*Yara Y. Mouselhy, Awatef I. Draz, Marwa M. El-Shafei, Dalia H. El-Rouby. Comparative immunohistochemical study on osteocalcin expression in fibrous dysplasia and ossifying fibroma. International Journal of Academic Research Part A; 2013; 5(1), 9-13. **DOI:** 10.7813/2075-4124.2013/5-1/2*

COMPARATIVE IMMUNOHISTOCHEMICAL STUDY ON OSTEOCALCIN EXPRESSION IN FIBROUS DYSPLASIA AND OSSIFYING FIBROMA

Yara Y. Mouselhy[1], Awatef I. Draz[4,2], Marwa M. El-Shafei[3]*, Dalia H. El-Rouby[4]

[1]Demonstrator of Oral Pathology, Faculty of Oral and Dental Medicine, Misr International University, Cairo **(EGYPT)**
[2]Professor of Oral Pathology, Faculty of Dentistry, King Abdul Aziz University **(KINGDOM OF SAUDI ARABIA)**
[3]Assoc. Professor of Oral Pathology, Faculty of Oral and Dental Medicine, Misr International University, Cairo **(EGYPT)**
[4]Professor of Oral Pathology, Faculty of Oral and Dental Medicine, Cairo University, Cairo **(EGYPT)**
***Corresponding author:** marwamokbel@gmail.com

DOI: 10.7813/2075-4124.2013/5-1/2

ABSTRACT

Objective: Fibrous dysplasia (FD) and ossifying fibroma (OF) show similar histological and radiological features, making distinction between them a diagnostic dilemma requiring clarification. **Study Design:** Forty (20 FD, 20 OF) paraffin-embedded specimens were immunohistochemically stained using the primary antibody Osteocalcin (OC). The immunostained area fraction was evaluated in the bone trabeculae alone and in both the bone trabeculae and the connective tissue stroma together. **Results:** All cases of FD and OF demonstrated OC immunostaining in both the bone trabeculae and the stromal cells. In FD, osteocalcin was more expressed in stromal cells, while in OF, immunoreactivity was more marked in bone trabeculae. The area fraction expressing OC in both the bone tarbeculae and fibrous stroma was significantly higher in FD than OF (p= 0.025). **Conclusion:** The stromal spindle-shaped cells in FD are of the osteoblastic lineage. Osteocalcin expression can be a significant support in differential diagnosis of FD and OF.

Key words: Osteocalcin, Fibrous dysplasia, Ossifying fibroma

1. INTRODUCTION

Benign fibro-osseous lesions of the jaws constitute a varied group of lesions with a common histological characteristic: the substitution of normal bone by tissue composed of collagen fibers and fibroblasts, with variable amounts of a mineralized substance that may be bone, cementum or both.[1] Among these lesions are fibrous dysplasia (FD) and ossifying fibroma (OF), both showing similar histological and radiological features, thus making distinction between the two a diagnostic dilemma.[2]

FD represents a bone developmental disorder, manifesting a defect in osteoblastic differentiation and maturation.[3] This dysplastic process is characterized by self-limited growth and apparent responsiveness to the hormonal changes of puberty.[4]

Cemento-ossifying fibroma is a benign fibro-osseous lesion of the jaws that consists of cellular fibroblastic tissue containing varying amounts of osteoid tissue, bone, cementum, and cementum like calcified tissue. This lesion seems to arise from the periodontal membrane, which contains pluripotential cells capable of forming cementum, bone, and fibrous tissue.[5] Ossifying fibroma is relatively slowly growing that may be present for a number of years before a diagnosis is made.[6]

Anubha et al,[7] stated that the dilemma in diagnosis of FD and OF rests in the bony trabeculae as well as in the fibrous stroma, where cases of FD, showing lamellated bony trabeculae and osteoblastic rimming have been reported and may confound diagnosis because of resemblance with OF. In their study, an attempt has been made to demonstrate the fibrous element of these two lesions using histochemical stains and the study revealed that the oxytalan fibers were more numerous in OF.

According to Toshihisa[8], type I collagen, osteonectin (ON), osteopontin (OP), and osteocalcin (OC) are bone matrix proteins. While also being phenotypic markers, they have characteristics that are often associated with osteoblastic differentiation. ON and OP appear in the early stages of osteogenic maturation, whereas OC appears in the late stages.

