Histopatological and immunohistochemical aspects (expression of matrix metalloproteinase 9) in polymyositis and dermatomyositis

ASPECTE HISTOPATOLOGICE ȘI IMUNOHISTOCHIMICE (EXPRESIA MATRIX METALLOPROTEINAZEI 9) ÎN POLIMIOZITĂ ȘI DERMATOMIOZITĂ

Keywords:
immunohistochemistry, matrix metalloproteinase 9, dermatomyositis, polymyositis

Abstract
Matrix metalloproteinases (MMP) are a family of structural and functional related endopeptidases, calcium-dependent, zinc-containing which are responsible for the tissue remodeling and degradation of the extracellular matrix. We performed a retrospective study on 20 patients with poly/dermatomyositis with the aim of detecting the expression of matrix metalloproteinase 9 in these patients. Immunohistochemistry showed that the expression of MMP-9 in degenerate muscle fibers was absent in 4 cases. All other cases (16 cases) had positive weak or moderate intensity in the cytoplasm of degenerate striated muscle fibers. In all cases analyzed of unaffected striated muscle fibers MMP 9 was negative. MMP 9 was intense expressed in lymphocytic inflammatory infiltrate, and intense or moderate expressed in the vessel walls.

The overexpression of matrix metalloproteinases 9 on degenerated muscle fibers may be an important event in the multistep process of dermatomyositis and polymyositis and may play an important role in the development of new therapeutic strategies.
Matrix metalloproteinazele sunt o familie de endopeptidaze înrudite structural și funcțional, calciu dependente, cu conținut de zinc, care sunt responsabile de remodelarea și degradarea matricei extracelulare. Am efectuat un studiu retrospectiv pe 20 pacienți cu poli/dermatomiozită cu scopul de a determina expresia matrix metalloproteinazei 9. Examenul imunohistochimic a arătat că expresia MMP 9 în fibrele musculare degenerate a fost absentă în 4 cazuri. Toate celelalte cazuri (16 cazuri) au prezentat imunomarcăj pozitiv cu intensitate slabă sau moderată la nivelul citoplasmei fibrelor musculare striate degenerate. Fibrele musculare striate neafectate au fost negative la MMP 9 în toate cazurile analizate. MMP 9 a fost intens exprimat în limfocitele din infiltratul inflamator și intens sau moderat exprimat în peretii vaselor. Imunoeexpresia crescută a MMP-9 poate fi un eveniment important în procesul patologic ce se desfășoară în mai multe etape în dermatomiozită și polimiozită. Supraexpresia MMP 9 la nivelul fibrelor musculare striate degenerate din dermato/polimiozită poate avea un rol important în dezvoltarea unor noi strategii terapeutice în cazul pacienților cu DM și PM.

Introduction

Polymyositis and dermatomyositis are conditions based on chronic inflammation of striated muscle (myositis) and characterized by multiple clinical features, predominated by symmetrical proximal weakness, associated with laboratory parameters changes (significant increases of activity muscle enzyme, morphological abnormalities in muscle biopsy and characteristic electromyogram appearance).

The annual incidence of these diseases is 2-10 new cases per million people, and the gender distribution is 2.5: 1 in favor of women report being unitary in children and in cases associated with malignancies (1,2).

Matrix metalloproteinases (MMP) are a family of structural and functional related endopeptidases, calcium-dependent, zinc-containing which are responsible for the tissue remodeling and degradation of the extracellular matrix (ECM), including collagens, elastins, gelatin, matrix glycoproteins, and proteoglycan. MMPs are regulated by hormones, growth factors, and cytokines, and are involved in ovarian functions. Currently 23 families of matrix metalloproteinase are known. They are classified in six categories: collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs and others (3).

Patients and methods

We performed a retrospective study on 20 patients with poly/dermatomyositis collecting biopsy pieces for histopathological examination in Dermatology Clinic of Clinical Emergency County Hospital of Craiova. The study was conducted in accordance with ethical and moral principles of the “Declaration of Human Rights” Helsinki approved by the local ethics committee. Patients signed informed consent, they met diagnostic criteria and had no exclusion criteria. Histopathological and immunohistochemical examamination were performed in the Department of Pathology of the same hospital.

