

CASE REPORT

Ectopic osteogenesis in the rectus sheath

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Abstract

Although was published many cases of ectopic osteogenesis of traumatic, neurogenic cause or hereditary form, the etiology of ectopic osteogenesis remaining unknown. We present ectopic osteogenesis in the rectus abdominal sheath. The study material was represented from fragments of ectopic bones discovered in rectus sheath of four patients suffering iterative surgical abdominal interventions. The pieces of ectopic bone were decalcified and then were made to the standard techniques (paraffin inclusion, general techniques dyeing). The process of ectopic osteogenesis was analyzed through microscopically study to seriated sections of discovered piece, finding the presence of the hematopoiesis foci. We conclude that is important identifying and characterizing the osteoinductor agents because these allowed the study of osteogenesis to the cellular level and make an estimation of the abnormally bone developing mechanisms. A possible osteoinductor factor has been considerate the non-absorbable wound closure material.

Keywords: ectopic osteogenesis, rectus sheath, haematopoiesis foci.

Introduction

Heterotopic ossification is the presence of the bone in soft tissue where bone normally does not exist. This condition should not be confused with metastatic hypercalcification and dystrophic calcification.

Reidel first described heterotopic ossification in 1883, and in 1918 Dejerne A and Ceillier A [1] reported that heterotopic ossification frequently occurred among soldiers who had experienced spinal cord trauma as combatants in World War I. The differentiation of the unspecialized mesenchymal cells population in bone soft tissue initiates the process named bone induction.

Approximately sixty years ago, Higgins C. (1930) had demonstrated the bone induction through the experiments [2]. The formation of the ectopic bone may be experimental induced in any soft tissue through the implantation of demineralized bone or dentine, contained bone morphogenetic proteins.

Heterotopic bone formation in abdominal incisions is a recognized but uncommon sequel of abdominal surgery [3]. On the other hand, the formation of ectopic bone is a well-recognized complication following arthroplasty of the hip [4]. Heterotopic ossification of midline abdominal incisions is a subtype of *myositis ossificans traumatica* [5]. Ectopic bone formation of midline abdominal incisions may cause regional pain or discomfort in the patient after surgery.

For clinicians treating patients with formation of ectopic bone following arthroplasty, the fever, swelling, erythema and decreased joint motion typically seen in early heterotopic ossification may closely mimic the presentation of cellulites, osteomyelitis or thrombophlebitis [6–8].

Heterotopic ossification can even be confused with

the bone-forming tumors: osteosarcoma and osteochondroma [9]. To resolve such diagnostic uncertainty, clinicians often request bone scanning. Although the various presentations of heterotopic ossification have been recognized for many years and numerous methods of treatment applied, the complete knowledge of the etiology of ectopic ossification remain elusive.

Material and methods

The ectopic bone detected in the rectus sheath, obtained from four male patients, 56–64 years old who had multiple operation for peptic ulcer and gastric carcinoma, represents the study material.

In two cases the abdominal CT examination, a bone density lesion, with no relation to xiphoid, was seen in the mid-epigastric area and on the incision line.

The lesion was excised because it was causing pain. In the operation bony mass varying in size 6–8×3.5 cm, lying between subcutaneous soft tissue and peritoneum in the rectus sheath was removed (Figure 1).

The distance was approximately 4.5 cm from the xiphoid process to the most superior aspect of the bony mass.

The ectopic bone was decalcified and then we applied the general staining procedure. We prepared the sections of bone in the following manner:

- the decalcification was made with EDTA, during seven days;
- washing with 70% alcohol and glycerol – 24 hours;
- maintain the bone pieces in pure anhydrous glycerol – 24 hours;
- introducing the sections in 100% alcohol, two times in 12 hours, and three times in butylic alcohol, one hour.

Usual light microscopy was applied: the fragments of ectopic bone included in paraffin and 6 μm sections were stained with Hematoxylin–Eosin and VG examined (Nikon Eclipse E600).

☐ Results

The results of our study are shown in Figures 1–7, which comprise the macroscopic image of the fragments of ectopic bones discovered in rectus sheath of four patients suffering iterative surgical abdominal interventions, and the microscopic images of the seriated sections of the pieces.

