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Study of the Tissue Reaction of the Region of the Midpalatine Suture of Rats after Disjunction with Spring Anchored in Mini-Implants

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Abstract Midpalatine suture disjunction is a routine procedure in the orthodontic clinic; however, the cellular events involved in the procedure have not yet been completely explained. The aim of this study was to verify the tissue events, particularly those related to osteogenic differentiation, which occur in the region of the palatal suture after disjunction, by means of histologic and immunohistochemical analyses of the following noncollagenous proteins: BSP, OCC, OPN and ONC. In this study, 10 male Wistar rats aged two months and weighing approximately 250 g were used. The animals were divided into two groups of five animals: Group I – control (mini-implant placement) and group II – experimental (placement of mini-implant and disjunction spring made of 0.60mm steel wire, with an initial load of 2N (200 grams)). After 21 days, the animals were sacrificed and histological and immunohistochemical analyses were performed for the noncollagenous proteins. The use of the spring anchored to mini-implants promoted the disjunction of the median palatal suture, with a V-shaped opening, in which the base faced the superior region (at the nasal cavity) and the vertex was in the inferior region of the oral cavity (at the palatal bone). It could be concluded that after disjunction, there was presence of osteoblasts in an osteoid matrix in the region of the median palatal suture, and the immunohistochemical expression of the noncollagenous proteins increased in all the analyzed parameters, expression being most significant for ONC.

Keywords: *rapid maxillary expansion, anchorage, mini-implants, noncollagenous proteins*

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1. Introduction

The correction of malocclusion that presents maxillary atresia has been performed by means of devices that promote rapid maxillary disjunction [1]. Different devices can be used for maxillary disjunction, and may be of the tooth-tissue-born type, such as Haas, presenting greater difficulty in hygiene and mucosal irritation and tooth-born, such as Hyrax, with a higher propensity to perforate the buccal alveolar bone plate [2]. Among these devices the choice should be based on differential diagnoses and treatment goals.

Rapid maxillary disjunction promotes the opening of the midpalatine suture with subsequent skeletal changes in the anteroposterior and vertical directions, and may correct posterior crossbite [3]. Among the skeletal changes resulting from this therapy, there is an increase in the floor of the nasal cavity, with consequent increase in airflow and normalization of breathing, which is frequently obstructed in patients with maxillary atresia [4,5]. On the other hand, it may promote undesirable effects such as: buccal inclination of teeth, cortical anchorage, bite opening and root resorption [6].

There is a growing number of individuals looking for orthodontic treatment and frequently have a previous dental history limiting such as: absence of teeth, periodontal changes, temporomandibular joint (TMJ) dysfunctions, among others [7]. Thus, to minimize risks during treatment, specific mini-implants were developed for anchoring in orthodontics, generally having one end with support for metallic ligatures, elastic modules or segmented arches and others. In addition to being more practical for orthodontists, these specific mini-implants avoid injury to the surrounding gingiva and unwanted dental movement, decreasing or avoiding discomfort for the patient [8,9,10].

The use of mini-implants may prevent surgical treatment in some cases, since they promote a more effective anchorage for palatine disjunction when compared to

tooth-tissue-born or tooth-born anchorage [8]. PARR et al. [11] proposed the use of endosteal implants as pillars for anchoring the palatine disjunction, in order to control the undesirable teeth movement.

The clinical use of palatal disjunctions with anchorage by means of implants is already widely used [12], but the tissue events involved in this process are still not fully understood.

The process of bone neoformation is a complex phenomenon, involving the mechanism of several types of noncollagenous proteins. The main noncollagenous bone matrix proteins are osteonectin (ONC), osteopontin (OPN), bone sialoprotein (BSP) and osteocalcin (OCC). The functions of these proteins are related to the regulation of mineralization of collagen fibers and modulation of the division, migration, differentiation and maturation of bone cells and are synthesized in different stages of osteogenesis [13].

Although they represent only 10% of the organic matrix of the mineralized tissues, it is believed that the noncollagennous proteins are the main responsible for the exclusive properties that distinguish the several mineralized tissues from each other. ONC is associated with the formation of extracellular bone matrix, OPN is responsible for the adhesion of osteoblasts to the extracellular bone matrix, BSP stimulates the pre-osteoblast mitosis and promotes the differentiation of these cells into osteoblasts and OCC regulates the growth of the hydroxyapatite crystals [14].

According to MARTINEZ; ARAÚJO [15], the noncollagenous components of the extracellular matrix participate in all regulatory mechanisms that require matrix interference on cell dynamics. Thus, changes in the ratio of cells to the extracellular matrix may interfere with tissue integrity.

This work aimed to verify the tissue events, mainly those related to the osteogenic differentiation that occur in the region of palatine suture after disjunction, through histological and immunohistochemical analysis of the following noncollagenous proteins BSP, OCC, OPN and ONC.

2. Methods

The research was approved by the Ethics Committee of the Faculty of Dentistry Post-Graduation Center - São Leopoldo Mandic, Campinas, SP, under protocol No. 06/091.

Ten laboratory rats (Rattus norvegicus) of the albinus variety and Wistar line, male, aged two months and weighing approximately 250grs, were used. The rats were obtained from the Butantan Institute Animal Hospital (São Paulo - SP) and remained at the Experimental Laboratory of the Genetics Laboratory, at the same Institute, during the research. The specimens were randomly divided into two groups of five animals: Group I - control (placement of the mini-implant) and Group II - experimental (mini-implant placement and installation of the disjunction spring made with 0.60mm steel wire, whose load was 2N [200 grams]).

After random selection of the animals, they remained in individual cages for approximately two hours in order to reduce stress. For the surgical procedure, each animal was anesthetized with intraperitoneal administration of ketamine at the dose of 72 mg/kg and xylosin hydrochloride at a dose of 10 mg/kg, tricotomized at the frontal region and antisepsis performed with chlorhexidine digluconate solution at 2%.

The incisions were made in the frontal region, in the vertical direction, with scalpel blades # 15 until the periosteum. Subsequently the tissue was detached with detachment-periosteum.

Each animal received two titanium mini-implants for orthodontic anchorage with a diameter of 1.3 mm in circumference per 9mm (1.3 x 9mm CA), self-drilling, symmetrically and bilaterally installed to the midpalatine suture, using the Tufo wrench (mini-implant and installation kit of NEODENT® - Curitiba / Paraná / Brazil).

After the installation, mini-implants were covered with the flap and the sutured skin was wrapped with black nylon, monofilament nylon, sterilized by gamma rays, length 45 cm, needle for triangular suture 1.5 cm (stainless steel) 3/8 circle.

In the immediate postoperative period, oral liquid analgesic (paracetamol) and single dose intramuscular antibiotic (penicillin sodium) were administered to all animals. Then, the inter-mini-implants distances were measured with digital caliper, whose average was 1.91 mm, and parallelism in the installation of mini-implants was verified. Subsequently, the disjunction spring with 0.60 mm steel wire was installed on the mini-implants, only in Group II. All animals were kept for 21 days in individual cages, receiving feed and water (ad libitum).

