Dentistry
ISSN: 2161-1122

Goel et al., Dentistry 2019, 9:4

Research Open Access

Evaluation of α-Smooth Muscle Actin (α-SMA) in Intraosseous lesions of Jaw-An Immunohistochemical Study

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Received date: April 25, 2019; **Accepted date:** May 07, 2019; **Published date:** June 13, 2019

Abstract

Introduction: Fibroblasts associated with tumor stroma called as "peritumoral fibroblasts", "cancer-associated fibroblasts" or "myofibroblasts" encompass heterogenous and multifunctional cell populations, manifesting various phenotypes. Myofibroblasts are known to play a pivotal role in normal growth and development, wound healing, organ fibrosis and cancers. The role of myofibroblasts in the remodeling of ECM in various intraosseous lesions may affect the nature and growth of these lesions by influencing matrix formation, cellular proliferation, cellular migration, angiogenesis, and extracellular proteolytic activity.

Aim and Objective: The following study was undertaken to evaluate the expression of α- smooth muscle actin in intraosseous lesions of the jaw and to correlate their role with the pathogenesis or progression of these diseases if any.

Material and Methods: A total of 75 cases of Intraosseous lesions of the jaw formed the study sample. The study sample was further categorized into six groups as- Inflammatory lesions, Reactive Lesions, Benign Neoplasms, Malignant Neoplasms, Odontogenic Cysts, and Fibro-osseous Lesions. The sections were stained using Standard Hematoxylin and Eosin staining and Immunohistochemical staining for α-Smooth Muscle Actin (α-SMA). The sections were scanned at low magnification to identify positively stained areas (Hot Spots). Maximum of 10 hotspots (positive fields) were selected for quantitative scores.

Statistical Analysis: Data was examined for statistical significance (p-value) using One-way analysis of variance (ANOVA) for four groups and Post Hoc tests for multiple comparisons (Tukey HSD).

Results: Group II (Reactive lesions) shows highest immunoexpression (2.56) followed by Group IV (Malignant neoplasms-1.83), Group III (Benign neoplasms-1.67), Group VI (Fibro-osseous lesions-1.57), Group V (Odontogenic jaw cysts-1.50) and Group I (Inflammatory lesions-0.40).

Conclusion: The understanding of expression and activity of myofibroblasts may thus be necessary for the application of anti-Myofibroblastic drugs in the therapy.

Keywords: Myofibroblasts; Odontogenic; Smooth muscle actin; Benign; Malignant

Introduction

Intraosseous lesions of the jaw have been reported to show a varied behavior ranging from benign, localized, small well-circumscribed lesions to large, aggressive ones and even those involving extensive bone destruction to the extent of bony perforation. The coordinated activity between epithelial cells and their stroma is fundamental in controlling growth and differentiation in the physiological and pathological environment [1]. It is a well-known fact that malignancies are composed of tumor cells and tumor-associated host cells. The latter termed as 'tumor microenvironment' constituting half of the mass of malignancies, encompassing blood and lymphoid cells, endothelial cells, inflammatory cells, immunocytes, macrophages, fibroblasts and myofibroblasts [2]. Fibroblasts associated with tumor stroma called as "peritumoral fibroblasts", "cancer-associated fibroblasts" or "myofibroblasts" encompass heterogenous and multifunctional cell populations, manifesting various phenotypes. The disclosed 5 cytoskeletal phenotypes based on the three filaments namely, vimentin, desmin, myosin and α-smooth muscle actin are Phenotype V type (vimentin), VD type (vimentin and desmin), VAD type (vimentin, smooth muscle actin and desmin), VA type (vimentin and actin) and VAM type (vimentin, smooth muscle actin and myosin) [3]. During embryogenesis, myofibroblasts are differentiated from progenitor neural crest cells on the 13th gestational week. Transforming growth factor-β and Platelet-derived growth factors seem to be a central regulator of various embryonic events. The former is involved in the transdifferentiating process while the latter in cell activation, proliferation and cell motility [4]. Myofibroblasts are known to play a pivotal role in normal growth and development, wound healing, organ

fibrosis and cancers. The role of myofibroblasts in the remodeling of ECM in various intraosseous lesions may affect the nature and growth of these lesions by influencing matrix formation, cellular proliferation, cellular migration, angiogenesis, and extracellular proteolytic activity.

Therefore, the following study was undertaken to evaluate the expression of α-smooth muscle actin in intraosseous lesions of the jaw and to correlate their role with the pathogenesis or progression of these diseases, if any.

Materials and Methods

A cross-sectional study was conducted from 2012 to 2014, in the department of oral and maxillofacial pathology, microbiology and forensic odontology, MMCDSR, Haryana (India).

A sample size of 75 was estimated after setting the level of significance as 95% and power of study as 80%. A total of 75 cases of Intraosseous lesions of the jaw formed the study sample. Formalin-fixed paraffin-embedded blocks of previously diagnosed cases were retrieved from the archives of the department. Included cases were devoid of considerable inflammatory infiltrate. The total study sample was categorized into Six Groups as follows:-

Group 1: 10 cases of inflammatory intraosseous lesions

- 5 cases of Periapical granuloma (PG)
- 5 cases of Osteomyelitis (OM)

Group 2: 9 cases of reactive intraosseous lesions

- 9 cases of Central giant cell granuloma (CGCG)

Group 3: 27 cases of benign intraosseous neoplasms

- Non Aggressive Lesions: 5 cases of Adenomatoid Odontogenic Tumor (AOT)
- Aggressive Lesions: 1 case of Osteoblastoma (OB)5 cases of Unicystic Ameloblastoma (UA)11 cases of Multicystic Ameloblastoma (MA)5 cases of Keratocystic Odontogenic Tumor (KCOT)

Group 4: 12 cases of malignant intraosseous neoplasms

- 1 case of intraosseous Squamous Cell Carcinoma (OSCC) of the jaws
- 2 cases of Osteosarcoma (OS)
- 5 cases of Ameloblastic Carcinoma (Am carcinoma)
- 4 cases of Mucoepidermoid Carcinoma (MEC)

Group 5: 10 cases of odontogenic cysts

- Secondary Inflammation was excluded from the study
- Inflammatory Cysts: 5 cases of Radicular Cysts (RC)
- Developmental Cysts: 5 cases of Dentigerous cyst (DC)

Group 6: 7 cases of fibro-osseous lesions

- 3 cases of Fibrous Dysplasia (FD)
- 3 cases of Cemento-Ossifying Fibroma (COF)
- 1 case of Cemento-Osseous Dysplasia (COD)

From the paraffin-embedded tissue blocks, 2 tissue sections each of 4 μm thickness were obtained, using Semiautomatic microtome (Shandon Finesse E/ME). The first set of sections was taken on albumin coated slides for Hematoxylin and Eosin staining and the second set of sections on poly-L-lysine coated slides for immunohistochemical staining. The sections were stained using Standard Hematoxylin and Eosin staining and Immunohistochemical staining for α-Smooth Muscle Actin (α-SMA). IHC staining protocol was standardized by subject experts before conducting the study.