The process of ectopic osteogenesis was analyzed using the microscopic technique, in the same time following the identification and characterization of the osteoinductor agent that has been considerate the non-absorbable wound closure material.

☐ Discussions

Definition

Heterotopic ossification is the presence of bone in soft tissue where bone normally does not exist. This condition, the more common may occur after virtually any type of musculo-skeletal trauma, spinal cord injury or central nervous system injury. For example, the patient who suffered orthopedic procedures such as shoulder arthroplasty, or had paraplegia after spinal cord injury, had a high risk for development of the heterotopic ossification.

Classification and incidence of heterotopic ossification

There are two version of heterotopic ossification. The most common is the acquired form. In the acquired form, heterotopic ossification usually either is precipitated by trauma (such as direct muscular trauma fracture or total hip arthroplasty) or has a neurogenic cause (such as spinal cord injury or central nervous system injury). In addition, there is the rare hereditary form known as *myositis ossificans progressiva*. The more common acquired form of the heterotopic ossification may occur after musculo-skeletal trauma [7, 10, 11]. For example, heterotopic ossification may occur after orthopedic procedures such as hip, knee, and shoulder or elbow arthroplasty; joint dislocations, fractures or soft-tissue trauma with the musculus quadriceps femoris and musculus brachialis often involved [12].

Heterotopic ossification includes the specific posttraumatic variant *myositis ossificans* in which patients often have soft-tissue ossification at sites of trauma adjacent to long bones. Less commonly encountered sites of posttraumatic heterotopic ossification are: abdominal incisions such as cases presents in this article, wounds of kidneys, uterus, the *corpora cavernosa* and gastro-intestinal tract [13–15].

The other common traumatic form of heterotopic ossification occurs after injury to the nervous system and is therefore known as posttraumatic neurogenic heterotopic ossification.

Heterotopic ossification occurs among patients with recent spinal cord injury, frequently an adolescent or young adult of either sex: heterotopic ossification develops only in sites distal to the level of the spinal cord injury. Closed head injury, brain tumors, strokes also may lead to heterotopic ossification [10, 16, 17].

Less often encountered in most clinical patients are cases of heterotopic ossification after burns, hemophilia, sickle cell anemia, poliomyelitis, multiple sclerosis, tetanus and toxic epidermal necrolysis [18]. Some cases of idiopathic heterotopic ossification occur without a recognized precipitating condition.

Pathophysiology of heterotopic ossification

Heterotopic ossification is, by definition, the formation of bone within soft tissue. This morbid soft tissue ossification has a distinct pathogenesis from metastatic and dystrophic soft-tissue calcification. The transformation of primitive cells of mesenchymal origin, present in the connective tissue septa within muscle, into osteogenic cells is thought to be the pathogenesis of heterotopic ossification [19].

Chalmers J *et al.* [20] proposed three conditions needed for heterotopic ossification: osteogenic precursor cells, inducing agents and a permissive environment.

Urist MR *et al.* discovered that demineralized bone matrix could invoke bone formation ectopically and postulated a small (<0.025 μm) hydrophobic bone morphogenetic protein as a causative agent [19].

This protein is capable of changing the development of mesenchymal cells in muscle from fibrous tissue into bone [19]. It has been postulated that bone morphogenetic protein is liberated from normal bone in response to venous stasis, inflammation, or diseases of connective tissue attachments to bone, conditions that often accompany immobilization or trauma [19]. Some investigators proposed the presence of a centrally mediated factor [21, 22]. The role of prostaglandin E₂ has recently been suggested as a mediator in the differentiation of the progenitor cells [23].

Interestingly, experiments have also shown that muscle injury alone will not cause the ectopic ossification, concomitant bone damage also being required [16].

Kurer MH *et al.* [24] took sera from four paraplegic patients with heterotopic ossification and four patients without heterotopic ossification; the sera were incubated with human osteoblasts in tissue culture, and their metabolic activity was quantitatively measured.

These investigators found that the sera of ossifying patients had significantly greater levels of osteoblasts-stimulating factors, which may contribute to the pathogenesis of heterotopic ossification.

Other contributing factors include: hypercalcemia, tissue hypoxia, changes in sympathetic nerve activity, prolonged immobilization, remobilization and disequilibria of parathyroid hormone and calcitonin [22, 23].

