated cases were characterized by the presence of nervous fillets, S100 positive, thick,

in the adventitia, and a lower presence of these in the outer media (Figure 1d). In two of these cases, the morphological stains indicated a thinner adventitia than the media.

In patients with associated diabetes mellitus, a small number of S100 positive nerve structures were found in the adventitia- values of 3.33 and 4.33, respectively, compared to other cases (Figure 2a). The same S100 positive nervous elements were also noticed in the outer

media.

In one of these cases it was found the existence of isolated, S100 positive cells, with dendritic morphology, in the sub-endothelial layer and outer media, around the zones with inflammatory infiltrate (Figure 2b). The other case presented S100 positive elements in the outer media but no cells with dendritic morphology at the sub-endothelial level and in outer media were identified.

A particular aspect was noted in the areas with endothelial discontinuities. The presence of cells with dendritic morphology and S100 positive nervous fillets in the sub-endothelial layer (Figure 2c) was noticed in these. A concentration of smooth muscle cells around S100 positive structures was found at this level (Figure 2d).

DISCUSSIONS

The evaluation of corneal dendritic cell number in the murine experimental model indicated an increased number in the case of diabetic patients compared to the control group (5). Cherian et al. (6) analyzed stenotic saphenous vein grafts used in normal bypass and normal saphenous veins and noted the absence of dendritic morphology cells, S100 positive in the intima and media of venous wall, for the last one. Cells with dendritic morphology were present in stenotic grafts, in the intima and media.

