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International Journal of Current Research
Vol. 7, Issue, 07, pp.18487-18491, July, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

IMMUNOHISTOCHEMICAL EXPRESSION OF OSTEOCALCIN, TRANSFORMING GROWTH FACTOR BETA-1 AND BONE MORPHOGENETIC PROTEIN-7 IN FIBROUS DYSPLASIA AND OSSIFYING FIBROMA OF THE JAW BONES (A COMPARATIVE STUDY)

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ARTICLE INFO

Article History:
Received 02nd April, 2015
Received in revised form
08th May, 2015
Accepted 19th June, 2015
Published online 31st July, 2015

Key words:
Fibrous dysplasia,
Ossifying Fibroma,
Osteocalcin,
TGF-β1,
BMP-7.

ABSTRACT

Background: Fibrous Dysplasia (FD) & Ossifying Fibroma (OF) of the jaw are maxillofacial fibro-osseous lesions sharing an overlapping clinicopathological characteristics. This can be diagnostically challenging for pathologists.

Materials and Methods: A total of 30 retrospective formalin- fixed, paraffine - embedded tissue blocks were included in this study, 15 were diagnosed as FD and the other 15 were OF of the jaw bones. An IHC staining was performed using OC, TGF-β1 and BMP-7 monoclonal antibodies was performed.

Results: OC positive IHC expression was found in fibroblast-like cells in 4 cases(26.66%) of FD and in- 7 cases (46.67%) of OF, TGF-β1 was positively expressed in 8cases (53.3%) of FD and 10 cases (66.67%) of OF. BMP-7 showed positive expression in 2 cases (13.3%) of FD and 4 cases (26.7%) of OF.

Conclusion: TGF-β1expression in most of FD & OF cases suggests its role in the process of osteogenesis. OC may be a helpful marker to differentiate between these two entities. However, further studies are required to verify this fact.

***Citation*:** Farah G. Ibrahim, Seta A. Sarkis and Sami Kh. Jabbar, 2015. "Immunohistochemical expression of osteocalcin, transforming growth factor beta-1 and bone morphogenetic protein-7 in fibrous dysplasia and ossifying fibroma of the jaw bones (a comparative study)", *International Journal of Current Research*, 7, (7), 18487-18491.

INTRODUCTION

The term "fibro-osseous lesions" (FOLs) has gained wide acceptance as a general designation for a challenging group of pathologic conditions that often pose difficulty in diagnosis (Lasisi *et al*., 2014). Common to all these pathologic entities is the replacement of normal bone architecture by a benign fibrous tissue composed of fibroblasts and collagen, consisting of varying amounts of mineralized material (Riminucci *et al*., 2006). Categorization of the fibro-osseous lesions depends on correlation of the patient's history, clinical findings, radiographic criteria, and histopathology (Maheshwari *et al*., 2014). FD has its onset during early life, usually in late childhood or early adolescence although more severe forms can arise in infancy, whereas OF occur across a wide age range, with the greatest number of cases encountered during the third and fourth decades of life (AlamWg *et al*., 2003 and Neville *et al*., 2009). Gender prevalence of monostotic and polyostotic FD is equal, Whilefor OF a definite female predilection has been reported as high as 5:1 (Jung *et al*., 1999). The radiologic features of FD are diverse and are dependent upon the proportion of mineralized bone to fibrous tissue in the lesion, often described as ground glass appearance (Grasso *et al*., 2006). For OF The lesion usually has a distinct boundary and in the early stages it presents as a lucent area, as the lesion matures, bone densities appear, transforming the lesion into a radiopaque mass surrounded by a "halo" of less ossified tissue (Som and Curtin, 2003). Histopathologycally, The key histologic features of FD are delicate trabeculae of immature bone, with few or no osteoblastic rimming, enmeshed within a bland fibrous stroma of dysplastic spindle shaped cells without any cellular features of malignancy (Nelson and Thompson, 2003). OF lesions are composed of fibrous connective tissue stroma containing calcified structures, The calcified structures are composed of irregular trabeculae of osteoid or bone and lobulated basophilic masses of cementum or cementum-like tissue (Gnepp, 2011).