OC is a vitamin K-and vitamin D-dependent protein that has high affinity for calcium and exhibits a compact calcium-dependent α-helical conformation.[9] Osteoblasts synthesize OC which after production is partly

incorporated into the bone matrix and partly delivered to the circulatory system. Circulating OC is associated with changes in the rate of bone turnover and regarded as a specific marker for bone formation.[10]

The physiological role of OC is not precisely understood, but OC does serve as an inhibitor of crystal growth in vitro and has been postulated to play a role in osteoclast recruitment and bone resorption. Osteocalcin deposition in bone matrix is most likely a hallmark of mature, metabolically inactive bone, whereas OC itself may be a cell marker for osteoblastic differentiation (Sakamoto et al, 1999).[11] Immunohistochemical reactivity for OC was observed in the cytoplasm and plasma membranes in the stromal cells, calcified structures, and bone surrounding osteoblasts in neoplastic and bone-related lesions (Elias et al, 2010).[12]

Since fibrous dysplasia and ossifying fibroma show distinct patterns of disease progression, it is important to distinguish between the two. Therefore, this study was conducted to analyze the immunohistochemical expression of osteocalcin in FD and OF, in order to assess its potential role in differentiation between these two disease entities.

2. MATERIAL AND METHODS

Specimens collection

Forty formalin-fixed, paraffin-embedded specimens were included in the present study. These included 20 cases diagnosed as FD and 20 cases diagnosed as OF. All cases were collected from the archives of the National Cancer Institute and of the Oral Pathology Department, Faculty of Oral and Dental Medicine, Cairo University. Hematoxylin and eosin stained sections were used for confirmation of the diagnosis.

Immunostaining Procedure:

Four-µm-thick sections were deparaffinized with xylene, and rehydrated in graded ethanol. Endogenous peroxidase activity was blocked by immersion of slides in methanol with 0.03% hydrogen peroxide for 30 min. Sections were then microwaved in citrate buffer, pH 6.1, at 95°C for 10 min for antigen retrieval. Non-specific binding was blocked with 3% normal rabbit serum in phosphate-buffered saline, (DAKO, Glostrup, Denmark). The lyophilized primary antibody: Osteocalcin Rabbit anti-Human Polyclonal antibody (MyBioSource, LLC, San Diego, USA) was diluted in phosphate buffer saline (PBS), (10 mg antibody/ml PBS). Sections were incubated with the primary body overnight, then were stained according to the avidin-biotin complex method using the ready-to-use Ultravision Detection System Anti-Polyvalent (Lab Vision Corp., USA) and visualized using 3,3-diaminobenzidine (DAB). The specimens were subsequently counterstained with Mayer's hematoxylin.

Negative control sections were processed identical to experimental sections except that the primary antibody was omitted and replaced with phosphate buffer saline.

Immunohistochemical Evaluation:

In each slide, 5 microscopic fields showing the highest immunopositivity were selected and photomicrographed. Images were acquired using a digital video camera (Olympus, C5060, Japan), which was mounted on a light microscope (Olympus, BX60, Japan). The immunohistochemical reaction was evaluated by the image analysis software (Image J, 1.37v, NIH, USA). Computerized calculation of the total surface area of the immunoreaction was expressed as a fraction (percentage) of the total surface area of the microscopic field (immunostained area fraction, IAF). The immunostained area fraction was calculated twice, one time including both the bone trabeculae and the connective tissue stroma, whereas, the second calculation was made after clearing the fibrous tissue (to evaluate immunoreactivity confined to the bone trabeculae). The used method constitutes a time efficient and reproducible method for quantification of the immunoreaction.

Statistical assessment

Using the Statistical Package for Social Science (SPSS 15.0) Software, Student t test was performed for comparison of means. Comparison was made between immuno-positivity in bone trabeculae alone in FD and OF. Moreover, immunoexpression in both bone trabeculae and stroma in FD and OF were compared. The results were considered significant when p value was ≤ 0.05.

3. RESULTS

(I) Immunohistochemical findings:

All cases of FD (100%) demonstrated homogenous brown OC immunostaining positivity in both the bone trabeculae and the stromal cells. Immuno-positivity was more marked in the stromal cells than in the bone trabeculae (Fig. 1). Cells close to the bone trabeculae showed higher immuno-positivity than those away from the bone trabeculae *(Fig 2)*. OC expression was observed within the bone matrix, in osteocytes entrapped in bone trabeculae and in cells bordering the thin irregular Chinese letter-like trabeculae *(Fig 3)*.

All cases of OF (100%) showed OC immuno-positivity in both bone trabeculae and stromal cells; however the reaction was higher in the bone trabeculae than the stromal cells *(Fig 4)*. OC expression was observed within the bone matrix *(Fig 5)*, in osteocytes entrapped in lacunae and in the cytoplasm of the neoplastic fibroblasts *(Fig 6)*.