Procedure for Immunohistochemical Staining using A-Smooth Muscle Actin

- Tissue sections from paraffin-embedded, formalin-fixed tissue blocks were mounted on poly-L-lysine coated slides
- The sections were deparaffinized by keeping at 60 degrees centigrade in the oven for 15 minutes and then transferred to three changes of xylene for 10 minutes each
- The sections were hydrated through descending grades of ethyl alcohol (100% alcohol, 90% alcohol, 50% alcohol) for 5 minutes each
- The antigen retrieval was performed in EZ Retriever system in 2 cycles:
- Cycle 1: 980 C for 5 minutes
- Cycle 2: 950 C for 8 minutes
- The slides were then cooled for half an hour
- The slides were washed with phosphate buffer saline (3 cycles for 2 minutes each)
- The endogenous peroxidize was blocked by treating the sections for 10 to 15 minutes in NovocastraTM Peroxidase block
- The slides were washed again in phosphate buffer saline (3 cycles for 2 min each)
- NovocastraTM Protein block was added and incubated for 10 to 15 minutes. The excess liquid was wiped away from the surrounding surfaces of the tissue section with paper pads. The slides were laid flat and prediluted primary antibody was added
- One set of slides was stained with antibody for α-smooth muscle actin (Dako A/S, Denmark, clone 1A4), dilution 1:100, for 60 min in a moist chamber at room temperature
- The slides were then washed with phosphate buffer saline (3 cycles for 2 minutes each)
- NovocastraTM Post-primary antibody was added on the tissues and incubated for 30 minutes followed by NovolinkTM Polymer for 30 minutes
- The slides were washed with phosphate buffer saline (3 cycles for 2 minutes each)
- Liquid DAB+Substrate chromogen system was added for 10 minutes till the brown colour appeared
- The slides were put in distilled water for 5 minutes
- The slides were counterstained with NovocastraTM haematoxylin (2-3 dips)
- The slides were kept in running tap water for 5 minutes
- The slides were hydrated through descending grades of ethyl alcohol (100% alcohol, 90% alcohol, 50% alcohol) for 5 minutes each
- The slides were cleared in two changes of xylene for 5 minutes each
- The slides were mounted using DPX

Evaluation of Immunohistochemically Stained Tissue Sections (A-Smooth Muscle Actin)

All stained areas, demonstrating positivity for α-smooth muscle actin were assessed at a magnification of 20x of the light microscope. Non-inflammatory, non-endothelial stromal cells showing positive cytoplasmic immunostaining were recognized as α-SMA positive myofibroblasts. The analysis will be performed under a 20x objective and 10x ocular lens (x200 magnification). The sections were scanned at low magnification to identify positively stained areas (Hot Spots). Maximum of 10 hotspots (positive fields) were selected for quantitative scores.

Quantitative criteria

The whole section was evaluated at 20x objective to determine the percentage of a number of positively staining stromal cells.

Score 0: 1% to 25% positive stromal cells

Score 1: 26% to 50% positive stromal cells

Score 2: 51% to 75% positive stromal cells

Score 3: 76% to 100% positive stromal cells

Data was stored and analyzed. Mean, median and standard deviations for α-SMA were calculated for all the six groups. Data was further examined for statistical significance (p- value) using One-way analysis of variance (ANOVA) for four groups and Post Hoc tests for multiple comparisons (Tukey HSD). 'p' value <0.05 was considered as statistically significant (marked in tables by '*'; while p value <0.001 was considered as statistically highly significant (marked in tables by '**').

Results

- Group I Inflammatory intraosseous lesions showed the mean expression as 0.40 ± 0.699. The subgroups i.e. periapical granuloma and osteomyelitis showed the mean expression as 0.20 ± 0.447 and 0.60 ± 0.894 respectively
- Group II Reactive intraosseous lesions consisting of Central giant cell granuloma showed the highest mean expression as 2.56 ± 0.726
- Group III Benign intraosseous lesions showed the mean expression as 1.67 ± 1.144. It consisted of 5 subgroups: Unicystic Ameloblastoma 2.20 ± 0.447 showed the highest mean value (2.20 ± 0.447) followed by Osteoblastoma (2.00 ± 0), Keratocystic Odontogenic Tumor (2.00 ± 0.707), Multicystic Ameloblastoma (1.64 ± 1.362) and Adenomatoid Odontogenic Tumor showed the least value (0.60 ± 0.894)
- Group IV Malignant intraosseous lesions showed the mean expression as 1.83 ±1.115. Within subgroups Squamous cell carcinoma showed the highest value (3.00 ± 0) followed by Osteosarcoma (2.50 ± 0.707), Mucoepidermoid carcinoma (2.00 ± 0.816) and Ameloblastic carcinoma (1.20 ± 1.304)
- Group V Odontogenic intraosseous cysts showed the mean expression as 1.50 ± 0.972. In this group, Radicular cysts and Dentigerous cysts displayed the mean expression as 0.80 ± 0.837 and 2.20 ± 0.447 respectively
- Group VI Fibro-osseous lesions showed the mean expression as 1.57 ± 1.272. Out of three subgroups, Central ossifying fibroma (2.33 ± 0.577) displayed a maximum score followed by Fibrous dysplasia (1.33 ± 1.528) and Cement-osseous dysplasia (00)

Discussion

The present research was undertaken to evaluate and correlate the immunoexpression of α-SMA in intraosseous lesions of jaws.

A total of 75 cases formed the study sample, which were divided into six groups; Group I (10 cases) comprised of inflammatory lesions, Group II (9 cases) consisted of reactive lesions, Group III (27 cases) enclosed benign lesions, Group IV (12 cases) included malignant lesions, Group V (10 cases) included odontogenic jaw cysts and fibro-osseous lesions with 7 cases formed Group VI.

Inflammatory lesions were further divided into 2 subgroups which included 5 cases of periapical granulomas and 5 cases of osteomyelitis. The resident cells of any tissue are "stress shielded" by a matrix that they deposit and remodel.

During the past years, it has been accepted that myofibroblasts which are specialized contractile fibroblasts, plays an important role in establishing tension during wound healing.