After 21 days, all the animals were sacrificed in a carbon gas chamber with double gas infusion; the pieces were dissected and immersed in 10% formalin. The bone pieces were decalcified in EDTA solution in distilled water with pH 7.4 in a microwave oven, following the protocol established by the Department of Pathology of the Faculty of Dentistry São Leopoldo Mandic, which consists of placing the pieces in "becker" with the EDTA solution, partially immersed in a glass vat with distilled water and crushed ice to retard the increase of the temperature and, consequently, to increase the time of action of microwaves. It passed through ascending baths in absolute alcohol at 700, 800, 900, 950 and 1000, alcohol xylol 1.1 and later two baths of xylol 1 and xylol 2 and two baths of paraffin, being an hour and a half in each. Subsequently the pieces were regularly included in paraffin in the Tissue-Tek® TEC5Tm apparatus. Serial sections of 3 micrometers thickness (LEICA RM 2245 microtome) were obtained, which were stained with Hematoxylin/Eosin and Masson's Trichrome.

The slides were observed in the Department of Pathology of São Leopoldo Mandic School, Campinas-SP - Brazil, when images of the osteogenic differentiation of the control and experimental groups were obtained.

2.1. Immunohistochemical Technique

Immunohistochemical reactions were performed for the antigens of the following proteins: osteopontin, osteocalcin, osteonectin and bone sialoprotein in the control and experimental groups of the palatine suture region using the Streptavidin-biotin-peroxidase technique.

From each paraffin block containing the biopsy material, seven 3mm cross sections were obtained. The slices were laid on glass slides, previously washed in absolute alcohol, dried and immersed for 1 minute in 3-aminopropyltriethoxy silane solution (Sigma Chemical Co., St. Louis, MO/USA), 10% absolute alcohol.

The sections were dewaxed in two xylol baths: the first at 60°C for thirty minutes, and the second at room temperature for twenty minutes. Then, rehydrated in descending series of ethanol, from three passes in absolute ethanol, followed by 95%, 85% and 80% ethanol, for five minutes each. For the removal of the formalin pigment the cuts were immersed in 10% ammonium hydroxide solution for ten minutes, followed by washing in running water for ten minutes and two baths of distilled water.

After treatment, the sections were washed again under running water, followed by two passages in distilled water and endogenous tissue peroxidase blockade, for which two 15 minute baths in 6% hydrogen peroxide solution in methanol (1:1, v/v). Repeating the rinse with distilled running water, the cuts were submerged three times in TRIS (hydroxymethyl-amino-methane) solution pH 7.4, for 5 minutes each. For blockade of the non-specific reaction the slices were incubated in normal saline serum (Daco Corp., Carpenteria, CA, USA) 30% diluted in TRIS buffer solution. The incubation was carried out in a humid chamber at room temperature for sixty minutes for the reactions against OPN, and for ninety minutes for reactions against ONC, BSP and OCC. After this period a bath in TRIS buffer was performed. The slides were incubated with the primary antibody diluted in TRIS pH 7.4, plus 1% bovine albumin containing 0.1% sodium azide (BSA-Biotest S / A, São Paulo, Brazil) .

Dilution and incubation time were optimized for each antibody used, BSP 1:800; OCC 1:400; OPN 1:1500; ONC 1:500. The slides were then immersed in running water and in TBST buffer, pH 7.6 with secondary serum and tertiary complex the LSAB Peroxidase kit (Dako Corporation) was used. For the development reaction, the slides were immersed in solution of the chromogen diaminobenzidine (DAB, 3,3-diaminobenzidine, Sigma Chemical Co., St. Louis, MO / USA) for ten minutes. After this procedure the slides were washed in TBST buffer pH = 7.6 and distilled water and counterstained with Mayer's hematoxylin for five minutes and rinsed again in distilled water.

After this passage the slides were dehydrated in an ascending chain of ethanol and diaphanized in three xylol baths. The slides were then mounted in Permount (Fisher Scientific, Fair Lawn, NJ / USA) for examination under a light microscope.

Negative controls were subjected to the same procedures performed with the positive control, and subjected to the immunohistochemical reaction described previously except for the incubation with the primary antibody, which was replaced with a buffer solution.

2.2. Immunohistochemical Analysis

The slides were analyzed under a light microscope by a calibrated observer to identify the presence or absence of labeling for each protein and its expression pattern. The results obtained were tabulated by means of the positivity index, that is, the areas of the midpalatine suture of Group I and Group II were evaluated and received marks classified in (/) absent; (-) negative; (+) weakly positive; (++) strongly positive according to the impregnation intensity of the chromogen substance and the extent of labeling.

The designation (/) absent refers to the absence of marking; (-) negative tissue presence but no marking; (+) weakly positive was applied in cases where there was low intensity of the chromogen or when the marking was not uniform showing positivity in focal areas; (++) strongly positive there was a lot of intensity of the chromogen or when the marking was not uniform showing positivity in focal areas.

After tabulation the data obtained from Group I (control) were compared with Group II (experimental).

3. Results

3.1. Microscopic Descriptive Analysis

3.1.1. Masson Trichrome Staining

The Figure 1, a, b, c, d, e, f, represents the microscopic image of the midpalatine suture of Wistar rat. Images a, b, c represent group I (control), and images d, e, f, group II (experimental). In Figure 1, group I is a panoramic view of the region, the arrow points to a stratified keratinized squamous epithelium of the palate region. The midpalatine suture is filled by loose connective tissue, with blood vessels, osteoblasts (c-arrow), and fibroblasts. In b, osteocytes are observed in gaps within the mineralized bone matrix (arrow).

After disjunction (d), the central region of the palatine suture is filled by a non-mineralized bone matrix, cartilaginous matrix, osteoblasts (f-arrow), chondroblasts, chondrocytes, and blood vessels. The arrow in E demonstrates the presence of an osteocyte within the non-mineralized bone matrix.

3.1.2. Hematoxylin and Eosin Staining

Figure 2 a, b, c, d, e, f, represents the microscopic image of the midpalatine suture of Wistar rat. The images a, b, c, represent group I and, the images d, e, f, group II. The presence of dense connective tissue, with blood vessels inside (arrow in b) and fibroblasts, is observed. The collagen fibers are organized in bundles and between this connective tissue and the bone tissue there is the presence of loose connective tissue indicative of periosteum rich in fibroblasts, osteocytes and osteoclasts. The bone tissue presents osteocytes trapped in gaps organized in concentric lamellae around the osteones.