There are three prevailing theories for heterotopic ossification of abdominal scars [3]. According to the first theory, small particles from the periosteum or

perichondrium of the xyphoid process or symphysis pubis are inoculated during surgery into the surgical wound and lead to the formation of bone. This theory is supported by the fact that all reported incidences of heterotopic bone are from vertical incisions [3]. This theory does not sufficiently explain heterotopic ossification with any close anatomic relationship to osseous tissue. The second theory contends that heterotopic bone formation is a result of immature pluripotent mesenchymal cells differentiating to osteoblasts or chondroblasts as a reaction to local injury. It fails to explain the occurrence in vertical incisions only. The third theory is excessive suture line tension, which may lead to intramuscular implantation and ossification of periosteal particles torn from sites of muscular insertion into a bone [3, 5].

Early in course of heterotopic ossification, edema with exudative cellular infiltrate is present, followed by fibroblastic proliferation and osteoid formation [9].

Myositis ossificans shows ossification principally in the periphery, so that an ossified and radio-opaque peripheral rim surrounds a non-ossified and radiolucent center; the opposite is true of osteosarcoma, a malignant tumor that often forms dense central ossification [27, 28]. Mature ossified foci may even contain reticulo-endothelial cells [29].

The periphery of *myositis ossificans* shows mature lamellar bone surrounded by a capsule of compressed muscle fibres and fibrous tissue [28].

The development of heterotopic ossification is extra-articular and occurs outside the joint capsule. Ectopic bone forms in the connective tissue between the muscle planes and not within the muscle itself [9]. The new bone can be contiguous with the skeleton but generally does not involve the periosteum [9, 24].

Mature heterotopic ossification shows cancellous bone and mature lamellar bone, vessels and bone marrow with a minor amount of hematopoiesis [26, 30]. In our cases we observe the presence of hematopoiesis in the ectopic osteogenesis zone (Figures 2 and 3).

In the same time we discovered the presence of ectopic bone in rectus sheath from four patients who had suffered iterative surgical intervention (Figure 1). We discuss the presence of determinants factors of ectopic osteogenesis: the unabsorbable wound closure material had developed in peripherally zone a fibrous reaction of extracellular matrix (Figures 4 and 5).

According to literature data, for our ectopic bone discovered in rectus sheath was possible the intervention of mesenchymal cells and bone morphogenetic proteins.

The mesenchymal cells could differentiate into a variety of mesenchymal lineages, including adipocytes, chondrocytes and osteoblasts. The differentiation has been reported to induce the temporal expression of transcriptional factors. Overexpression of Sox-9 induced chondroblastic differentiation and overexpression of MyoD induced myoblastic differentiation. Overexpression of proliferate activated receptor γ 2 (PPAR) induced adipocytic differentiation whereas Cbfa1/Runx2 is necessary for osteoblastic differentiation [31].

Recently has been reported that the cartilage regeneration may be realized using the mesenchymal stem cells originated from bone marrow [32]. This mesenchymal stem cells can differentiated into a variety of mesenchymal lineages, including adipocytes, chondrocytes, and osteoblasts [33].

Bone morphogenetic proteins (BMP), members of the transforming growth factor- β (TGF- β) participate to migration, adhesion, multiplication and cellular differentiation [34]. The induction capacity of bone matrix is due to the presence of soluble matrix proteins known as bone morphogenetic proteins (BMP) or osteoinductive proteins, which show a high capacity to stimulate the morphogenetic phase of osteogenesis, leading to the cytodifferentiation of osteoblasts and ectopic bone formation.

The discoveries have resulted in the use of allogenic bone matrix previously subjected to special demineralization and preparation procedures to completely eliminate matrix cells and antigenic determinants, as graft material for inducing new bone formation in permanent bone defects or defects that are difficult to repair [35–37].

BMP are able to promote osteogenesis, chondrogenesis and adipogenesis, whereas they inhibit myogenesis of mesenchymal progenitor cells [38]. Bone morphogenetic proteins, members of the transforming growth factor- β (TGF- β) superfamily are expected to be applied to the treatment of various orthopedic diseases including bone fracture and spinal fusion [39, 40].