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Recent advances in biological research have brought the search for markers that can help to classify, characterize or understand the behavior of groups of pathologies. These markers include a variety of proteins usually including growth factors, and hormones among others (Sobral *et al*., 2014). Osteocalcin (OC) secreted exclusively by osteoblasts and its high serum levels are correlated with increased bone mineral density. It is therefore, used as biomarker for bone formation process and also has a role in regulation of osteoblast function (Lee *et al*., 2007). TGF-β1 is secreted by osteoblasts and is very abundant in bone matrix, it is a regulator of cell growth which will either stimulate or inhibit proliferation of mesenchymal cells depending on the presence of other growth factors (Massague, 1996). Bone morphogenetic protein -7 (BMP-7), is a member of transforming growth factor–b (TGF-b) superfamily, it is widely expressed during embryonic growth, and is an essential morphogen in renal, skeletal, and eye development (Jena *et al*., 1997). Since fibrous dysplasia and ossifying fibroma show distinct patterns of disease progression, it is important to distinguish between them. Therefore, this study was conducted to analyze the IHC expression of OC, TGF-β1 & BMP-7 in FD and OF, in order to assess its potential role in differentiating between these two disease entities.

MATERIALS AND METHODS

Sample: The study sample included thirty formalin -fixed, paraffin -embedded tissue blocks, which have been diagnosed as follows: fifteen FD of jaw bones dated from (1979 till 2013), fifteen OFof jaw bones dated from (1986 till 2013). The specimens were obtained from the archives of the department of Oral & Maxillofacial Pathology/ College of Dentistry/ University of Baghdad and Al-Shaheed Ghazi Hospital / Medical City / Baghdad. Four-micrometer-thick sections were cut from each paraffin tissue block and stained with hematoxylin (Mayer′s) and eosin for diagnostic confirmation. Another three 4-μm sections were cut from each tissue block and mounted on positively charged slides (Fisher super frost , USA) to be stained with monoclonal antibodies to OC , TGF-β1and BMP-7(Abcam). Negative and positive tissue controls were included into each immunohistochemical run (according to the manufacturer).

Immunohistochemical staining procedure: The sections were deparaffinized and rehydrated for IHC staining by OC, TGF-β1and BMP-7monoclonal antibodies. Heat mediated antigen retrieval was done for BMP-7 using citrate buffer PH (6.0) then the sections were immersed in hydrogen peroxide (H2O2) to block the endogenous peroxidase activity, washed in phosphate-buffered saline (PBS), and then protein blocking reagent was added and incubated for 20 minutes at 37°C within humid chamber to reduce the non-specific staining. The tissue sections were incubated with OC, TGF-β1and BMP-7 antibodies for one hour at 37°C. After that the slides were kept at 4°C overnight in humid chamber. The bounded antibodies were detected by the streptavidin-biotin complex method, after an immunoreaction, the sections were counterstained with Hematoxylin (Mayer's).

Assessment of immunohistochemical results: The staining analysis was evaluated by taking into consideration the number of positive and negative cells in the FD and OF samples. Quantitative analyses were evaluated in the stromal fibroblast-like cells. The number of cells in series of cases of FD and OF counted by selecting five microscopic fields at (40x) magnifying power and counting the cells at five fields. The total number of each power field was determined and then the summation was divided by five to acquire the average number of cells per high power field and converted into scores as follows: for OC, 0 (-ve),1(<25%),2 (25%-50%),3 (>50%) [15]; for TGF-β1, 0 (-ve),1(1–25%),2 (26%-50%),3 (50– 75%),4 (>75%) [16].While BMP-7, 0 (-ve),1(<25%),2 (25%-50%), 3 (50–75%),4 (>75%) (Haque et al., 2005 and Rauch et al., 2000).

Statistical analysis: The data was compiled into statistical software, statistical package of social sciences (SPSS) version 17. All variables were compared using Chi- square test. While spearman's coefficient correlation test was applied to plot a correlation matrix among the different IHC markers expression values altogether. P values of less than 0.05 were considered statistically significant.

RESULTS

The results revealed that most of the cases were females (73.33%) for each FD & OF (Table1). The age range was (8-35) years for FD and (7-50) years for OF. Most of FD cases presented in the maxilla (66.76%) while for OF most of the cases presented in the mandible (73.33%) (Table2). FD cases were more predominant in molar area (60%) whereas OF cases were more predominant in premolar & molar area (33.33%). Statistically significant difference was found between FD & OF regarding jaws & site distribution (P=0.028 &0.04) respectively (Tables 2&3). Positive OC expression was found in the fibroblast-like cells of 4 cases(26.66%) of FD and 7 cases (46.67%) of OF, chi-square test revealeda statistically significant difference regarding OC expression between both lesions (P=0.048). TGF-β1 was positively expressed in 8cases (53.3%) of FD and 10 cases (66.67%) of OF. BMP-7 was positive in 2 cases (13.3%) of FD and 4 cases (26.7%) of OF (Table4) Figures (1,2,3,4,5,6).

