MYOSITIS OSSIFICANS – A CASE REPORT AND REVIEW OF LITERATURE

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ABSTRACT

Associated with abnormal bone formation with various localizations, myositis ossificans is both a diagnostic and a therapeutic challenge due to its rare occurrence and clinical presentation. We examined a case of a 14 year old male patient who was diagnosed with the clinical suspicion of myositis ossficans. Contrast substance MRI revealed the presence of a strictly intramuscular mass located in the 1/3 proximal area of the left thigh, at the level of the vastus lateralis muscle. Macroscopic examination of the specimens revealed multiple, irregular tissue fragments, forming a mass of about 5,5/6/1,2 cm. Additional immunohistochemical analysis was made using the following panel of markers: SMA, Vimentin, Desmin, protein S100, NSE and GFAP. Vimentin was intensely positive in osteoblasts, partially in osteocytes, in the stromal fibroblasts/fibrocytes, in adipocytes and in the vascular wall, in endothelial and smooth muscle cells. GFAP and NSE were negative.

INTRODUCTION

Associated with abnormal bone formation with various localizations [1, 2, 3], myositis ossificans is both a diagnostic and a therapeutic challenge due to its rare occurrence and clinical presentation.

Congenital myositis ossificans occurs in pediatric patients [2] and is regarded as an autosomal dominant disease with irregular penetrance that leads to ectopic bone formation and motion disfunctionalities [2, 4]. Morbidity and early mortality in children is due to respiratory difficulties and improper treatment [2] very few cases being curable by means of surgical intervention [5]. Literature data refers to the association between myositis ossificans and different skeletal anomalies such as microdactily of the first digits, exostosis, the absence of the two upper incisors and anomalies of the great toe [2, 6].

Myositis ossificans traumatica is rarely encountered in children and does not imply genetic mutations with the exception of sporadic cases that involve the head and neck region in the pediatric population [1]. Its mechanism may imply osteoblast stimulation following bone and/or soft tissue damage resulting in dystrophic calcification and formation of calcified chondroid matrix [7]. Such cases are known to present frequent recurrence and require patient long-term follow-up [8]. Less frequent etiologies are represented by burns, infections, surgical interventions and chronic diseases such as diabetes [9, 10, 11, 12, 13].

Regardless of the etiologic type, myositis ossificans that develops around joints leads to deformities and complete restriction of motion due to the occurence of an enlarged mass consecutive to repeated trauma [14]. Other complications include different limitation degrees of the mouth opening in case of orofacial involvement, extra-articular temporomandibular joint ankylosis and compressions of the adjacent structures [4, 11, 15, 16, 17].

CASE REPORT

We examined a case of a 14 year old male patient who was diagnosed with the clinical suspicion of myositis ossficans. Contrast substance MRI revealed the presence of a strictly intramuscular mass located in the 1/3 proximal area of the left thigh, at the level of the vastus lateralis muscle. The 5,2/2,8/2,7 cm lesion had well circumscribed borders and exhibited heterogeneous, solid-cystic features, with septae and microcalcifications. Diffuse inflammation, including edema, was observed in its vicinity. No changes in the femur structure and no signs of lesions in the profound femoral vascular-nervous pack were noticed. No inghino-crural lymphadenopathy was present and the mass did not overcome the posterior fascia. Due to imagistic suspicions of malignancy a biopsy was solicited in order to exclude a sarcoma or a mixoma. Macroscopic examination of the specimens revealed multiple, irregular tissue fragments, forming a mass of about 5,5/6/1,2 cm. The smallest fragment measured 0,7/0,5/0,2 cm and the largest one measured 5/2,2/2 cm. The specimens exhibited variable consistencies, from elastic to bone tissue and presented a gray-brown color in case of 1-2 soft fragments. Specimens were processed for histopathologic diagnosis using H&E, Mallory trychrome and PAS-AA reaction.