When recombinant human BMP-2 is applied to a carrier protein and implanted subcutaneously, mesenchymal cells infiltrate into the matrix from surrounding tissues; then the matrix is degraded and replaced by trabecular bone and cartilage [37]. However, the bone formation by BMP-2 is a self-limiting process, suggesting the presence of endogenous inhibitors of bone formation [41].

TGF- β is stored abundantly in bone, as has potent effects on osteoblasts. Three different isoforms of TGF- β , that is, TGF- β ₁, β ₂ and β ₃, with essentially similar bioactivities, have been identified in mammals [42]. TGF- β has been reported to exhibit both positive and negative effects on bone. Exogenously injected TGF- β induce bone formation on periosteum [43, 44], whereas transgenic mice over-expressing TGF- β ₂ in bone exhibited an osteoporotic phenotype characterized by increased activities of osteoblasts and osteoclasts and impaired matrix mineralization by osteoblasts [45].

Alliston T *et al.* [46] reported that TGF- β inhibits osteoblast differentiation through modulation of expression and transcriptional activity of Runx2. However, the role of endogenous TGF- β in bone formation has not been fully elucidated.

Members of the TGF- β superfamily transduce their signals through two types of serine/threonine kinase receptors, termed type I and type II [47–49].

The type II receptors are constitutively active kinases, which phosphorylate type I receptors upon ligand binding. Seven type I receptors termed activin

receptor-like kinase (ALK)-1 though -7 have been identified in mammals study. In the present study, the microscopic sections of decalcified ectopic bone show the differentiation of osteoid tissue from extracellular matrix elements, the conjunctivo-vascular tissue with vasculogenesis, and the presence of osteoblasts (Figures 6 and 7).

According to Reddi AH [50] and Kale AA *et al.* [51], this inflammatory reaction characterized by the recruitment, activation and interaction between polymorphonuclear leukocytes and the graft matrix, represents a critical factor for activation of the cascade of events leading to osteogenesis.

For this reason, some investigators have questioned the use of anti-inflammatory drugs such as indomethacin and corticoids during the initial post-implantation period since these drugs reduce the initial inflammatory response and, consequently, the migration and proliferation of undifferentiated mesenchymal cells essential for osteoinduction and osteoblast differentiation. This formation of new bone in subcutaneous or intramuscular connective tissue stimulated by BMP mimics bone development during embryonic and fetal life [52, 53].

Clinical presentation and differential diagnosis

The clinical signs and symptoms of heterotopic ossification may appear as early as three week or as 12 week after musculoskeletal trauma, spinal cord injury or other precipitating event [54].

The commonly involved sites of heterotopic ossifications in decreasing order of frequency are the hip, the knees, the shoulders, the elbows, and even very rarely, the feet [55, 56].

Loss of joint mobility and resulting loss of function are the principal complication of heterotopic ossification [55–58]. Largely because of the non-specificity of the patient's signs and symptoms, diagnosis of heterotopic ossification in its initial stages is difficult.

Early in the course of the disease, heterotopic ossification may cause pain, fever, swelling, erythema and decreased joint mobility. In this early inflammatory phase, the condition may mimic cellulitis, thrombophlebitis, osteomyelitis or tumor [6–8, 55].

Generally, ectopic bone formation in midline incision scars takes place within a few months and almost always within the first year after surgery. In our patient, heterotopic ossification was observed within eight months of the second surgical procedure. The size of heterotopic bone varies greatly in the literature, with the largest piece, reported by Pearson P *et al.*, being 15×4.5 cm [59, 60]. In our cases, the largest piece was 8×3.5 cm (Figure 1).

The distance was approximately 4.5 cm from the xyphoid process to the most superior aspect of the bony mass. Microscopically, the lesion consisted of cartilage and mature bone tissue, within hematopoiesis was present.

Dystrophic calcification of soft tissues is a condition in which there is an abnormal deposition of amorphous calcium. This condition can be found in the calcification

of bursae and hematomas, neoplasms and collagen diseases [4].

Traumatic *myositis ossificans* has been repeatedly reported in the literature. This usually follows a contusion to a muscle caused by a sports injury [61].

