Original Article

Immunohistochemical evaluation of myofibroblasts in oral epithelial dysplasia and oral squamous cell carcinoma

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Abstract

Aim: The aim of the study is to evaluate the presence of myofibroblasts quantitatively in oral epithelial dysplasia, oral squamous cell carcinoma (OSCC).

Materials and Methods: Formalin-fixed, paraffin-embedded blocks were retrieved from the institutional archives. The sample size is 35 and included 15 cases of oral epithelial dysplasia ($n = 15$), 15 cases of squamous cell carcinoma ($n = 15$) and 5 cases of normal oral mucosa which served as the control ($n = 5$). Histologic sections were subjected to immunohistochemical analysis using alpha-smooth muscle actin, and the mean number of myofibroblasts was evaluated.

Results: There were no myofibroblasts in the stroma of normal oral mucosa and oral epithelial dysplasia. Whereas all cases of OSCC showed myofibroblasts (mean ± standard deviation: 21.49 ± 9.76). This difference of myofibroblasts between OSCC and oral epithelial dysplasia was statistically significant with a $P < 0.05$. There was no statistically significant difference in the mean number of Myofibroblasts(MF) between 3 histologic grades of OSCC.

Conclusion: The presence of myofibroblasts in the stroma of OSCC and their absence in normal oral mucosa and epithelial dysplasia reveals that these cells may play a role in cancer cell invasion and progression so the treatment strategies targeting the myofibroblasts and their by products may be beneficial in OSCC patients.

Keywords: Myofibroblasts, oral epithelial dysplasia, oral squamous cell carcinoma

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common malignant neoplasm of the oral cavity with a high mortality rate and is characterized histologically by the invasion of the malignant epithelial islands into the connective tissue stroma.[1] The progression of carcinomas has been attributed to step-wise accumulation of genetic changes in the epithelium, with the appearance of epithelial hyperplastic lesions, to dysplasia and frank carcinomas.[2] As the normal oral epithelium becomes a seat of malignancy, numerous changes are thought to occur in the connective tissue stroma which changes from activated to tumor associated.[3]

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A continuous molecular cross talk between epithelial and mesenchymal cells is required during embryonic development and probably plays an important role in pathologic process such as wound healing and tumor progression. Many of the epithelial tumors are characterized by the local accumulation of connective tissue cells and various extracellular components called as the stroma reaction. One of the stromal reactions is the appearance of specialized fibroblasts called myofibroblasts.[4]

Myofibroblasts are specialized fibroblasts with ultrastructural features of both fibroblasts and smooth muscle cells.[5] They are extremely heterogeneous and multifunctional cell population exhibiting a different phenotype. Their presence has been reported in normal oral tissues and pathologic conditions such as reactive lesions, benign tumors, locally aggressive tumors and malignancies affecting the oral cavity. Myofibroblasts play a key role in extracellular matrix synthesis, wound healing and pathologic process like tumorigenesis.[4] Tumor-derived cytokines such as transforming growth factor-beta (TGF-β) and platelet-derived growth factor are responsible for the differentiation of fibroblasts to myofibroblasts[6] and inturn these myofibroblasts were thought to secrete numerous growth factors that stimulate neoplastic epithelial cell proliferation. The aim of the present study is to evaluate immunohistochemically the presence of myofibroblasts in oral epithelial dysplasia, OSCC and to compare with that of normal oral mucosa.

MATERIALS AND METHODS

Sample selection

This is a retrospective study. Formalin-fixed, paraffin-embedded blocks were retrieved from the institutional archives. The sample size is 35 and included 15 cases of oral epithelial dysplasia (n = 15) and 15 cases of squamous cell carcinoma (n = 15) and 5 cases of normal oral mucosa which served as the control (n = 5). Normal mucosa was obtained from the patients undergoing crown lengthening and orthodontic tooth extraction procedures after signing written consents. Diagnosis was given using hematoxylin and eosin-stained sections based on the WHO classification of cancerous and precancerous lesions. Epithelial dysplasias were graded according to as mild, moderate and severe, and squamous cell carcinomas were graded according to the WHO as well, moderate and poorly differentiated.[7]