Routine microscopic examination showed a proliferative tumor formation exhibiting a solid growth pattern surrounded by a fibrous capsule containing mature bone lamellae (Fig.1.a, b, d) lined by numerous osteoblasts(Fig.1.c). Spindle shaped cells presenting a storiform and trabecular layout, with relatively

monotonous, round nuclei were observed in the center of the lesion. Also, associated mixoid areas and osteoid lamellae lined by osteoblasts were observed along with hyalinized blood vessels, a focal perivascular inflammatory infiltrate containing lymphocytes and plasma cells and small areas of hemorrhage.
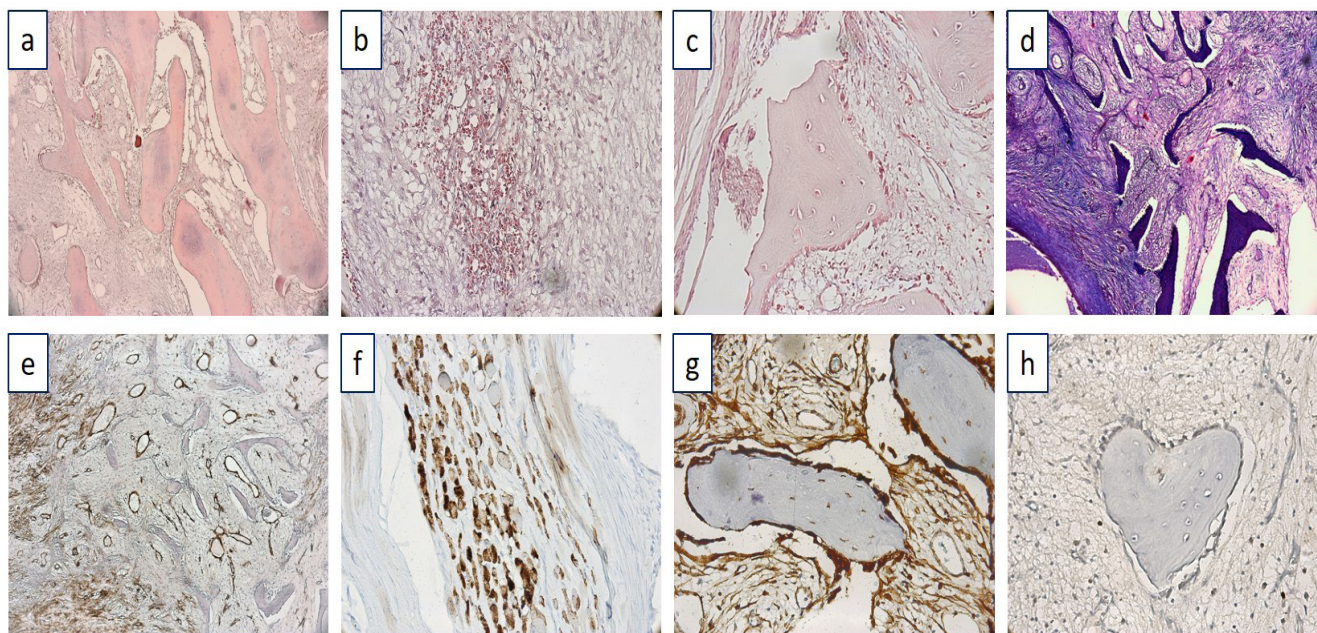
In an attempt to elucidate the mechanisms of bone formation in myositis ossificans we applied an immunohistochemical assay of the markers used to identify progenitor cells, namely: BMP4, Runx2 and OCT-3/4. All three markers were positive and exhibited a nuclear pattern of reaction.