The excised tissue fragments were fixed in buffered formalin 10% and processed using the classical paraffin embedding technique. The paraffin blocks were sectioned in 3-4 microns thick and sections were initially stained with the usual Hematoxylin-Eosin staining. Subsequently, serial sections were carried out which were displayed on glass slides coated with Poly-L-lysine for the immunohistochemical examination. The immunohistochemical technique used was the two-step technique with streptavidin-biotin as secondary antibody (LSAB plus kit, DakoCytomation, Denmark), and the chromogen used for visualizing the immunoreactions was 3′-3′-diaminobenzidine. The primary antibody used was represented by mouse monoclonal MMP 9 antibody; clone IIA5, Novus Biological, dilution 1:50. Sections were counterstained with haematoxylin.

Results

The study group consisted of 14 women and 6 men. Patients age was between 6 years to 79 years. In our study lot 4 patients associated another...
Clinical Study

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Figure 1. Skin with degeneration of basal layer of epidermis and lymphocytic perivascular infiltrate HE staining, x100

Figure 2. Perifascicular atrophy of muscular fibres, HE staining, x40

Figure 3. Perifascicular atrophy of muscular fibres and nuclei internalization, HE staining x100

Figure 4. Degenerated skeletal muscle fibers with loss of transverse striations, HE staining X 100

All patients complained of pain and weakness of the proximal musculature, with varying degrees of functional impotence. Skin lesions were present in 8 cases as facial and chest rash. In two of these cases were described Gottron papules with the available feature. Laboratory tests have shown significant increases in muscle enzyme levels in 13 cases and 17 patients had a moderate biological inflammatory syndrome.

Histopathologic examination of skin biopsy in dermatomyositis, showed epidermal atrophy with vacuolar degeneration of basal keratinocytes and lymphocytic inflammatory infiltrate located predominantly perivascular in the superficial dermis (figure 1). Histological examination of the muscle fragments showed in striated muscle fibers the presence of perifascicular atrophy with enlarged nuclei and internalized nuclei in striated muscle fibers.(figure 2, 3, 5). In some places the muscle fibers showed degenerative lesions with intracytoplasmic vacuolation along with loss of transverse striations (figure 4). At distance of atrophic muscle fibers, in perimysium and sometimes in endomysium was present lymphocytic inflammatory infiltrate arranged perivascular (figure 6). The blood vessels located in the perifascicular atrophy areas were numerical reduced and had sometimes turgid endothelial cells and reduced lumen (figure 7). The blood vessels located near of muscle fibre atrophy were sometimes dilated.

Immunohistochemistry showed that the expression of MMP-9 in degenerate muscle fibers was absent in 4 cases. All other cases (16 cases) had positive weak or moderate intensity in the cytoplasm of degenerate striated muscle fibers (figure 8). In all cases of unaffected striated muscle fibres analyzed MMP 9 was negative. The MMP 9 immunoeexpression in the interstitial inflammatory infiltrate was detected in all cases. MMP 9 expression in inflammatory cells was high but the percentage...
of positive inflammatory cells was relatively low. Thus, MMP 9 was detected in the cytoplasmic level of the lymphocytes (figure 9).

Expression of MMP 9 was constantly present in the endothelial cells of blood capillaries and into the smooth muscle layer of arteriolar blood vessels. Blood vessels presented moderate and high intensity expression of MMP 9 in the cytoplasm of endothelial cells (figure 10) and in the smooth muscle cells (figure 11).

Discussions

Dermatomyositis and polymyositis are inflammatory myopathies with symmetrical muscle weakness that develops in weeks or months. Associate multiorgan involvement (lung, gastrointestinal tract, heart, joints) and both diseases have an increased risk of cancer (ovarian, lung, breast, pancreas, lymphoma non-Hodgkin) (4).