Though myofibroblasts are present in granulation tissue during wound closure or pathological contracture tissue as the traction forces generated by fibroblasts in vivo though are sufficient to initiate wound closure but subsequent resistance in surrounding tissue induces myofibroblastic differentiation which generates greater contractile forces and hence, the initiation of contraction [5].

The periapical granuloma lesions showed a mean α-SMA immunoexpression as 0.20+0.447 per high power fields.

The number of α-SMA positive cells on the periphery of the lesion was greater than in the central region as an attempt to limit the surface area of the damaged underlying tissue (Figure 1).

This is accomplished because the myofibroblasts are connected to each other in a syncytium (because of fibronexus adhesion complex) and presence of α-smooth muscle actin and myosin isoforms [6].

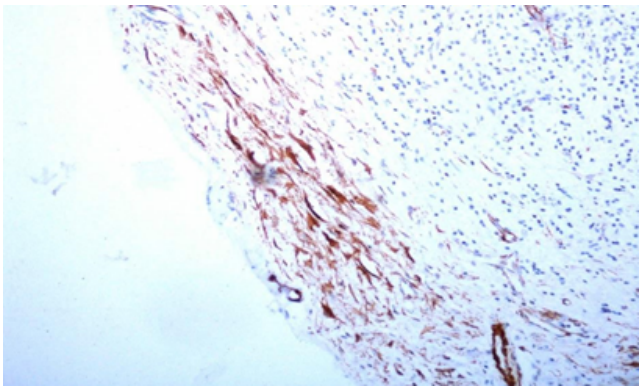

Figure 1: Photomicrograph showing α-SMA immunoexpression (Periapical Granuloma) Original magnification x200.

In the present study, osteomyelitis lesions showed a mean score of 0.60+0.894 per high power fields (Figure 2). Tissue inflammation has been shown to modulate the matrix by inducing transdifferentiation of fibroblasts into myofibroblasts. Myofibroblasts are known to modulate inflammatory response by secreting chemokines, proinflammatory cytokines, and prostaglandins such as COX-1 and COX-2 products, adhesion molecules as VCAM and ICAM. Also, following infection, osteoblasts display high expression of cytokine-like IL-6 inducing both COX-1, COX-2 and, ICAM and VCAM for cross-linking with the opposing cells to facilitate bone resorption [7].

In the present study, Group II (reactive lesions) enclosing central giant cell granulomas showed a mean α-SMA immunoexpression of 2.56+0.726 with minimum value of 1 and maximum value of 3 (Figure 3). Central giant cell tumors of bone are benign but aggressively

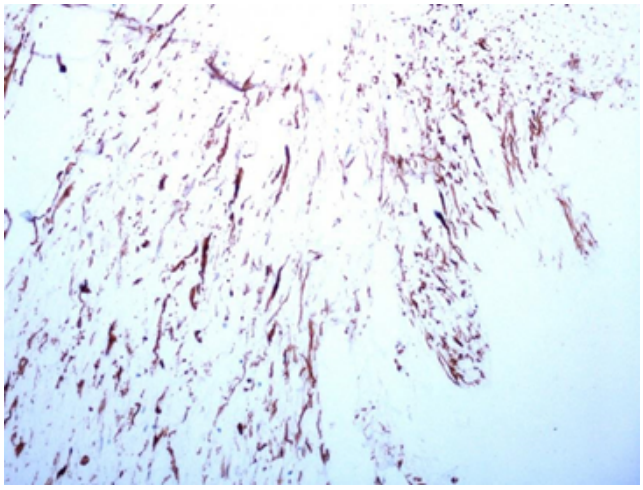

Figure 2: Photomicrograph showing α-SMA immunoexpression (Osteomyelitis) Original magnification x200.

destructive osteolytic lesions El labban and Lee KW, 1983 performed an ultrastructural study to observe the role of myofibroblasts in CGCGs of jaws [8]. They suggested that the majority of stromal cells resembled fibroblasts with intracytoplasmic myofilaments and dense bodies, the so-called Myofibroblasts. They further added that these myofibroblastic cell types were found close to giant cells in lesions and signs of early fusion explained the increased size of giant cells.

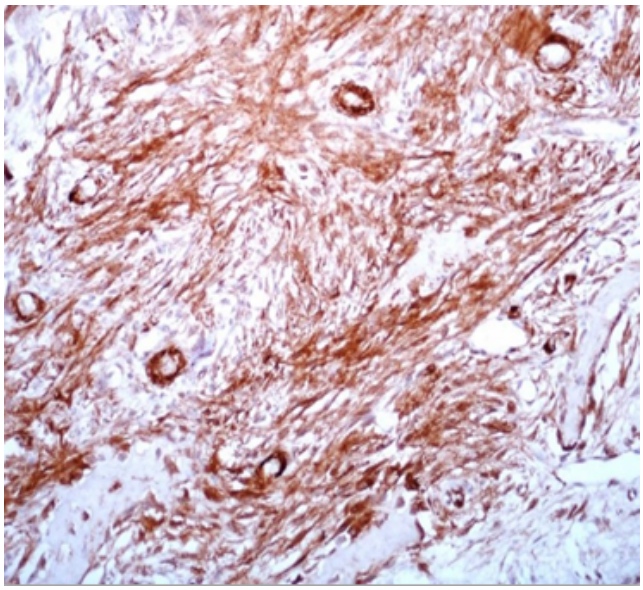

Figure 3: Photomicrograph showing intense α-SMA immunoexpression (central giant cell granuloma) Original magnification x200.

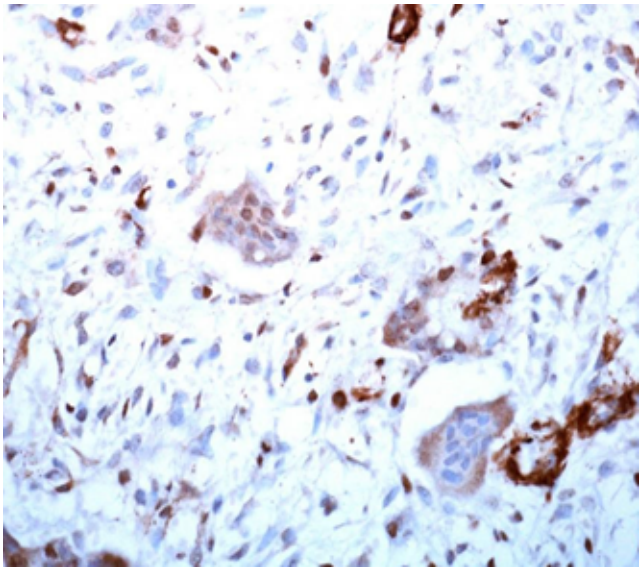

Figure 4: Photomicrograph showing α-SMA immunoexpression in giant cells (central giant cell granuloma) Original magnification x400.