Fig.1.*Morphology and immunohistochemistry of myositis ossificans*

The periphery showed striated muscle tissue with reactive changes. The lesions was found in the profound excision limit. Additional immunohistochemical analysis was made using the following panel of markers: SMA, Vimentin, Desmin, protein S100, NSE and GFAP. Protein 100 was positive in the majority of osteoblasts, in a few osteocytes, in the striated muscle cells, in fibroblasts, adipocytes, in the adventitia of a few blood vessels and in the endothelial cells of the small vessels. The wall of the blood vessels, fibroblasts and myofibroblasts exhibited a positive reaction for SMA(Fig.1.e). Desmin expression was restricted to the striated muscle fibers, predominantly found at the periphery of the lesion(Fig.1.f). Vimentin was intensely positive in osteoblasts(Fig.1.g), partially in osteocytes, in the stromal fibroblasts/fibrocytes, in adipocytes and in the vascular wall, in endothelial and smooth muscle cells. GFAP and NSE were negative(Fig.1.h). The main diagnostic issue was whether it was a case of myositis ossificans or a case of ossifying fibromyxoid tumor. The histopathologic features revealed by the preliminary diagnosis, correlated with the immunohistochemical results were suggestive for a case of myositis ossificans. Unlike myositis ossificans, ossifying fibromyxoid tumors are negative for SMA and positive for protein S100 [60% of cases], vimentin, GFAP [focal reaction] and desmin [13 % of cases]. Microscopic analysis was correlated with clinical and imagistic data in order to improve diagnostic accuracy.

Rare, isolated osteoblasts expressed Runx2 while Oct-3/4 was exhibited by osteoblasts and partially by osteocytes. The endothelial cells of capillary vessels presented an intense, positive reaction. A weak expression for Oct-3/4 was found in the striated muscle fibers.

DISCUSSIONS

It is well known that BMP4 plays a critical role in heterotopic bone formation [18, 19]. Myoblasts differentiate into osteoblasts following exposure to aberrant BMP4 signaling [18, 20]. BMP4 is known to inhibit myoblast alignment and fusion which are required for the formation of myotubes [21]. Studies carried on myositis ossificans cases have reported a genetic disruption of BMP4 expression due to inflammatory factors and increased levels of heparan sulfate proteoglycans [22, 23, 24, 25]. Inflammatory cells are present in this disease in all evolutionary stages [26]. It has been evidenced that myositis ossificans associated lymphocytes constitute a particular cell group in which the BMP-p38 MAPK pathway is affected [27]. Besides lymphocytes, mast cells seem to undergo mobilization and activation in myositis ossificans specimens, being concentrated at the periphery of the affected tissue [26]. Abnormal bone formation in soft tissue where it is normally absent is stimulated either by an increased level of BMP4 or by the lack of BMP4 antagonists [28]. Despite its strong implication in the differentiation of bone progenitor cells, BMP4 is influenced by other molecules. Pitx2 appears to

suppress BMP4 signaling in myoblasts while FOXC2 is a myogenesis inhibitor and an osteogenesis promoter via BMP4 signaling pathway [18, 21]. A notable fact is that BMP4 expression does not influence osteoblast maturation, its action being restricted to progenitor cell orientation towards the osteoblast lineage [18]. Runx2 is an osteogenic gene that is expressed by mesenchymal cells [29]. Its influence has been evaluated on osteoblast differentiation, and on the function of osteoclasts and chondrocytes [29, 30]. Bone marrow mesenchymal cells are oriented towards the osteoblast lineage following Runx2 action [30]. Moreover, Runx2 interacts with a series of osteogenesis related genes such as Osterix gene, a transcription factor that induces osteoblast differentiation [18, 30]. Despite its important role as a bone formation molecule, the implication of Runx2 in myositis ossificans has not yet been elucidated. It is well known that BMPs signaling pathway, namely Smad 1/5/8, sometimes influences Runx2 in order to induce osteogenesis [30]. Through the BMPs/Runx2 axis, BMP4 seems to upregulate Runx2 expression thus inducing the osteogenic differentiation of bone marrow mesenchymal cells [30]. Similarly to BMP4, Runx2 expression is decreases after osteoblast inducement [30]. The transcription factor Oct-3/4 is expressed by the primitive inner cell mass and by embryonic cells and its influence is exerted on pluripotent cells via Sox2/Oct-3/4 axis [31, 32]. Okumura-Nakanishi et al. [31] have evidenced that quantitative levels of Oct-3/4 are essential in determining the fate of primitive cells, in maintaining their pluripotent state and ensuring their self-renewal capacity [32, 33]. An increased Oct-3/4 level induces the formation of the endoderm and the mesoderm while its suppression induces loss of pluripotency [32]. Abu-Remaileh et al. showed that Oct-3/4-beta-catenin interaction influences the epithelial-mesenchymal transition and maintains stem cell identity [33]. Unlike BMP4 and Runx2, Oct-3/4 levels are maintained after osteoblast formation [34] thus indicating its potential role in bone cell maturation.