After disjunction (d) a dense connective tissue with blood vessels, non mineralized bone tissue, blood vessels and mature bone tissue is observed at the center of the midpalatine suture. In the image and, there is a microscopic detail of the upper half of the region of the palatine suture, there is a connective tissue with presence of collagen fibers, scarce blood vessels, with the presence of fibroblasts. At the periphery, cuboidal cells, indicative of osteoblasts, are observed in an osteoid matrix. Neighboring this matrix is a neoformed bone tissue, with

the presence of blood vessels. It is not organized and the osteocytes are trapped in gaps. Attached to this tissue, we observe the mature bone tissue with organized osteones. Microscopically in the inferior region (f) of the palatine suture the presence of dense connective tissue, with the presence of collagen fibers and few blood vessels, is observed. There are also fibroblasts and scarce inflammatory infiltrate.

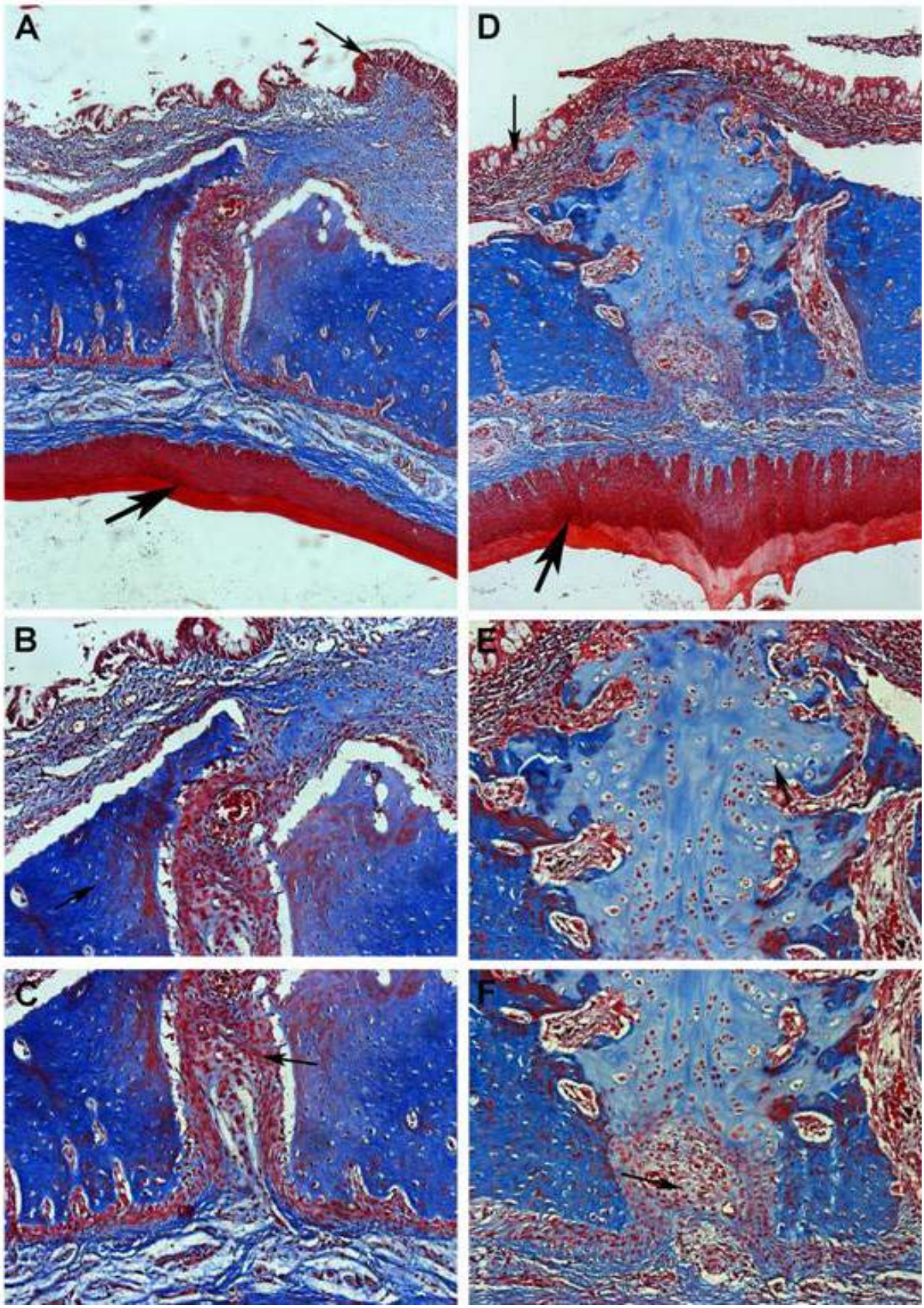

Figure 1. Group I a, b, c and Group II d, e, f; (a, d) panoramic view of the region of the medial palatine suture and arrow in the epithelial tissue, increase 100X; (b, e) detail of the upper region, with arrow in osteocyte; (c, f) detail of the lower region with arrow indicating osteoblast, 200X magnification, Masson's Trichrome