Muscle injuries associate the issues of destruction and regeneration, afterwards muscular atrophy with perivascular inflammatory infiltrates and perifascicular dysplasia. In chronic forms fibrosis is observed. In perimysium and sometimes in endomysium was observed a perivascular lymphocytic inflammatory infiltrate. The main histopathologic characteristic in dermatomyositis is the degenerate myofibrils and atrophy who are disposed perifascicular. These changes arranged perifascicular result from the destruction of capillaries that populate this region. This capillary depletion leads to hypoxia and injury of myofibrils. Also, in muscle biopsy (in particular juvenile dermatomyositis) areas of neovascularization can be observed, which are induced by the increase in the concentration of endothelial growth factor in serum and muscle. (VEGF). This increase of VEGF is induced by hypoxia- produced by decreasing capillaries (5).

As for the pathogenesis of these diseases, immunohistochemical studies show that the muscle fiber necrosis derived from the activation of T helper lymphocytes, T suppressor lymphocytes...
accompanied the presence of macrophages in the inflammatory infiltration. Regarding dermatomyositis, it has been demonstrated that the deposition of immunoglobulins and complex membrane attack C5b9 in the muscle’s blood vessels, suggesting that humoral damage initiate primitive microangiopathies, which are responsible for a secondary tissue ischemia especially muscle and mucocutaneous.

MMPs are excreted by a variety of connective tissue cells and pro-inflammatory cells such as fibroblasts, endothelial cells, osteoblasts, macrophages, lymphocytes and neutrophils. All these enzymes are also responsible for tissue damage in several pathological conditions including acute and chronic inflammation, skin changes that occur as a consequence of the aging process following acute ultraviolet exposure and also in the destruction of connective tissue in tumor invasions. Most of the MMP’s are secreted as inactive proteins which are activated when are cleaved by extracellular proteases. The encoded enzyme of this gene, degrades type IV and V collagen and other extracellular matrix proteins.

MMPs are regulated by hormones, growth factors, cytokines and are involved in ovarian function. MMP inhibitors (MMPIs) and tissue inhibitors of MMPs (TIMPs) strictly control these enzymes. MMP overexpression is determined by a disorder of balance between MMP and TIMPs, and this leads to a variety of pathological conditions. MMP 9 has several important functions including neutrophil activation and extracellular matrix degradation, IL-1β activation and cleavage of several chemokines. This, together with elastase, appears to be a factor in regulating neutrophil migration around the basement membrane. MMP-9 is highly expressed in atrophic myofibers in all inflammatory myopathies. In dermatomyositis the perifascicular atrophy showed pronounced MMP-9 immunoreactivity, probably reflecting denervated patterns of myofibers. According to some studies performed through PCR, MMP-9 is significantly increased in polymyositis and dermatomyositis and in a lesser extent in inclusion body myositis, while the expression of TIMP remained unchanged compared with controls. We found a higher expression of MMP 9 in the cytoplasm of the lymphocytic inflammatory infiltrate in patients with dermatomyositis or polymyositis. These findings are similar with those of Kieseier BC which concluded that in inflammatory myopathies MMP-1 could be localized around the sarcolemma of...
diseased muscle fibres and to cells resembling fibroblasts, whereas MMP-9 seemed to be expressed primarily by invading T lymphocytes.

Our findings showed that blood vessels presented moderate and high intensity expression of MMP 9 in the cytoplasm of endothelial cells and in the smooth muscle layer of arteriolar blood vessels. This positivity to MMP 9 is the most likely consequence of structural and functional alterations of vascular walls in patients with dermatomyositis and polymyositis. A series of other changes were identified in the blood vessels walls in patients with inflammatory myopathy. Therefore, the up-regulation of adhesion molecules (VCAM-1 and E-selectin) has been reported in muscle biopsy of patients with inflammatory myopathies. These molecules are secreted mainly from activated endothelial cells and are expressed from the endothelial cells after stimulation with cytokines in patients with dermatomyositis.

Conclusions

MMP 9 was intensely expressed in lymphocytic inflammatory infiltrate, intensly and moderately expressed in vessel walls, moderate and poor positive in degenerated skeletal muscle fibers and negative in normal muscular fibres in patients with dermatomyositis and polimyositis. MMP 9 seems to be expressed more intense in inflammatory infiltrate lymphocytes and less in sarcoplasma of degenerated muscle fibers.

The overexpression of matrix metalloproteinases 9 on degenerated muscle fibers may be an important event in the multistep process of dermatomyositis and polymyositis and may play an important role in the development of new therapeutic strategies.

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