Staining procedure and evaluation of myofibroblasts

The histologic sections subjected to immunohistochemical staining with alpha-smooth muscle actin (α-SMA) mouse anti-human antibody (Biogenex, clone IA4). Brown staining was considered as positive staining. Human colon was used as the external positive control. Whereas, α-SMA positive cells within the blood vessels served as the positive control for the specificity of the stain. Number of positive stromal cells was counted for quantitative analysis. Counts were performed on olympus microscope using image pro plus image analysis software. Representative fields were randomly selected and 10 fields were chosen for each section at 40x magnification. Each α-SMA-positive cell, excluding those surrounding blood vessels, was counted, and the total number of positive cells for all 10 examined fields per case was calculated. This allowed calculation of the mean number of α-SMA-positive cells per field. Results were presented as the mean number of α-SMA positive cells per field.

The distribution pattern of myofibroblasts was evaluated by the criteria given by Kellerman *et al.*[8]

Statistical analysis

The Statistical Package for the Social Sciences software (SPSS 17, IBM, 2008) was used for computations. Differences in the mean number of α-SMA-positive cells per field among epithelial dysplasias and squamous cell carcinomas were analyzed using one-way ANOVA. Statistical significance was at $P < 0.05$.

RESULTS

A total of 35 cases including 15 OSCC, 15 oral epithelial dysplasias and 5 cases of normal oral mucosa were evaluated in the study. Out of 15 cases of OSCC 5 cases were well differentiated, 5 cases were moderately differentiated and 5 cases were poorly differentiated. Epithelial dysplasias consisted of mild 5 cases, moderate 5 cases and severe 5 cases.

There were no myofibroblasts in the stroma of normal oral mucosa and oral epithelial dysplasia [Figure 1]. Whereas all cases of OSCC showed myofibroblasts in the connective tissue stroma surrounding the epithelial islands and near the invasive front [Figures 2 and 3]. The mean number of MF in OSCC was 21.49 ± 9.76. There was no statistically significant difference in the mean number of MF between 3 histologic grades of OSCC (one-way ANOVA).

Table 1 shows morphology and arrangement of MF in different grades of OSCC. Of 15 cases of OSCC, 20% showed focal arrangement whereas 40% showed network arrangement and 40% showed network and spindle arrangement. Well-differentiated cases of OSCC showed focal arrangement [Figure 4], whereas moderate and

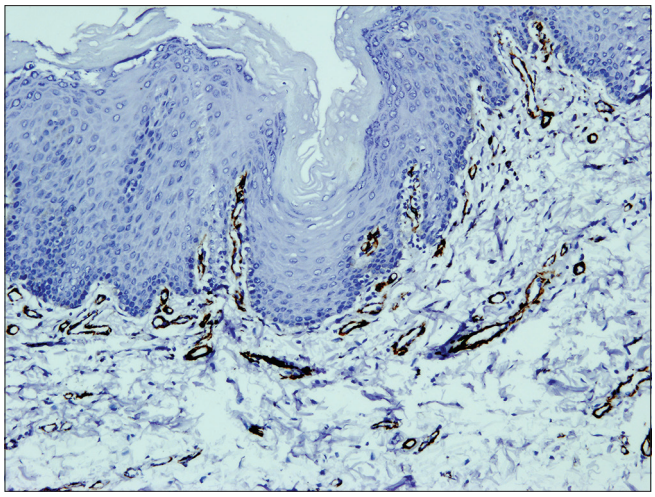

Figure 1: Moderate epithelial dysplasia showing only α-SMA within the blood vessel walls. No α-SMA immunostaining corresponding to stromal myofibroblasts (x100)