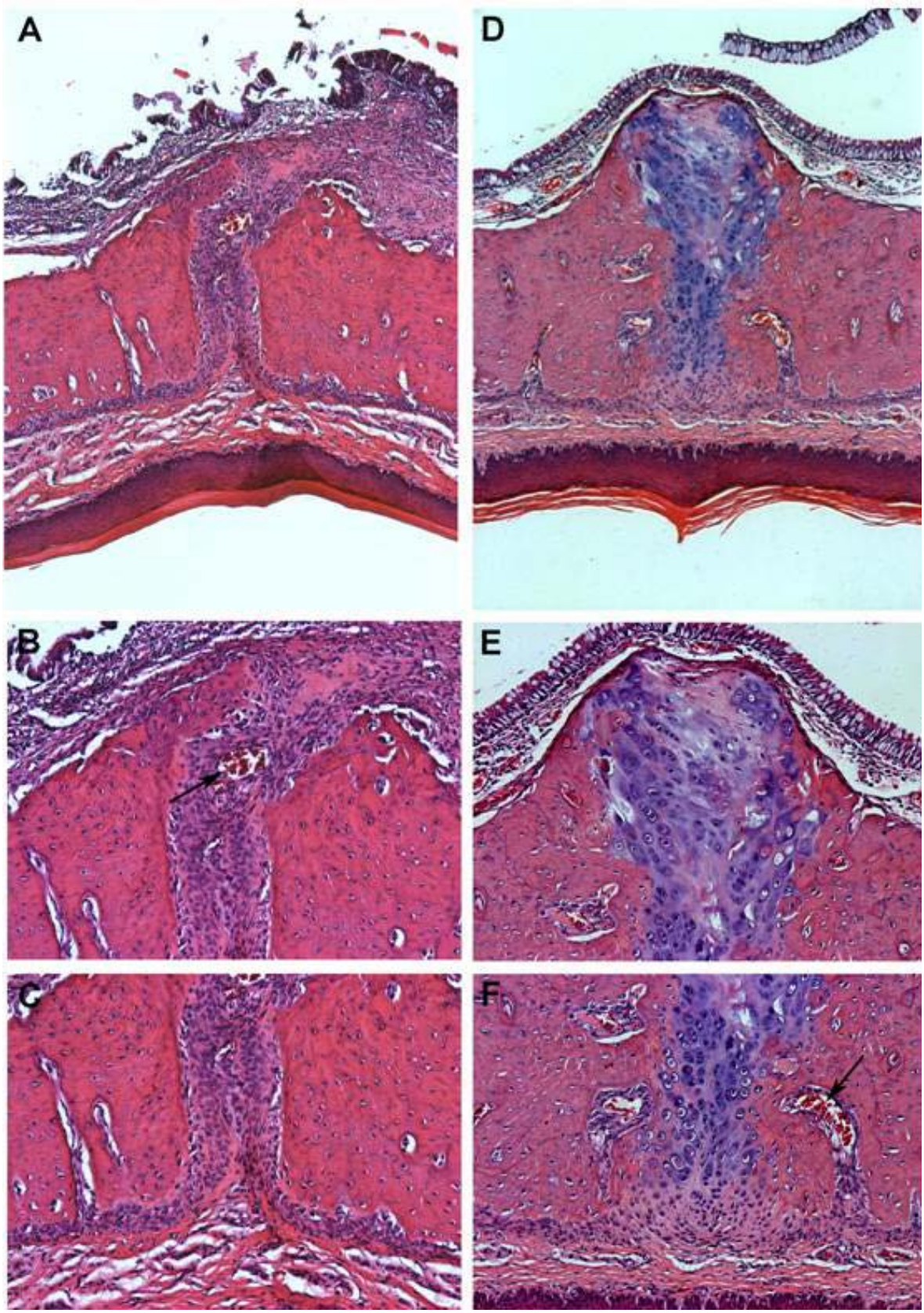

Figure 2. Group I a, b, c, and Group II d, e, f; (a, d) panoramic view of the region of the midpalatine suture, increase 100X; (b, e) detail of the upper region; (c, f) detail of the lower region; (b, f) presence of blood vessels, arrows increase 200X, staining Hematoxylin and Eosin

3.2. Microscopic Analysis of Immunohistochemical Reactions

The analysis of the immunohistochemical reactions for Bone Sialoprotein (BSP); Osteocalcin (OCC); Osteopontin (OPN) and Osteonectin (ONC) in the palatine suture region was performed by means of positivity index, comparing Group I to Group II, with the markings classified as (/) absent; (-) negative; (+) weakly positive; (++) strongly positive according to the impregnation

intensity of the chromogen substance and the extent of labeling (Table 1).

Table 1. Expression of BSP, OCC, OPN and ONC, in groups I and II

NONCOLLAGENOUS PROTEINS	BSP		OCC		OPN		ONC	
GROUPS	I	II	I	II	I	II	I	II
Osteoblasts	+	++	+	++	-	+	+	++
Osteocytes	+	+	-	+	-	+	-	+
Osteoid Matrix	/	++	/	+	/	+	/	++
Mineralized Matrix	/	++	+	+	+	+	+	+
Chondroblasts	/	++	-	++	-	+	+	++
Chondrocytes	/	+	/	+	/	+	/	++
Hypertrophic chondrocytes	/	+	/	++	-	/	-	++
Cartilaginous Matrix	/	+	/	+	-	+	/	++

(/) absent; (-) negative; (+) weakly positive; (++) strongly positive.

Figure 3 and Figure 4 represent the expression of BSP. In group I, a weakly positive marking was observed only in osteoblasts, osteocytes, chondroblasts and chondrocytes. This condition was maintained in group II in osteocytes and chondrocytes. For the osteoblasts, osteoid matrix, mineralized matrix and chondroblasts there was an increase in the protein with strongly positive labeling. Already for the osteoid matrix, hypertrophic chondrocytes and cartilaginous matrix BSP was found only in group II.

Figure 5 and Figure 6 demonstrate the expression of OCC. For group I, the protein showed a negative marking on the osteocytes, mineralized matrix and chondroblasts; absence in the osteoid matrix, hypertrophic chondrocytes and cartilaginous matrix. Only for the osteoblasts there was a strongly positive mark. OCC expression was found to be higher in all parameters analyzed in group II, and in the osteoblasts, chondroblasts and hypertrophic chondrocytes the labeling was strongly positive.