Table 1. sex distribution of the study sample

	FD		OF	
	No.	%	No.	%
Male	4	26.67	4	26.67
Female	11	73.33	11	73.33
Mean	19.3		25.2	
SD	7.961		10.923	

Table 2. Jaws distribution of the study sample

	FD		OF	
	No	%	No	%
Max.	10	66.67	4	26.67
Mand.	5	33.33	11	73.33
Fisher 'Exact test	Chi-square	P-value	Coefficient of Association	
0.033	4.821	0.028	0.498	

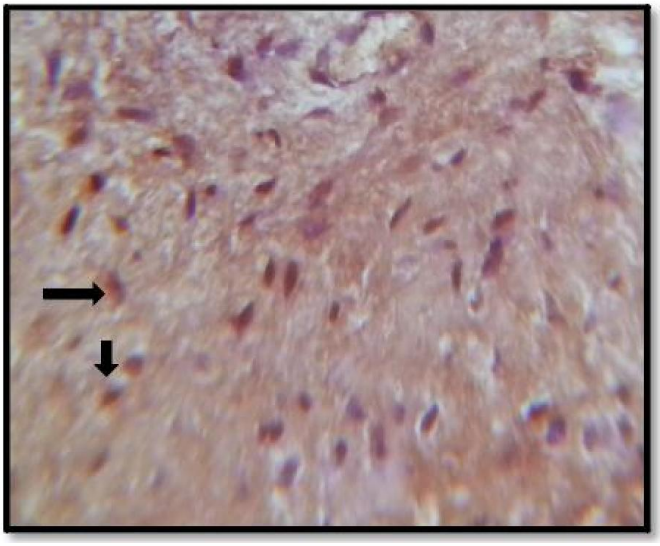

Figure 1. Positive OC cytoplasmic expression of the spindle fibroblast-like cells in FD (400x).

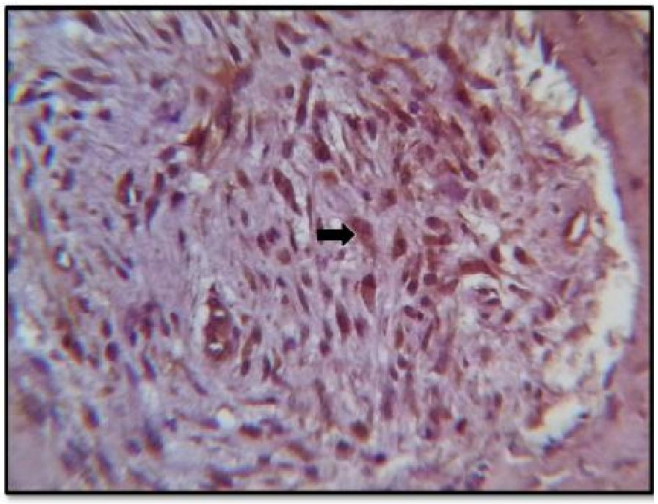

Figure 2. Positive OC cytoplasmic expression of the spindle fibroblast-like cells in OF (200x)

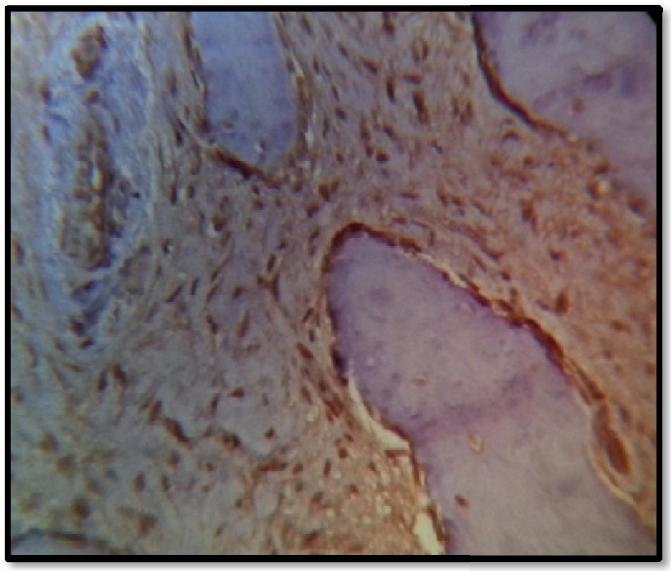

Figure 3. Positive TGF-β1cytoplasmic expression of the spindle fibroblast-like cells in FD (200x).