It appears that in the early stages of myositis ossificans, lesional stromal cells are recruited from the bone marrow vessels [35]. These cells of vascular origin exhibit the potential to differentiate towards an endochondral ossification pathway [35], thus highlighting the possible implication of endothelial-mesenchymal transition in heterotopic bone formation. With regards to this aspect, Lounev et al. [22] have stated that myositis ossificans is partially composed of cells with vascular origin. Sun et al. [36] demonstrated that the early stages of heterotopic bone formation caused by trauma are characterized by the activation of the endothelial-mesenchymal transition mechanism and by the presence of endothelial precursors. The presence of endothelial precursors is legit, considering the fact that vascular damage is physiologically followed by angiogenesis with the occurrence of vascular buds. Vascular buds are not yet oriented towards a specific profile, arterial, venous or capillary. It is well known that the richest source for osteoblasts in normal conditions is the mesenchymal stem cell [24]. In pathologic situations such as myositis ossificans, stromal fibroblasts seem to be controversial entities. Fibroblast plasticity is a well known fact, these cells being able to differentiate themselves not only into fibrocytes but also into neural stem cells, glia cells, adipocytes and even osteoblasts [37, 38, 39]. The capacity to form other cell types that present a totally different function compared to the precursor cell is a characteristic of embryonic fibroblasts [39]. These cells present a Sox9 and Sox10 positive profile which is required for their conversion [39]. Fibroblast are also positive for alpha-SMA, thus showing their ability to migrate [40] and possibly line the bone lamellae before differentiating into osteoblasts.

CONCLUSIONS

The mechanism of bone formation in myositis ossificans is complex and implies the participation of a series of activated osteogenic genes along with differentiation molecules for progenitor cells. Our results support previous studies carried on heterotopic ossification specimens regarding the expression of markers used to evidence progenitor cells.
Runx2 expression was weak and restricted to a few osteoblasts thus supporting its implication in bone cell differentiation but not maturation. Oct-3/4 is implicated in the self renewal of undifferentiated stem cells, an aspect that is supported by the numerous positive osteoblasts. The decrease in Oct-3/4 levels in osteocyte may suggest the loss of the precursor phenotype as the cells undergo maturation. This aspect is sustained by the low number of Oct-3/4 positive osteocytes.

The source of newly formed bone tissue in cases of myositis ossifcans may be represented by endothelial cells and striated muscle fibers. A high density of blood vessels was observed at the periphery of the lesional tissue. Most of these vessels were of small caliber and numerous capillary vessels were identified. Also, the immunohistochemical reaction for SMA revealed the presence of a great number of myofibroblasts, which were recognizable due to their elongated shape and network-like disposition. These findings support the occurrence of posttraumatic angiogenesis. Through the endothelial-mesenchymal transition, precursor endothelial cells may be activated and recruited by osteogenic genes in order to differentiate into osteoblasts that will line the surface of the bone lamellae and secrete the bone matrix. Striated muscle cells are known to possess an important regenerative capacity via satellite cells. During the differentiation process, myoblasts may be subject to osteogenic gene influence, thus being oriented towards a bone cell phenotype. These aspects are supported by the positive reaction for Oct-3/4 found in the wall of capillary vessels and in the striated muscle cells. SMA

positive fibroblasts support the hypothesis according to which lesional stromal cells found in myositis ossificans may be of vascular origin. However, none of bone precursor markers was positive in the stroma cells. Osteoblasts originating from fibroblasts in this disease is disputable and in need of further investigation.

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