In the present study, we observed the immunoexpression of α-SMA in mononuclear stromal cells in all the 9 cases and in multinucleated giant cells in 3 cases out of 9 cases of CGCG (Figure 4) which may be related to the aggressive nature and bony resorption associated with these lesions. Also, Omalley M et al. proposed CGCGs as fibroblastic and myofibroblastic tumors in which macrophages appear to occupy a secondary role [9].

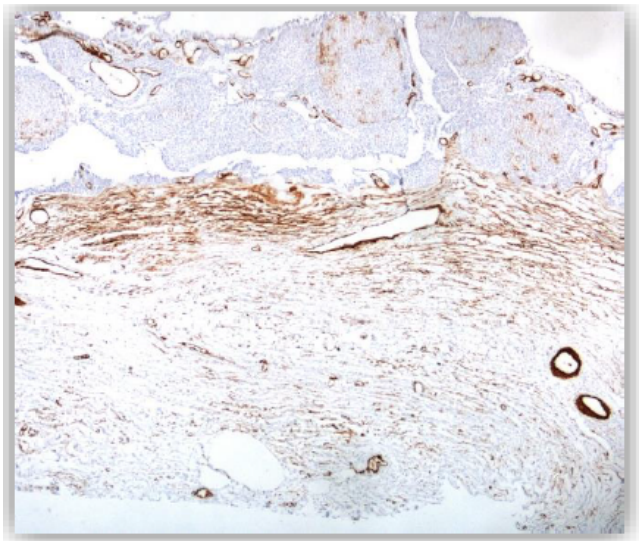

Figure 5: Photomicrograph showing α-SMA immunoexpression close to tumor islands (Adenomatoid odontogenic tumor) Original magnification x40.

In the present study, the benign intraosseous lesions showed mean α-SMA immunoexpression of 1.67 ± 1.144. This group further

encompass of five cases of Adenomatoid odontogenic tumor, one case of Osteoblastoma, five 6 cases of Unicystic ameloblastoma, 11 cases of Multicystic ameloblastoma and five cases of Keratocystic odontogenic tumor.

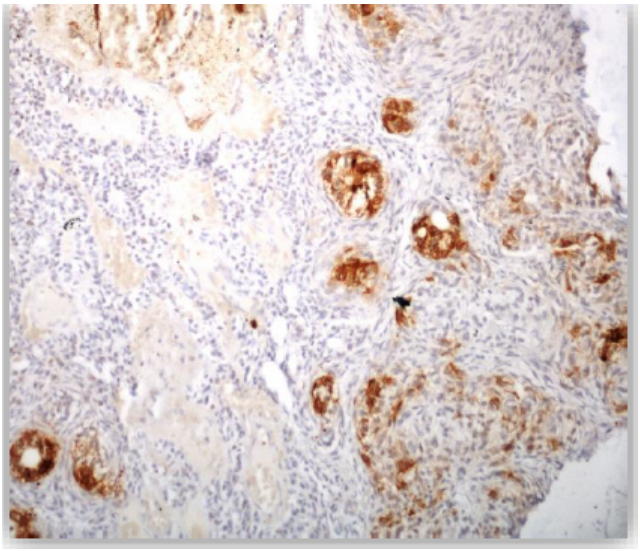

Figure 6: Photomicrograph showing α-SMA immunoexpression in pseudoglandular ducts (Adenomatoid odontogenic tumor) Original magnification x200.

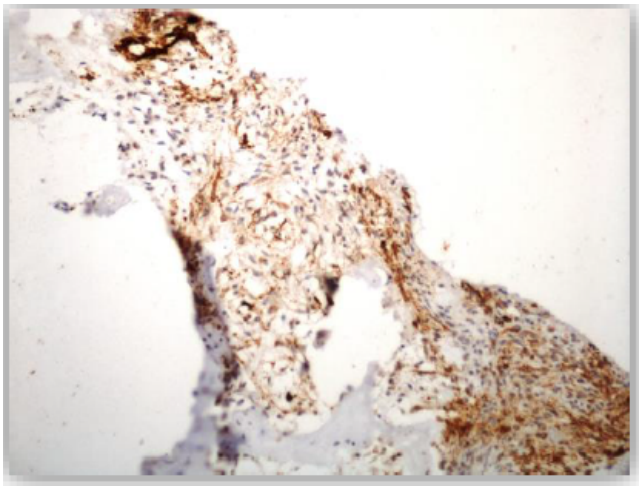

Figure 7: Photomicrograph showing α-SMA immunoexpression (Osteoblastoma) Original magnification x200.

In an adenomatoid odontogenic tumor, the mean α-SMA immunoexpression of 0.60 ± 0.894 with a minimum value of 0 and a maximum value of 2 was observed. In our study, two cases showed the distribution of α-SMA positive myofibroblasts in two planes as inner zone adjacent to tumor islands (Figure 5) and outer peripheral zone approximating osseous trabeculae. One case showed a diffuse pattern of distribution in the stroma. The presence of mild epithelial cell positivity was seen in two cases especially the pseudoglandular cells lining the duct-like structures (Figure 6).

To support, we propose that as MFs produce various extracellular matrix proteins and basement membrane components as laminins, type IV collagen, heparin sulphate, prostaglandins, fibronectin, enamel matrix proteins like amelogenin and enamelin, which are also seen to be constituent of the eosinophilic material or the hyaline ring that lines the periluminal surface of the ductal spaces in AOT [10].

In the present study, osteoblastoma showed mean α-SMA immunoexpression of 2 ± 0 with minimum value as 2 and maximum value as 2 (Figure 7). The high immunoexpression of α- SMA positive cells in the region adjacent to active bony trabeculae compared to rest of the stroma, directs the involvement of these myofibroblasts in the reactive and inflammatory zone of the tumor, as seen during initial stages of tumor progression. The mean immunoexpression in unicystic ameloblastoma observed was 2.20+0.447 with minimum value as 2 and maximum value as 3 and in multicystic ameloblastoma with mean α-SMA immunoexpression of 1.64+1.362 with a minimum value of 0 and maximum value of 3. In the present study, one case of unicystic ameloblastoma and two cases of multicystic ameloblastoma showed positive immunoexpression of α-smooth muscle actin in few neoplastic epithelial cells in both inner layer and the outer layer (Figures 8 and 9).