Ectopic bone formation is a more advanced process than dystrophic calcification and traumatic *myositis ossificans*. There is definite osteoblastic activity, cartilaginous and myelogenous elements in the ectopic osseous matrix. The presence of cartilaginous and bony elements distinguishes this entity from dystrophic calcification [3, 62].

Histologically, the ectopic bone formations are composed of mature bone with marrow and cartilaginous elements surrounded by fibrous tissue [5, 63].

A striking male prevalence has been noted, and it is estimated that male to female ratio is approximately 10 : 1 [63].

Ectopic bone formation in midline abdominal surgical scars has been noted to occur only within vertical incisions. In incisions with both horizontal and vertical components, ossification always occurs in the vertical component [3]. In our cases, the ectopic bone formation in rectus sheath has been noted only within vertical incisions.

Imaging of ectopic bone formation in abdominal midline incisions appears typical. Nuclear medicine studies employing Tc-pyrophosphate have been reported to show increasing activity within the incision before ossification shows on plain films [59].

A lateral plain film will demonstrate a calcifying or a bone density linear structure within the abdominal wall [63].

Sonography may demonstrate a hyper-echoic mass posterior acoustic shadowing [59].

CT can show complete ossifications indicative of mature phase of the pathologic process and may help in planning surgical resection [64].

Ossified components show densities equivalent to bone, with intra-lesional fatty components representative of marrow sometimes present [5].

Although it is rare, ectopic bone formation in midline abdominal incisions may cause regional pain or discomfort [63].

Treatment of heterotopic ossification

Treatment should consist of complete excision with primar closure. In the literature, three cases of recurrent ectopic bone in surgical scars have been reported.

In our cases, no recurrence was observed in the eight months following excision of the ectopic bone. Recurrent ectopic bone formation is treated with re-excision and postoperative radiotherapy. Non-steroidal anti-inflammatory medication could be used to prevent recurrent heterotopic bone formation [3].

Diphosphonates and non-steroidal anti-inflammatory drugs (such as indomethacin and ibuprofen) have been used for prophylaxis or treatment of heterotopic ossification. However, there is no consensus on which drug should be used and when treatment should begin.



Figure 1 – Ectopic osteogenesis. Bone fragment discovered in the rectus sheath of a 67-years old male patient who had had two operations previously

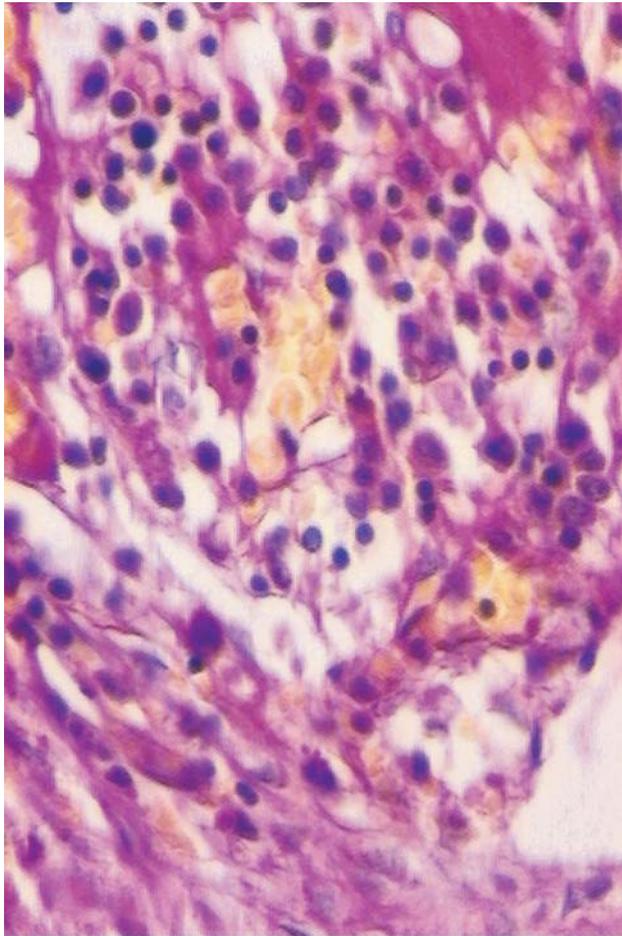


Figure 2 – Ectopic osteogenesis. Haematopoiesis foci appeared in the inter-mesenchymal ossification zone (HE staining, $\times 140$)