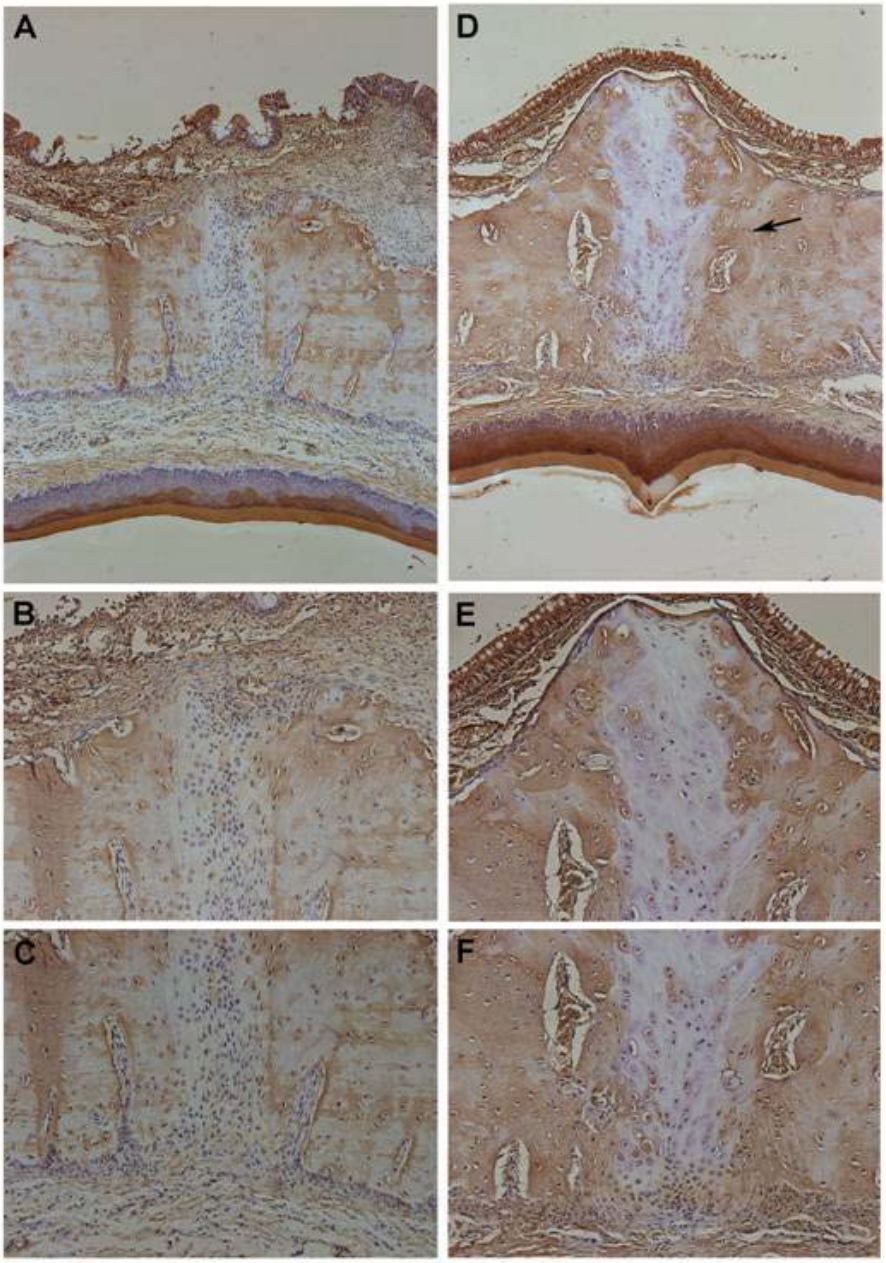

Figure 3. BSP expression. Group I a, b, c, and Group II d, e, f; (a, d) panoramic view of the region of the midpalatine suture, increase 100X; (b, e) detail of the upper region; (c, f) detail of the lower region; Osteocyte indicated on the arrow at d, increase 200X

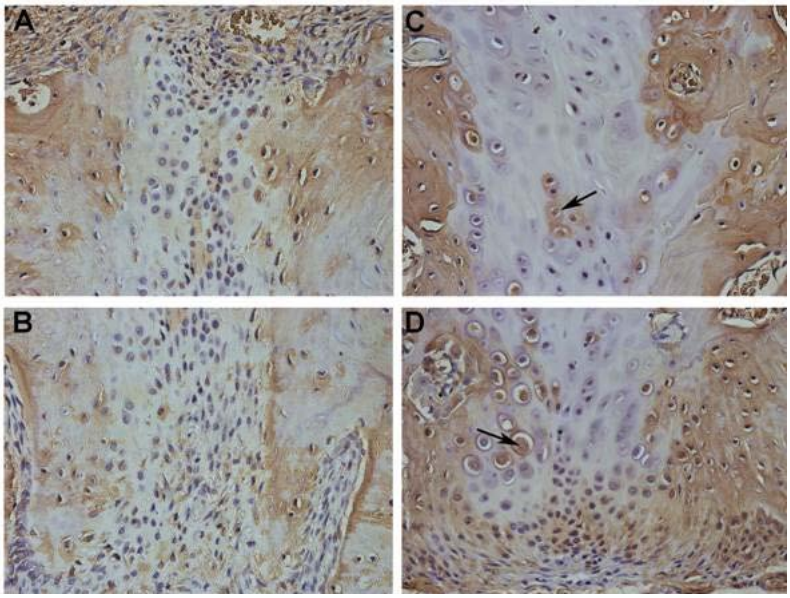

Figure 4. BSP expression in the midpalatine suture. Group I a, b, and Group II c, d, increase 400X; Osteoblast indicated on the arrow at c and presence of chondroblast at the arrow at d

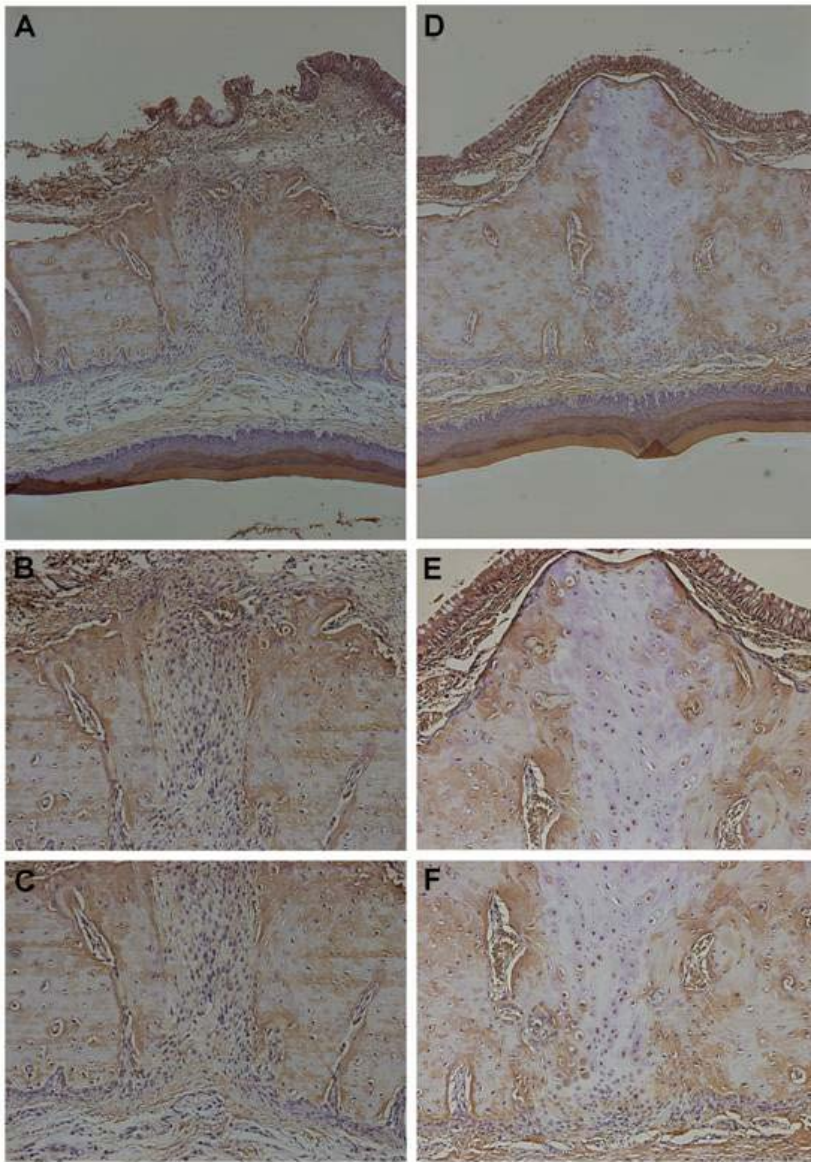

Figure 5. Expression of OCC. Group I a, b, c, and Group II d, e, e; (a, d) panoramic view of the region of the midpalatine suture, increase 100X; (b, e) detail of the upper region; (c, f) lower region detail, 200X magnification