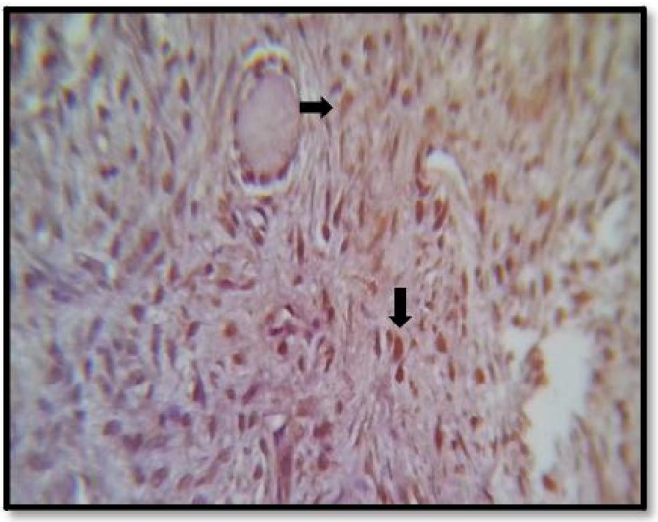

Figure 4. Positive TGF-β1cytoplasmic expression of the spindle fibroblast-like cells in OF (200x).

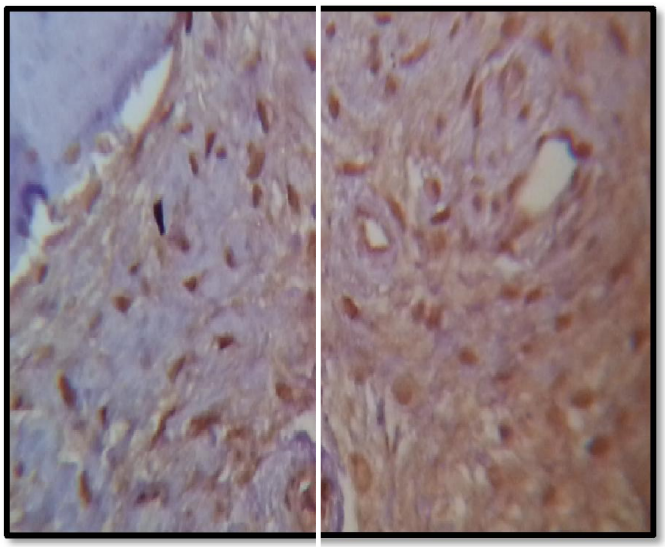

Figure 5. Positive BMP-7cytoplasmic expression of the spindle fibroblast-like cells in FD) (400x).

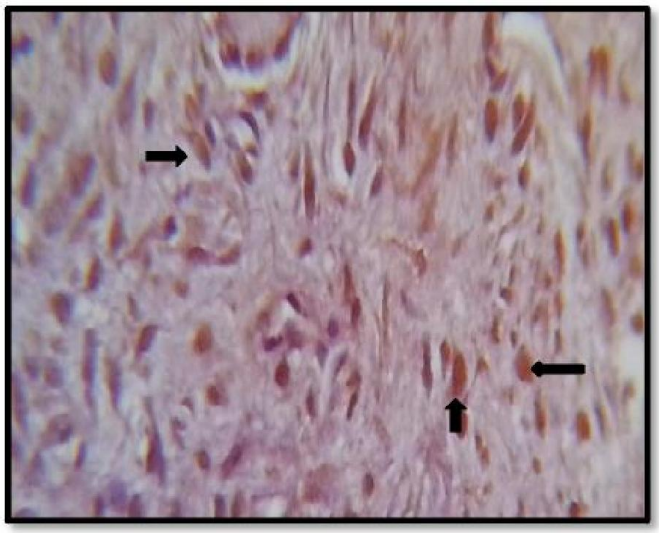

Figure 6. Positive BMP-7cytoplasmic expression of the spindle fibroblast-like cells in OF (400x).