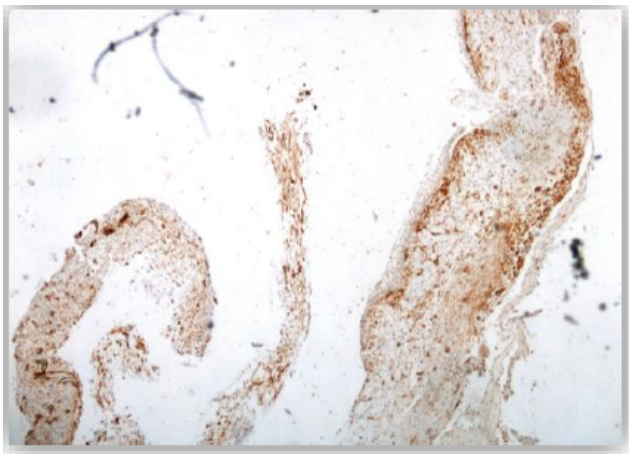

Figure 8: Photomicrograph showing α-SMA immunoexpression (Unicystic ameloblastoma) Original magnification x40.

In accordance with a study done by Vered et al. [11] we observed a higher immunoexpression of α-SMA positivity in the stroma adjacent to multicystic ameloblastic tumor islands when compared to rest of the stroma, whereas in unicystic variant, though the immunoexpression was diffuse but two distinct positive zones can be appreciated in two cases namely the inner subepithelial zone and outer zone approximating bony trabeculae, which may signify role of myofibroblasts in degradation of adjacent matrix facilitating in tumor island growth, expansion and finally to invasion surrounding structures.

Some studies demonstrated that the stromal component of ameloblastoma is composed of myofibroblasts which are associated with collagen and basal lamina material and proposed their contribution to aggressive behavior [12]. Thus, neoplastic cells derived from transdifferentiation of myofibroblasts are involved in the local invasion of ameloblastoma, as the myofibroblasts create a "pre-invasive

niche" in the vicinity of invading tumor cells and islands which may be related to intraosseous growth.

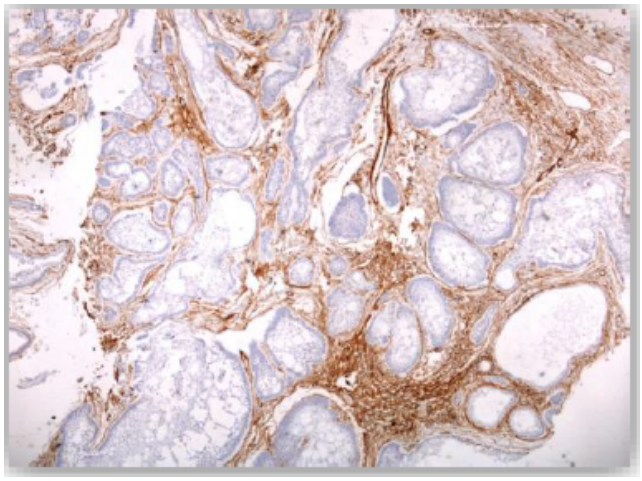

Figure 9: Photomicrograph showing α-SMA immunoexpression (Solid multicystic ameloblastoma) Original magnification x40.

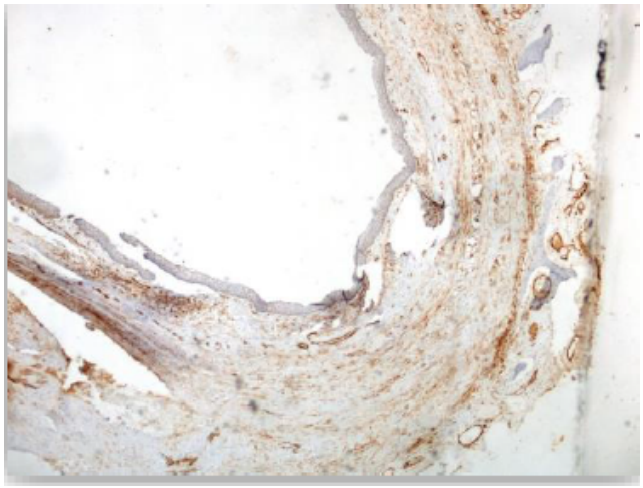

Figure 10: Photomicrograph showing α-SMA immunoexpression (Keratocystic odontogenic tumor) Original magnification x40.

The keratocystic odontogenic tumor in the present study showed the mean α-SMA immunoexpression of 2.00 ± 0.707 with minimum value as 1 and maximum value as 3. In our results, four cases showed α-SMA positive fibroblasts in two zones in cyst wall capsule being subepithelial zone and other deeper zone or outer zone approximating osseous trabeculae (Figure 10). One case showed intense positivity for myofibroblasts in the deeper stroma. Same results were obtained by Lombardi T and Morgan PR [13]. They contributed this pattern of distribution of myofibroblasts to the cyst wall elasticity restraining cyst growth and expansion. While other authors [10] suggest that the stroma of KCOT is not just structural support to the cyst wall but rather accentuates the neoplastic behavior of the cyst. Further, the authors observed the higher myofibroblasts in para keratinized KCOT than SCC cases. This aggressive biological potential may promote the growth of neoplastic epithelial cells and further invasion by the molecular cross-talk between cystic epithelium and the stroma by the well-known growth factors as TGF-β and PDGF.

The presence of high immunoexpression of myofibroblasts at the invasive front and near osseous trabeculae clearly explains its role in promotion than as a part of host defense mechanism against invasion, as was thought earlier.

Thus, to summarize myofibroblasts play a dominant role through various downstream mechanisms regulated by an array of molecular factors in the modulation of growth, enlargement, and invasion of KCOT into the surrounding tissues. Thus, contributing to the destructive biological behavior of these lesions, comparable to lesions like ameloblastoma and squamous cell carcinoma.

In this study, malignant intraosseous lesions show mean α-SMA immunoexpression of 1.83 ± 1.115. This group is comprised of 4 subgroups i.e. ameloblastic carcinoma, squamous cell carcinoma, osteosarcoma, and mucoepidermoid carcinoma.

The Ameloblastic Carcinoma (AC) showed mean α-SMA immunoexpression of 1.20 ± 1.304 with minimum value as 0 and maximum value as 3. Two cases showed an intense and diffuse pattern of distribution of positive stromal cells (Figures 11 and 12) while other cases showed mild immunoexpression. Four cases showed α-SMA positivity in the neoplastic epithelial islands. Moreover, this immunoexpression was not restricted to the peripheral cells only but also observed in the central cells. A plausible explanation proposed by authors for this is the acquisition of myofibroblastic phenotype by the malignant epithelial cells through epithelial-mesenchymal transition. This contributes to the increase in relative aggressiveness as the density of myofibroblasts increases.