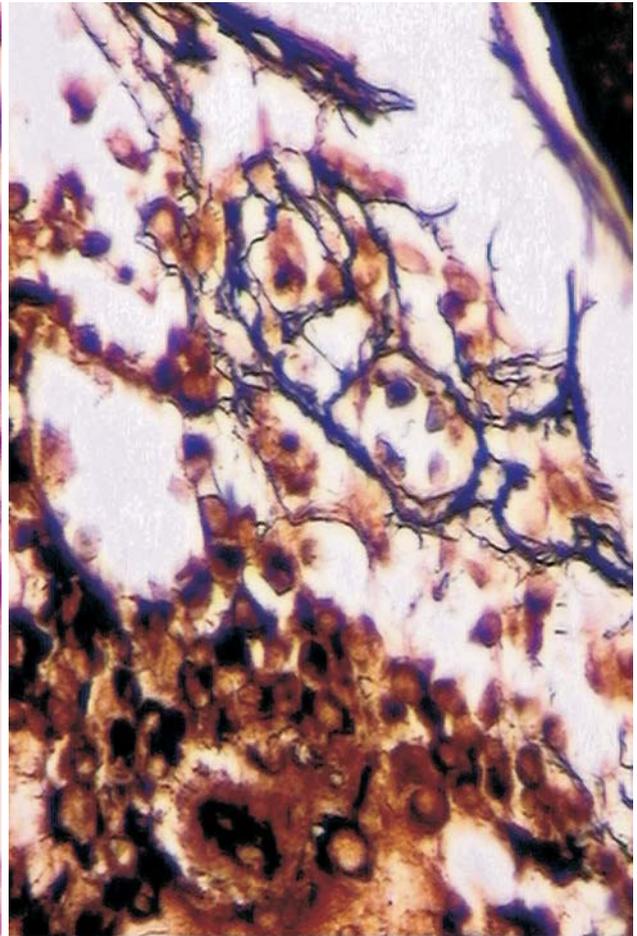


Figure 3 – Reticular fiber network in the haematopoiesis foci (HE staining, $\times 280$)

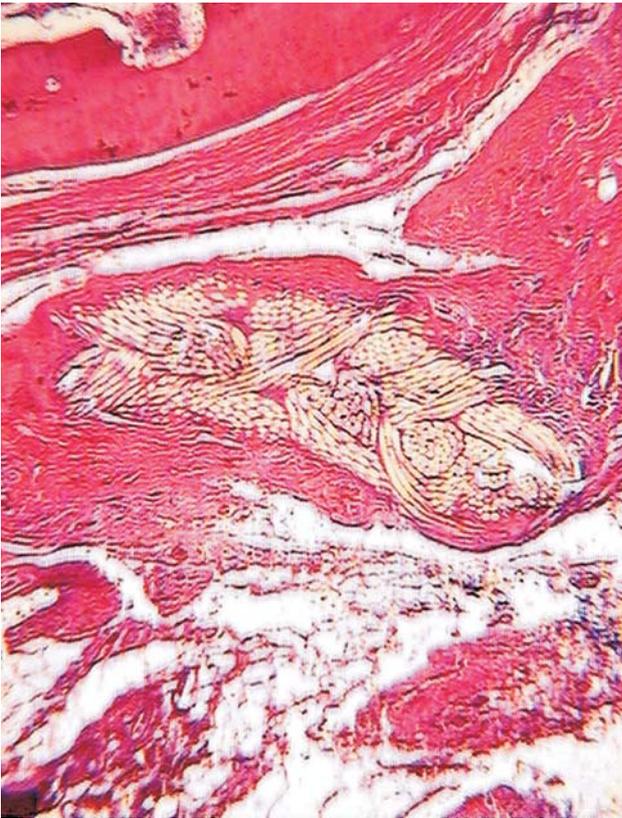


Figure 4 – Bone tissue fragments discovered in the rectus sheath of a 67-years old patient. In the centre of the image we observed the presence of material wound closure (surgical non-absorbable multifilament silk suture) with three knots (HE staining, $\times 140$)