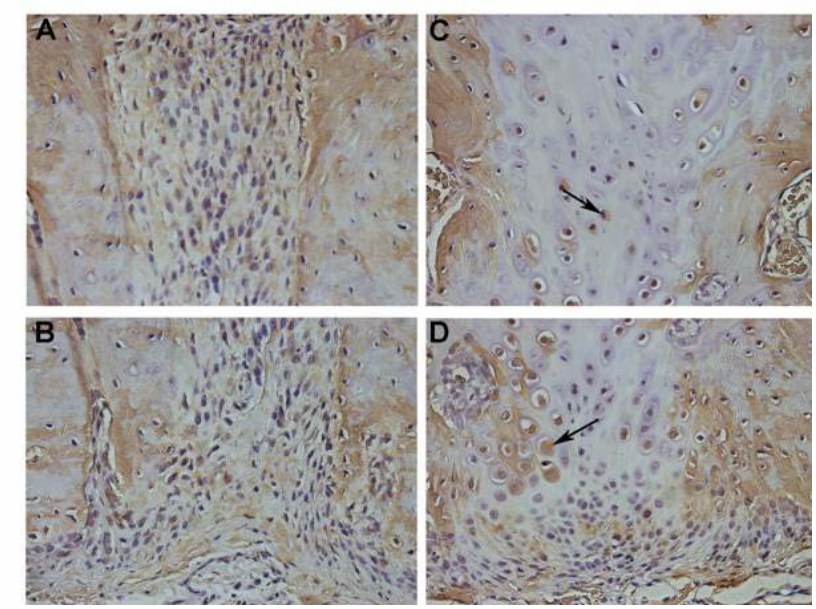

Figure 6. OCC expression in the midpalatine suture. Group I a, b, and Group II c, d, increase 400X; Osteoblast indicated on the arrow at c and presence of chondroblast at the arrow at d

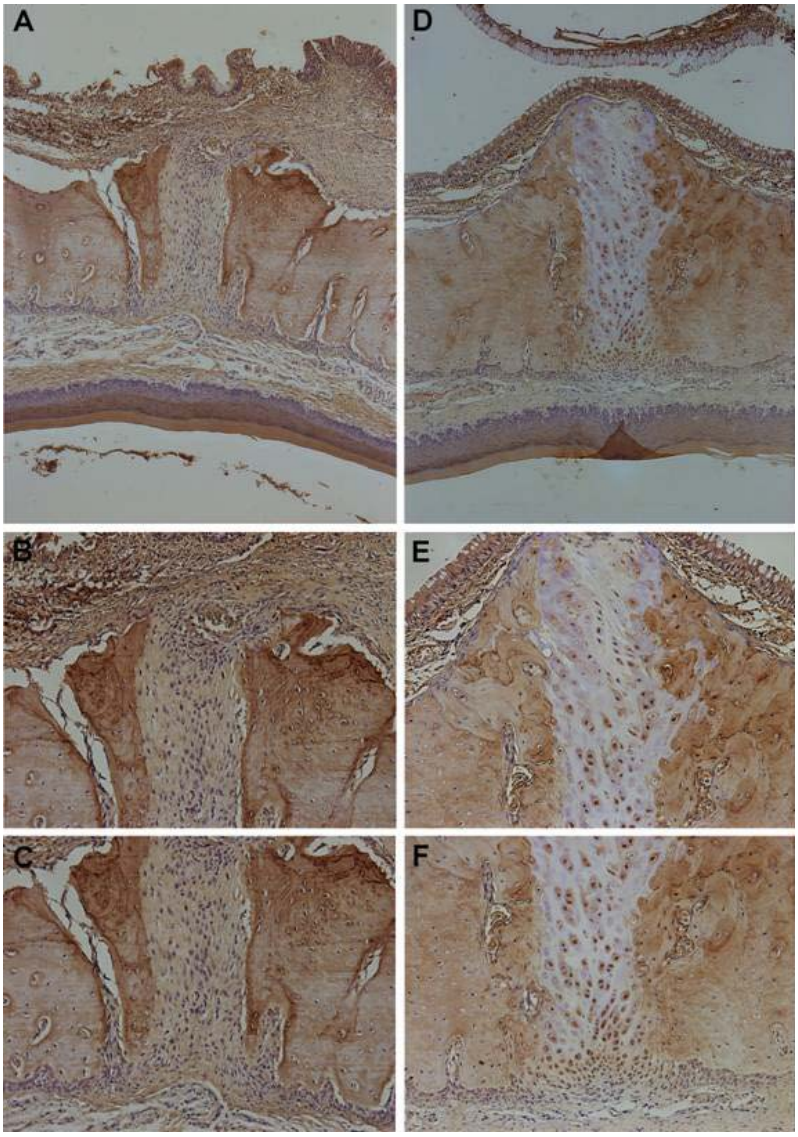

Figure 7. OPN expression. Group I a, b, c and Group II d, e, f; (a, d) panoramic view of the region of the medial palatine suture, increase 100X; (b, e) detail of the upper region; (c, f); lower region detail, 200X magnification

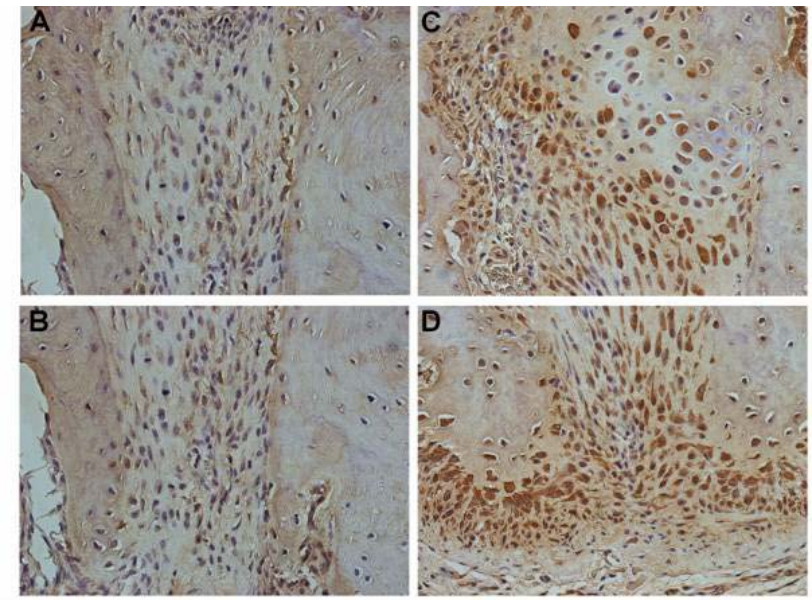

Figure 8. OPN expression in the midpalatine suture. Group I a, b, and Group II c, d, increase 400X