DISCUSSION

Fibro-osseous lesions encompass a variety group of biologically and morphologically diverse bone tumors. They are all related to one another by the presence of pathologic ossifications and calcifications in association with a hypercellular fibroblastic marrow elements (Eversole *et al*., 2008).

Assessment of OC Immunohistochemistry: Osteocalcin, is secreted by fully differentiated osteoblasts and osteocytes and is functional in inhibiting crystal growth and enhancing recruitment of osteoclasts. It is a late marker of osteogenic cell maturation. This study showed OC positive immunostaining in (26.66%) of FD, and (46.67%) of OF cases. Since FD and OF contain osteoid producing cells which are not necessarily morphologically typical osteoblasts (Maheshwari *et al*., 2014) this could explain the negative expression of this marker in the fibroblast-like cells in most of our cases. Nevertheless, these pathologic cells may share differentiation steps with osteoblasts at the molecular level (Marie *et al*., 1997), this suggests that the fibroblast-like cells share some phenotypic features with osteoprogenitor cells of normal osteogenic tissues (Riminucci *et al*., 1997 and Sakamoto *et al*., 1999).

In the present study, OFs showed higher expression of OC staining than FD. Other authors also reported low OCexpression in FD. It seems that OF have the ability for forming hard tissue more than FD (Neville *et al*., 2009 and Zafar *et al*., 2012). Moreover FD usually exhibits mild to moderate cellularity while OF show moderate to severe cellularity (Sakamoto *et al*., 1999) this could be another explanation for this finding. However (Zafar *et al.*, 2012) reported strong OC IHC in the stromal fibroblast-like cells of FD &moderate expression in OF, and (Mouselhy *et al*., 2013) found positive OC in all cases of FD and OF in the stromal fibroblast-like cells. There was a statistically significant difference regarding OC expression of both lesions, suggesting that OC maybe a helpful marker to differentiate between these two entities. However, further studies are required to clarify many aspects of this perplexing group of lesion. The present findings pave the way for additional approaches to determine the mechanisms of mineralized tissue formation in this group of lesions.

Assessment of TGF-β1 Immunohistochemistry: Most of the cases showed positive expression of TGF-B1. This could be due to that fibroblast-like cells in these lesions share some phenotypic features with osteoprogenitor cells of normal osteogenic tissues (Riminucci *et al*., 1997 and Sakamoto *et al*., 1999). Additionally the expression was higher in OF rather than FD, thereby suggesting that fibroblast-like cells of OF have greater bone-forming ability than those of FD, resulting in the osteosclerosis in OF characterized in the roentgen graphs. Similarly (Sakamoto *et al.*, 2007) reported lower expression of TGF-ß1 in the fibroblast-like cells of FD. However (Sobral *et al*., 2014) reported negative expression of TGF-B1 in Fibrous dysplasia cases. Furthermore (Slootweg, 1996) reported that TGF-B1 provides a significant advantage for tumor formation, and (Neville *et al.*, 2009) stated that TGF-B1 was expressed more highly in areas of osteogenesis. All the aforementioned facts possibly justify the more positive cases of the ossifying fibroma due to its neoplastic nature.

Assessment of BMP-7 immunohistochemistry: BMP-7, a protein relateded to the BMP family which are identified by their ability to induce de novo bone formation at non-bone sites (Eisenberg *et al*., 1997 and Brannon *et al*., 2001). This unique osteoinductive property is due to activation of the canonical BMP signaling pathway in mesenchymal cells and to date, all BMPs that activate canonical BMP signaling have been shown to possess osteoinductive activity (Riminucci *et al*., 1997). Since Fibroblast-like cells in OF and FD share some phenotypic features with osteoprogenitor cells of normal osteogenic tissues (Suizbacher *et al*., 2002 and Riminucci *et al*., 1997) these facts explain the presence of labeling in this study even in low percentage. Furthermore previous studies have shown that BMP-7 generally was expressed more highly in osteosarcoma (OS) lesions. The findings described in the literature suggest that the BMP-7expression is more related to tumor progression of malignant cells, this agrees with (Suizbacher *et al*., 2002) whom reported high expression of BMP-7 in OS. These facts possibly justify the absence of labeling in most of our cases.

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