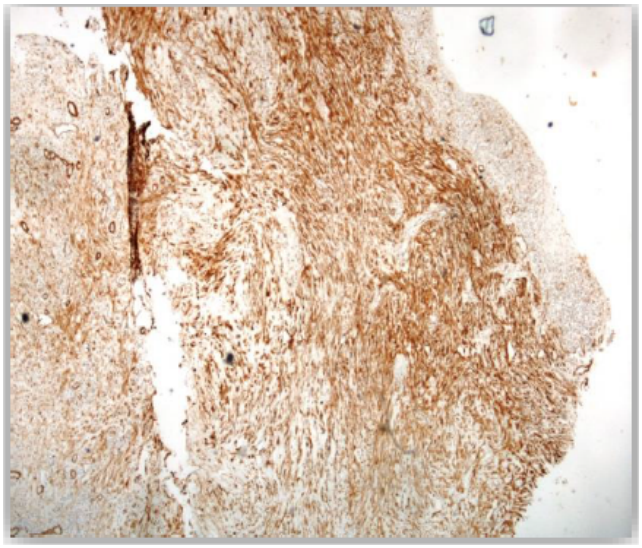

Figure 11: Photomicrograph showing intense α-SMA immunoexpression in the stroma (Ameloblastic carcinoma) Original magnification x40.

Other supporting studies also reveal the presence of α-SMA in the odontogenic epithelium in stellate reticula like cells and the stroma of ameloblastic carcinoma whereas, in the case of ameloblastoma, it was found only in the stromal part. The association of myofibroblasts with

the neoplastic epithelium is attributed to epithelial-mesenchymal transition facilitating in distant metastasis [14].

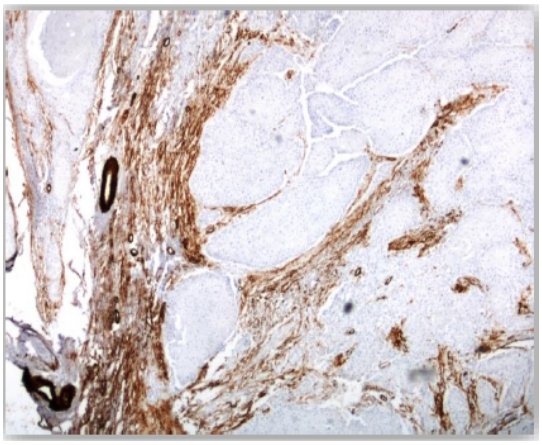

Figure 12: Photomicrograph showing intense α-SMA immunoexpression around tumor islands (Squamous cell carcinoma) Original magnification x40.

To add further, Bello et al., [15], have also stated that immunoexpression of α-SMA in the epithelial odontogenic islands in these lesions is virtually diagnostic of a spindle-shaped variant of AC. In their study, α-SMA immunoreactivity was also found within the epithelial islands, the quantity had higher scores and they were haphazard in the arrangement.

The presence of CAFs in the stroma in relation to benign ameloblastoma may be one explanation for its relatively aggressive behavior as a benign tumor. Thus, myofibroblasts would serve as a predictor model for assessing prognosis in ameloblastomas and in differentiating ameloblastic carcinoma from its benign counterpart.

The squamous cell carcinoma showed intense immunoexpression with mean α-SMA immunoexpression of 3 ± 0. The two well-known morphologic patterns of myofibroblasts exist in the tumor stroma, namely diffuse network pattern and localized peripheral spindle pattern [16].

In the present study, the prominent structural pattern appears to be diffuse network pattern scattered in the stroma.

Also, high frequency of α-SMA positive myofibroblasts was found around the periphery of tumor islands or at the invasive front suggests a vital role in predicting prognosis and the stage of progression.

According to one model, epithelial invasion beyond the basement membrane is essential for myofibroblastic transdifferentiation [17]. Kawashiri S et al. proposed that increased production of extracellular components and collagen fibers at the invasive front constitutes the desmoplastic reaction which predisposes tumor to advance stages of progression [18].

In another model, SCC displayed variable staining for α-SMA but it was generally noted that they were more numerous at the invasive front than at the superficial parts of the tumor and that the quantity stained decreased when there was increased inflammatory cell infiltration in the area around the tumor [15].

Myofibroblasts synthesize type III collagen and these contractile properties contribute to the firm consistency and retraction of carcinomas. The myofibroblastic induction thus represents an imperative host stromal response toward restraining the extent of invasive and/or metastatic carcinoma.

This response was thought to be important in neoplasms with weak antigenicity and/or slow doubling times [19]. Thus, the proliferation of myofibroblasts in the tumor stroma may be used as a stromal marker of malignancy. The autocrine and paracrine effects of transformed myofibroblasts and of the stromal-epithelial interaction are associated with the creation of a permissive environment for tumor invasion in OSCC.

In this study, the mean α-SMA immunoexpression of osteosarcoma was 2.50+0.70 with minimum value as 2 and maximum value as 3. The pattern of immunoexpression is intense and diffuse throughout the sarcomatous stroma (Figure 13). To support, the dysregulated expression of growth factors such as TGF, Insulin-like Growth Factor (IGF) and Connective Tissue Growth Factor (CTGF) which are the major secretory products of myofibroblasts are also known to affect the osteosarcoma malignant cells. The major cytokines secreted by α-SMA positive myofibroblasts include IL-1, 4, 6, 8 and IL-11, of which IL-6 and 8 are seen to be significantly higher in malignant bone tumors as osteosarcoma. The degraded bone matrix release TGF-β which activates IL-6 and IL-11 leading to osteoclastic activation, facilitating further invasion and the release of pro-resorptive cytokines [20].

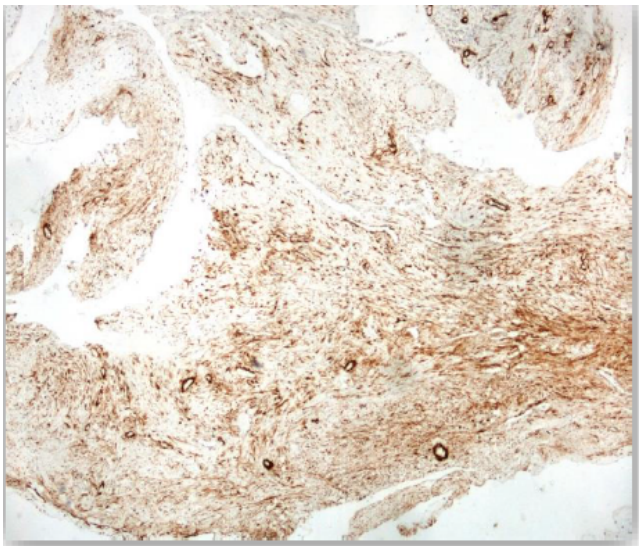

Figure 13: Photomicrograph showing intense α-SMA immunoexpression in the stroma (Osteosarcoma) Original magnification x40.