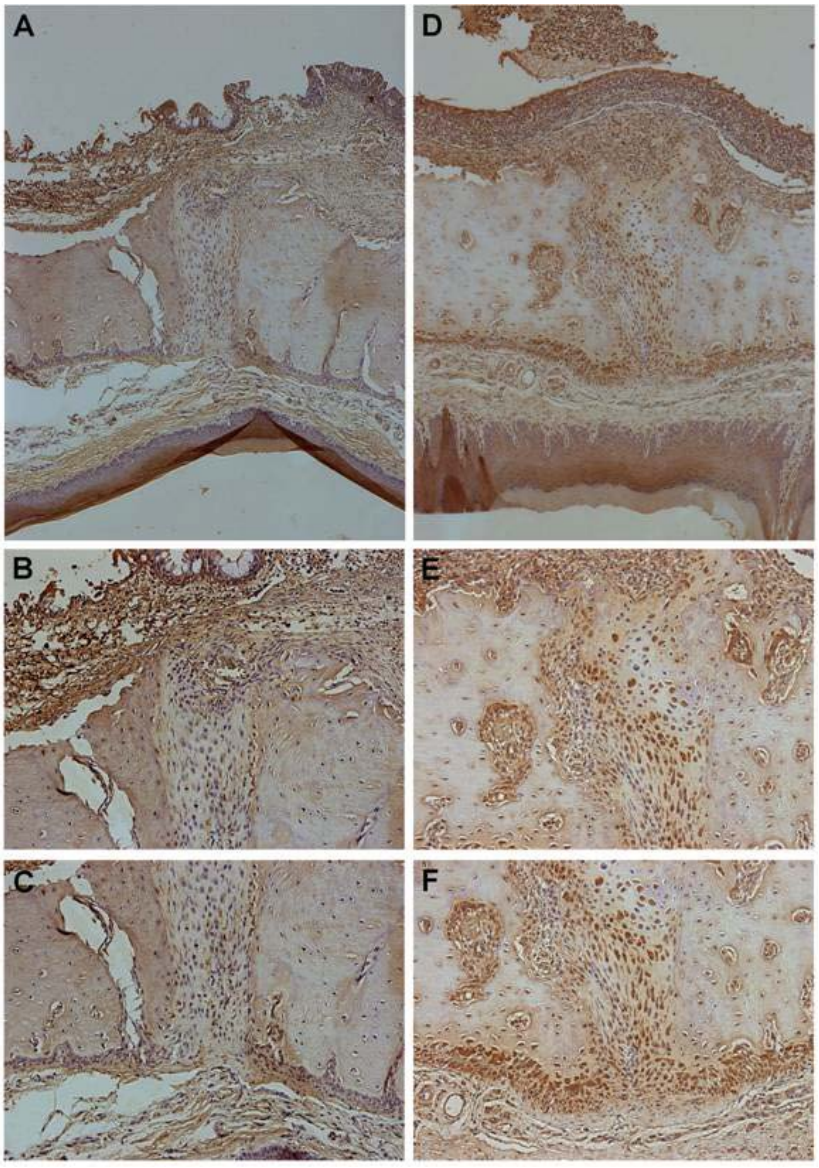

Figure 9. Expression of ONC. Group I a, b, c, and Group II d, e, f; (a, d) panoramic view of the region of the midpalatine suture, increase 100X; (b, e); detail of the upper region; (c, f) lower region detail, 200X magnification

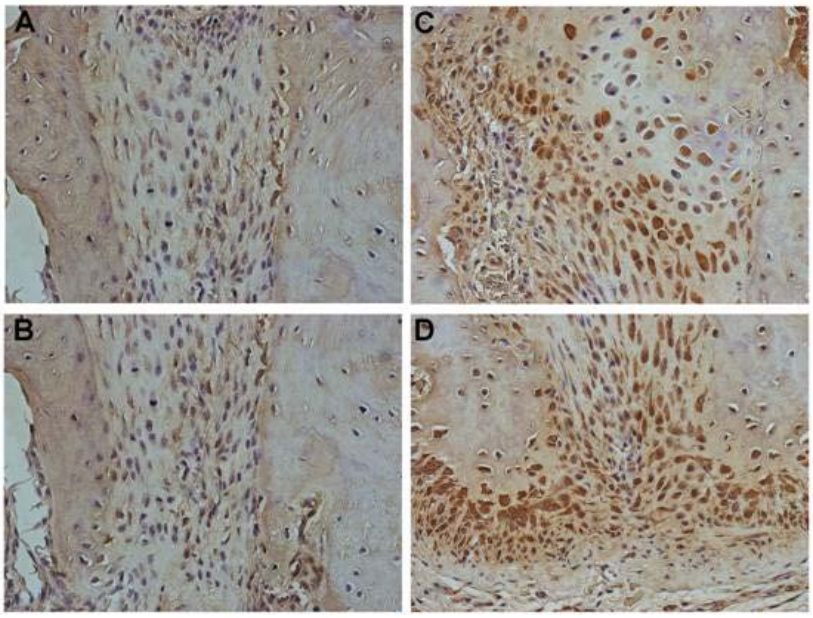

Figure 10. ONC expression in the midpalatine suture. Group I a, b, and Group II c, d, increase 400X

For OPN (Figure 7 and Figure 8) a negative mark was observed in all parameters analyzed in group I, with the exception of absence in the osteoid matrix. After the disjunction, the OPN was observed with a weakly positive marking in all analyzed parameters, except for the mineralized matrix that remained with the negative marking.

ONC (Figure 9 and Figure 10) was found to be weakly positive in osteoblasts and chondroblasts; with negative marking for osteocytes, osteoid matrix, mineralized matrix, chondrocytes and hypertrophic chondrocytes; absence in the cartilaginous matrix. In group II, its expression increased in all analyzed parameters. In the osteocytes and mineralized matrix the marking was weakly positive and in the remaining parameters, strongly positive.

4. Discussion

The procedures of disjunction of the maxilla by means of different devices that make possible the separation of the palatine suture have been much discussed, in animals as well as in human beings. HAAS [1,16] reported that maxillary disjunction allows palatal opening and CAPELOZZA FILHO et al. [17] agree and affirm that this opening presents in triangular form with base oriented towards the central incisors.

The maxillary growth occurs in two ways, by bone apposition and superficial remodeling [18]. According to CONSOLARO et al. [19], the sutures present great osteogenic and growth capacity, especially when stimulated by tension forces, which is confirmed by ARAÚJO; BUSCHANG [20] and DEL SANTO JR [21].

According to VIAZIS [22], the description of the palatine suture morphology provided an understanding of the effectiveness of maxillary disjunction. The separation of the median suture would be the most appropriate method to achieve transverse maxillary enlargement [23], and for this procedure some devices have been presented throughout history such as the Haas type considered tooth-tissue-born [1,3,16,24,25] and devices such as the Hyrax type [2,6] differ from the Haas type because they do not present acrylic on the palate.

In addition to the orthopedic effect, represented by the opening of the palatine suture, the maxillary disjunction can provide orthodontic effects [1,17,22,26,27], such as: inclination of alveolar processes; buccal inclination of the maxillary posterior teeth; extrusion of maxillary molars used as anchorage, which was confirmed by SILVA FILHO; CAPELOZZA FILHO, [27]; MARTINS et al., [28]; CAPELOZZA FILHO et al., [29]; GARIB et al., [5].