Figure 5 – In the peripheral border of the foreigner body it was developed the fibrous reaction of the extracellular matrix (HE staining, $\times 280$)

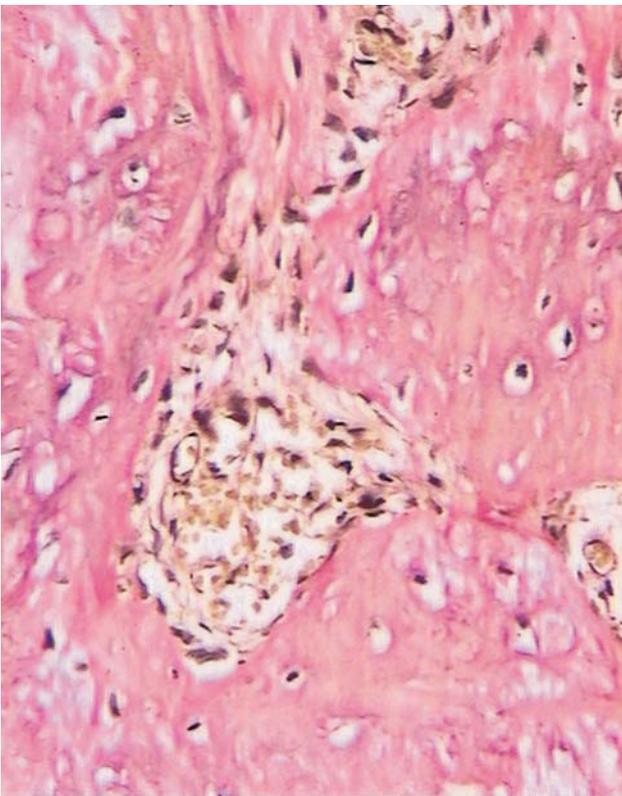


Figure 6 – Ectopic osteogenesis. Osteoid tissue differentiated from extracellular matrix elements and conjunctive-vascular tissue with vasculogenesis process limited from the osteoblasts (HE staining, $\times 140$)

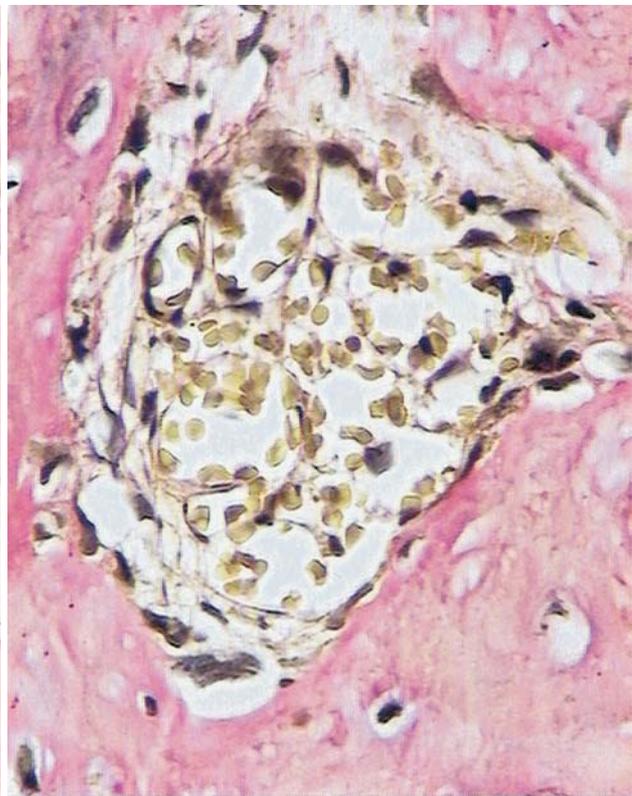


Figure 7 – Presence of the osteoblasts in the peripheral border of the osteoid tissue, differentiated from extracellular matrix elements (HE staining, $\times 280$)

☐ Conclusions

We consider importantly identifying and characterizing the osteoinductive agents because they allowed the study of the osteogenesis to cellular level, allowing the estimation of the mechanisms of abnormal bone growth.

The ectopic bone formation in rectus sheath was associated with hematopoietic foci, a possible osteoinductor factor being considerate the non-absorbable wound closure material.

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