Thus, we can outline the pivotal direct or indirect role of myofibroblasts in the molecular pathogenesis of osteosarcoma oncogenesis through its diverse key molecular factors affecting osteosarcoma cells and endothelial cells in the tumor progression correlating with poor prognosis.

In mucoepidermoid carcinoma (Figure 14), the α-SMA positive cells showed a mean immunoexpression of 2.00 ± 0.816 with minimum value as 1 and maximum value as 3.

One case showed the α-SMA positivity in both neoplastic epithelial or epidermoid cells and the stromal fibroblasts, indicating to process of epithelial-mesenchymal transition favoring cell growth, motility, invasion, tumor progression, and thus poorer prognosis. One case showed intense positivity throughout the stroma intervening malignant islands.

Sobral AP et al., [21] on the contrary found increased immunoexpression of myofibroblasts in lower grades of mucoepidermoid carcinoma as compared to intermediate and higher grades and proposed that inflammatory infiltrates in the stroma inhibit myofibroblastic transdifferentiation.

They thus suggested an inverse relation between myofibroblastic differentiation and density of lymphocytic infiltration.

To conclude, myofibroblasts have a dual role i.e. host defense mechanism in limiting the extent of the lesion by desmoplasia and progression of the lesion by stromal modulation including the growth of the lesion, degradation of the extracellular matrix, angiogenesis, and metastasis.

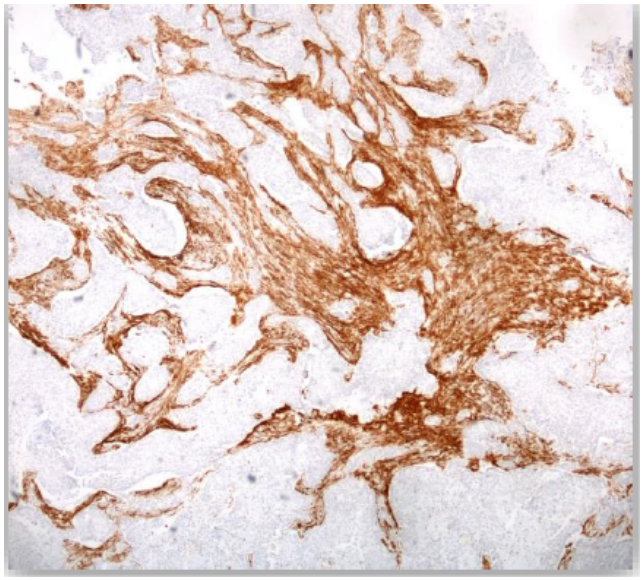

Figure 14: Photomicrograph showing intense α-SMA immunoexpression in the stroma (Mucoepidermoid carcinoma) Original magnification x40.

In our study, odontogenic cystic lesions consisted of 2 subgroups namely; Radicular cysts and Dentigerous cysts.

This group showed mean α-SMA immunoexpression of 1.50 ± 0.972 with minimum value as 0 and maximum value as 3. The radicular cysts showed the mild mean α-SMA immunoexpression of 0.80 ± 0.837. In this subgroup α-SMA, positive cells are distributed from sparse to cohesive groups (Figure 15).

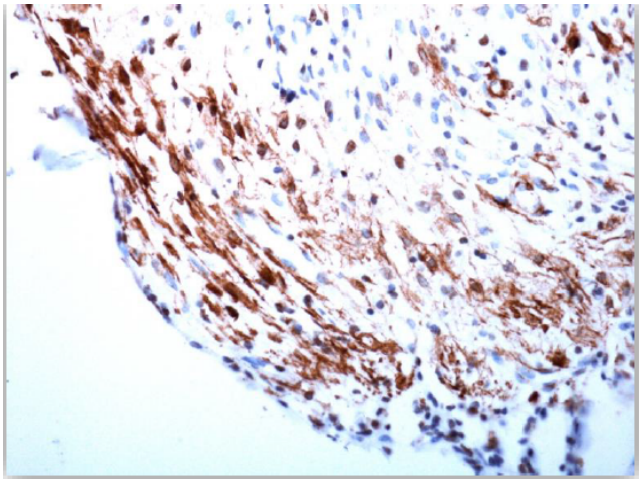

Figure 15: Photomicrograph showing α-SMA immunoexpression in the stroma (Radicular cysts) Original magnification x400.

In this study, Dentigerous cysts showed high mean α-SMA immunoexpression of 2.20 ± 0.447. The pattern of distribution was divided into three zones as subepithelial zone with few stained cells lined elongated and parallel to the epithelium, intermediate zone with no positive staining and outer or peripheral zone with intense positivity of α-SMA positivity in both diffuse random and cohesive bundles (Figure 16). Lombardi T and Morgan PR [13], suggested that strain exerted by positive hydrostatic pressure from cystic fluid on the cyst wall provides the environment for the conversion of stromal fibroblasts to myofibroblasts which neoexpress cytoplasmic microfilaments with specialized stress fibers for generating tensile force, thus providing host response to cystic distension. Authors further add that the fibroblasts from dentigerous cyst show collagen gel contraction at decreased rates compared to other cysts.

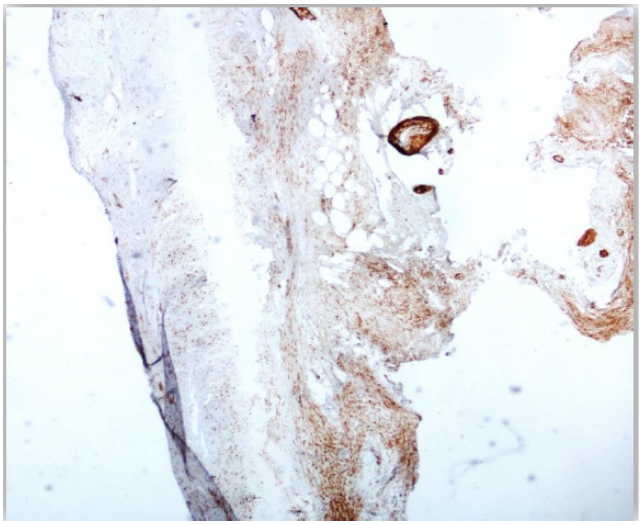

Figure 16: Photomicrograph showing α-SMA immunoexpression in the stroma (Dentigerous cysts) Original magnification x40.