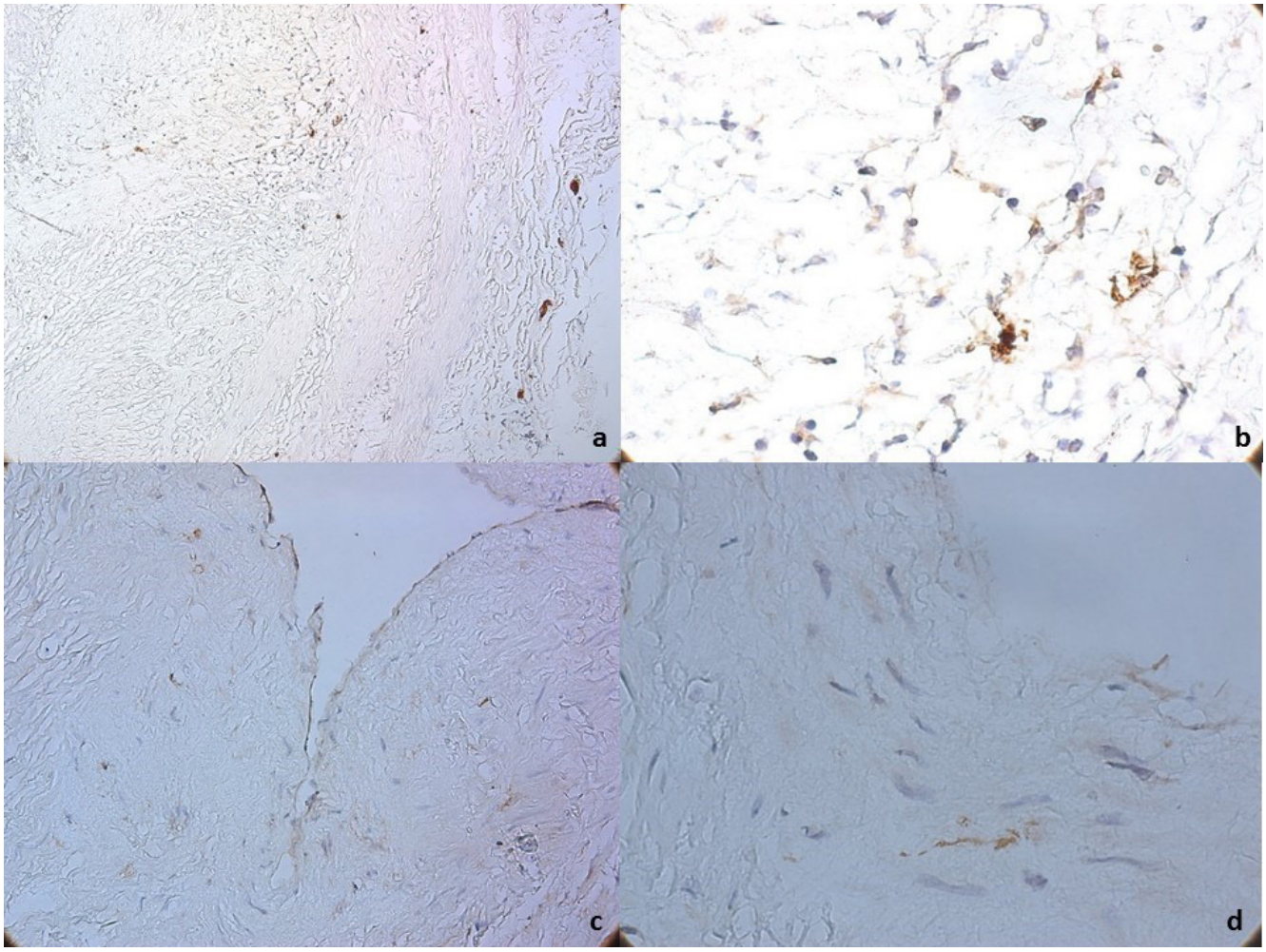

Figure 2

a). S100 positive structures in the vein adventitia of the patients with associated diabetes mellitus, S100 immunostaining, ob.X10

b) The S100 positive cells, with dendritic morphology in the vein sub-endothelial layer of patients with diabetes, S100 immunostaining, ob.X40

c,d.) S100 immunoexpresion in the vein subendotelial layer, ob. X40 (c), X100 (d)

In our study, one of the patients with associated diabetes showed an increase in the number of cells with dendritic morphology, S100 positive in the media and sub-endothelial space of the venous wall.

Using venous fragments from the sapheno-femoral junction, varicose veins from the same junction and recurrent veins in the vicinity of the junction belonging to the deep thigh venous system, Rewerk et al. (7) noticed numerous S100 positive nerve fibers in the adventitia of all evaluated cases. In our study, we found the S100 expression in the nerve fibers of the adventitia and the outer media with variable values. A decrease in the number of S100 positive nerve fibers in media and adventitia was showed in both cases with associated diabetes mellitus.

Several integrin subunits have been identified on the surface of the smooth muscle cells in the wall of the saphenous veins, such as: beta1, alpha2, alpha5 and alpha v beta3. It has been shown that integrins are required for the PDGF and extracellular matrix proteins induced smooth muscle cells migration. Agonists intended for inhibition of integrin functions may be used to reduce smooth muscle cell migration and suppression of intimal hyperplasia (8).

The impaired endothelium exhibits a decrease in vascular relaxation, impaired as a result of the reduction of endothelial nitric oxide synthesis and NO production (9).

It was shown that smooth muscle cells, in venous grafts, expressed different growth factors such as PDGF, TGF-β, vascular endothelial growth factor, endothelin 1 that play an essential role in intimal hyperplasia (10). PDGF-B is a mitogenic and survival factor for Schwann cells and has trophic activity on neurons (11).

The growth of blood vessels has been shown to be regulated by signals of nervous origin. Desert hedgehog (Dhh), one of the family members, is expressed by peripheral nerve Schwann cells. It has been demonstrated in a murine model that Dhh did not promote angiogenesis by direct activation of endothelial cells but promoted peripheral nerve survival in the ischemic muscle, maintaining the level of pro-angiogenic factors derived from the nerves. Foot denervation, immediately after ischemia, severely affected angiogenesis induced by ischemia, with decreased VEGF-A expression, angiopoietin 1 in ischemic muscle (12). In the present study, the presence of cells with dendritic morphology and S100-positive nerves in the sub-endothelial area was noticed in the immediate vicinity of some clusters of smooth muscle cells.

CONCLUSIONS

The present study showed some particularities of S100 expression in patients with associated metabolic disease. The patients with diabetes mellitus showed reduced average values of peripheral nerve in the vein adventitia, the existence of the cells with dendritic morphology, with S100 positive profile, in the outer media and sub-endothelial space. A focal co-existence of S100 positive nervous fillets, dendritic cells and smooth muscle cells in the sub-endothelial space, predominantly in endothelial discontinuous areas was found.

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