In an attempt to minimize the undesirable effects of anchorage teeth on palatal disjunction, an alternative came about through the use of mini-implants to avoid the use of the teeth as anchorage, minimizing these undesirable effects [11,12]. It was proved that direct disjunction in mini-implants prevents buccal inclination in posterior teeth. The clinical use of palatal disjunctions with anchorage by means of implants is already widely used [12], but the tissue events involved in this process are still not fully understood. According to CONSOLARO et al. [19], the sutures present great osteogenic and growth capacity, especially when stimulated by tension forces, which is confirmed by ARAÚJO; BUSCHANG [20] and DEL SANTO JR [21].

In the present study, the disjunction of the midpalatine suture was observed in Group II when compared to Group I. This region presented a dense connective tissue with blood vessels, non mineralized bone tissue and apart mature bone tissue. This connective tissue had collagen fibers, scarce blood vessels and fibroblasts. At the periphery, cuboidal cells, indicative of osteoblasts, were observed in an osteoid matrix. Neighboring this matrix is a neoformed bone tissue, with the presence of blood vessels. The same did not appear organized and the osteocytes were imprisoned in gaps. Mature bone tissue revealed organized osteones. These data were compared with the reports of PARR et al. [11] and ENLOW; HANS [30]. They report that the bone tissue formed in the region of the suture presents a great amount of spinal spaces. ENLOW; HANS [30] also reported that in the sutures an intense osteogenic process is found comparable to the growth of the periosteal bone and there is the replacement of soft tissue after disjunction by bone, connective tissue, epithelium and blood vessels.

VARDIMON et al. [31] described that the mineralization in the suture after disjunction is similar to a "closed zipper" with postero-anterior direction, confirming the report by CONSOLARO et al. [19] that the maxilla presents adaptive and reactive capacity of the periosteum obtained after the disjunction. The space between the two palatine processes will initially be filled by inflammatory exudate and blood clot that are then invaded by cells from the inner layers of the upper and lower periosteum. The primary bone matrix is deposited and is gradually replaced by mature bone, which confirms that presented by THILANDER et al. [32] found that the traction produced by orthopedic forces on the sutures causes a marked increase of osteoblasts, leading to bone formation, allowing the suture to recover a normal histological picture.

The presence of osteoblasts, osteoclasts and osteocytes, which are cells normally found in a bone tissue, were reported in Group II, as reported by CHAMBERS et al. [33], MEGHJI [34], RIGGS et al. [35] and HILL [36] in whose tissues the osteoblasts produce the matrix that mineralizes in a well-regulated way and this can be removed by the activity of osteoclasts, when activated. According to MEGHJI [34], the undifferentiated mesenchymal cells of the bone matrix can differentiate into osteoblasts, which then synthesize and secrete components of the bone matrix, such as osteopontin, osteocalcin, osteonectin and bone sialoprotein.

In this work, the immunohistochemical analysis was used for antigen of noncollagenous proteins: ONC, OPN, BSP and OCC in the samples of Group I and II of the midpalatine suture region. Biochemical tests play an important role in the evaluation and diagnosis of bone metabolism according to SEIBEL [37] and HIRAKI [14]. According to SEIBEL [37], the isolation and identification of cellular and extra-cellular components of the bone matrix have resulted in the development of molecular markers that are considered in the participation of bone resorption and formation. Among these markers, noncollagenous proteins are observed.

For BSP expression, a poorly positive labeling was observed in Group I only in osteoblasts and osteocytes. This condition was maintained in Group II in osteocytes. For the osteoblasts, osteoid matrix, mineralized matrix and chondroblasts there was an increase in the protein with strongly positive labeling. The increase in BSP expression, after disjunction, may be justified by the fact that this protein participates in the process of cell differentiation and activities, as well as maturation and mineralization of the bone matrix [13,38].

According to HIRAKI [14], BSP marking showed in most cases positivity in undifferentiated cells, osteoblasts, osteocytes, osteoid matrix and cartilaginous matrix, whereas in the mineralized matrix the marking ranged from negative to weakly positive, as found in this study.

The OCC expression was found to be higher in all parameters analyzed in Group II when compared to Group I, and in the hypertrophic osteoblasts, chondroblasts and chondrocytes the labeling was strongly positive. The study by MEYER et al. [39] suggested an important role of this protein in the process of mineralization stimulated by mechanical loading, as observed in Group II, which was mechanically stimulated by means of a disjunction spring. The strongly positive labeling in Group II osteoblasts can be explained by CHRISTENSON [40]. The author reports that OCC in its primary structure is a protein produced by osteoblasts during the bone mineralization phase, with a high affinity for mineral ions such as Ca2 + and hydroxyapatite crystals. VIEIRA [41] also stated that osteocalcin is produced almost exclusively by osteoblasts and its measure is highly specific for bone tissue, thus, correlates well with histological analyzes of formation and mineralization, as confirmed by WARREN et al. [42].

Group I showed absence of OCC in the osteoid matrix, which was later found in Group II. SEIBEL [37]suggested that this protein is involved in the process of osteoid mineralization during the bone formation phase.

UEMURA et al. [43] reported that OPN acts on bone remodeling through release by osteoblasts. In Group II of the present study a weakly positive labeling of this protein was found, whereas, before the disjunction, it was not observed. In a similar study, MORINOBU et al. [44] evaluated the expression and role of osteopontin in bone formation in vivo in rat calvaria when submitted to suture disjunction. The authors concluded that OPN expression increases during bone formation in palatal expansion and that its presence is a positive factor for bone neoformation.

It was also found a weakly positive marking on chondroblasts, chondrocytes, hypertrophic chondrocytes and cartilaginous matrix for Group II of the present study. PERRIEN et al. [45] suggested that in areas of endochondral ossification, OPN was strongly expressed in oval cells. Intramembranous ossification was expressed in pre-osteoblasts and osteoblasts, but not in the osteoblasts of the border of the osteoid matrix. According to the authors, OPN was virtually expressed in all osteocytes of neoformation and bone matrix, and this noncollagenous protein would be linked to the reception of mechanical stimuli of the bone tissue.

According to MARTINEZ; ARAÚJO [15], ONC can play an important role in bone mineralization in vivo, being the most abundant organic constituent of the non-collagen portion of the bone. In the present study, ONC expression showed a strongly positive marking in all parameters analyzed in Group II with the exception of the mineralized and osteocyte matrix, confirming the report of the previous study. The weakly positive ONC marking on the mineralized matrix and the osteocytes can be explained by the fact that this protein is more found in cells in the process of calcification when compared to already mineralized tissues [46].

5. Conclusion

By means of the results obtained in this study, it can be concluded that:

a) The use of a spring anchored in mini-implants promoted the disjunction of the midpalatine suture, with the presence of osteoblasts in an osteoid matrix, neoformed bone tissue with blood vessels and osteocytes;

b) The immunohistochemical expression of the following noncollagenous proteins: BSP, OCC, OPN and ONC increased after disjunction of the midpalatine suture in all analyzed parameters, and for ONC the expression was more significant.

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