As radicular cysts are indolent lesions characterized by low growth potential and less aggressive biological behavior, this may account for low immunoexpression of α-SMA positive cells. The higher immunoexpression of myofibroblasts in dentigerous cysts compared to radicular cysts accounted for the different degree of aggressiveness in their biological behavior. Thus a positive link can be suggested; the higher immunoexpression of myofibroblasts, more aggressive behavior of the odontogenic cyst.

The Fibro-osseous lesions included in the study comprised of 3 cases of fibrous dysplasia, 3 cases of ossifying fibroma and one case of cement-osseous dysplasia. The group showed a mean α-SMA immunoexpression of 1.57 ± 1.272. Ossifying fibroma showed the highest immunoexpression and cement-osseous dysplasia showed the least. The subgroup Fibrous dysplasia displayed a mean score of 1.33 ± 1.528 with a minimum value of 0 and a maximum value of 3. One case showed intense positivity with a diffuse pattern of distribution (Figure 17) and one case showed negligible immunostaining of α-SMA. These lesions show the arrest of maturation at the woven bone stage. They tend to grow slowly with the ability to stabilize in early life. Thus, the nondestructive nature and mild clinical course explain lower immunoexpression compared to ossifying fibroma. Hemingway F et al. [22], showed the same results and concluded that α-SMA was strongly and diffusely expressed by mesenchymal stem cells in fibrous dysplasia.

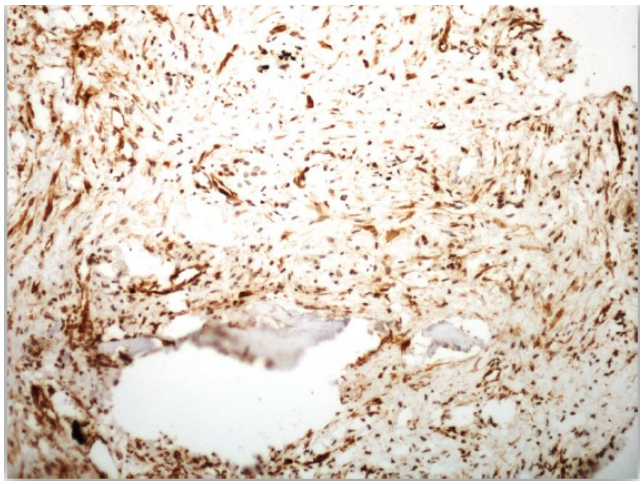

Figure 17: Photomicrograph showing α-SMA immunoexpression in the stroma (Fibrous dysplasia) Original magnification x200.

In the present study, the second subgroup Cemento-ossifying fibroma showed high α-SMA immunoexpression of 2.33 ± 0.577 with a minimum value of 2 and a maximum value of 3. The α-SMA positive plump spindle cells are arranged in the form of cellular fascicles and bundles in the stroma was seen (Figure 18). Thus, the high intensity of α-SMA positive myofibroblasts around bony trabeculae is a response to modified cellular stroma contributing to the intraosseous expansion and aggressiveness of these lesions.

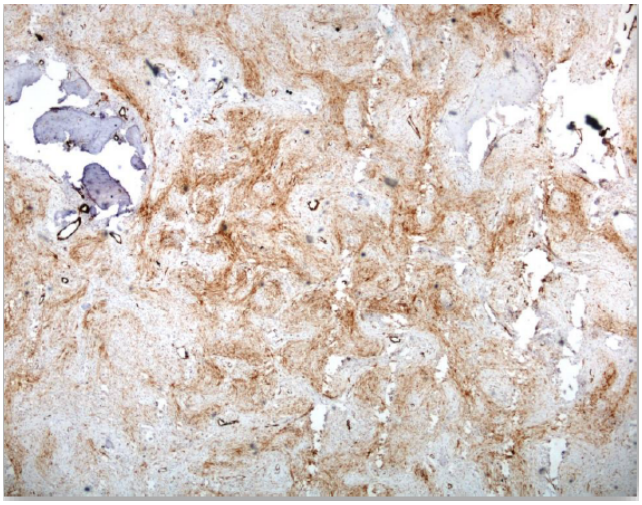

Figure 18: Photomicrograph showing intense α-SMA immunoexpression in the stroma (Ossifying fibroma) Original magnification x40.

In the present study, one case of Cement-osseous dysplasia was taken which showed no α-SMA immunoexpression in their stroma, indicating no role of myofibroblasts in the pathogenesis of these lesions (Figure 19). The cells involved in the pathogenesis are neither neoplastic nor premalignant. Thus, these small asymptomatic multiples well defined sclerotic lesions have a non-aggressive course of progression which correlates with the negligible α-SMA immunoexpression.

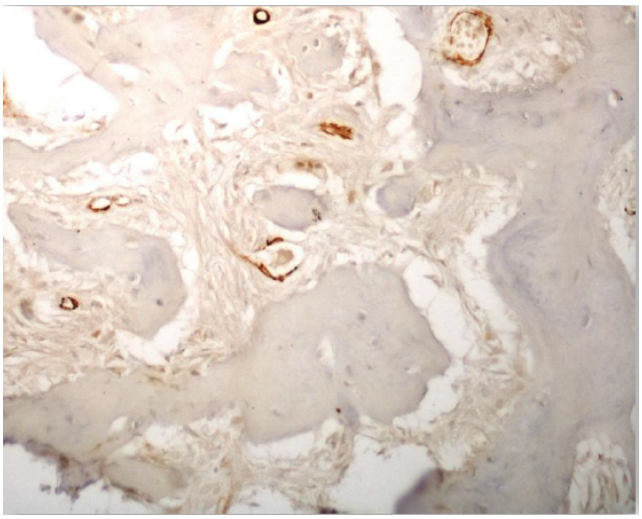

Figure 19: Photomicrograph showing no α-SMA immunoexpression in the stroma (Cement-Osseous dysplasia) Original magnification x200.

Conclusion

Through this study, we attempted to summarize the role of myofibroblasts by using α-smooth muscle actin in various intraosseous lesions of the jaw and it is suggested that they may play a pivotal role in modulating the biological behavior of these lesions. The understanding of expression and activity of myofibroblasts may thus be necessary for the application of anti-Myofibroblastic drugs in the therapy.

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