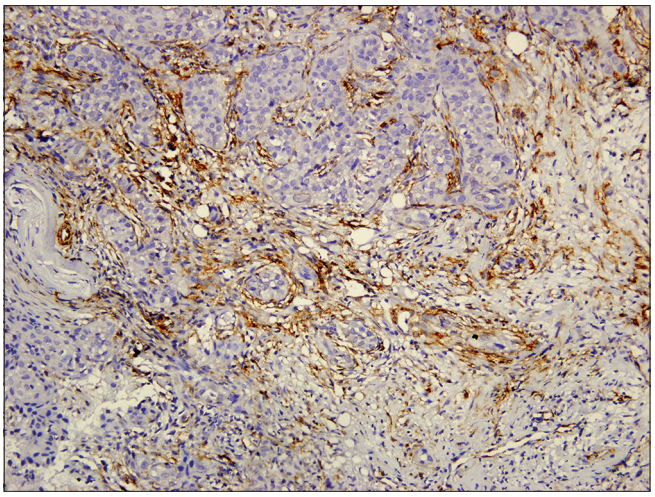

Figure 2: α-SMA positive myofibroblasts surrounding the tumor islands in oral squamous cell carcinoma (x200)

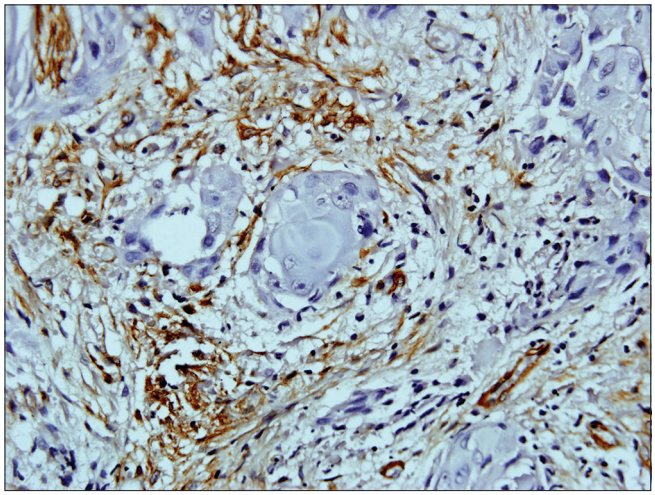

Figure 3: High power view showing α-SMA positive myofibroblasts surrounding the malignant epithelial cells in oral squamous cell carcinoma (x400)

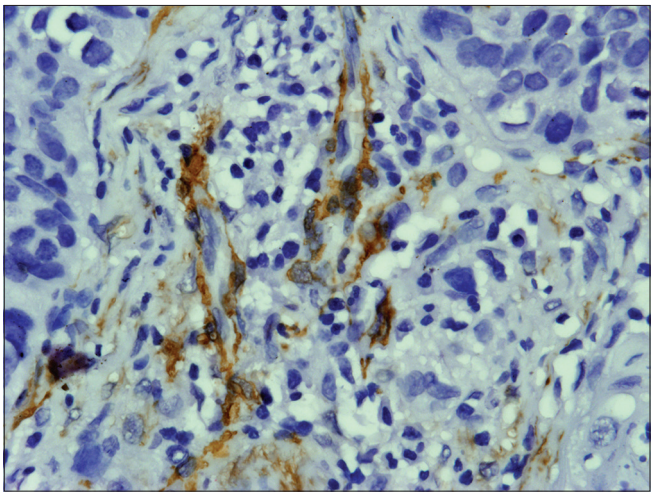

Figure 4: Focal arrangement of myofibroblasts in Well differentiated cases of OSCC

Table 1: Morphology and arrangement of myofibroblasts

Grade of OSCC	Focal	Network	Network and spindle
Well-differentiated OSCC (*n*=5)	3	2	0
Moderately differentiated OSCC (*n*=5)	0	2	3
Poorly differentiated OSCC (*n*=5)	0	2	3

OSCC: Oral squamous cell carcinoma

poorly differentiated cases showed network and spindle arrangement [Figure 5].

DISCUSSION

The activity of epithelial cells with their stroma is important for controlling the growth and differentiation in normal and pathologic situations. During the tumor evolution, many changes take place in the connective tissue stroma called the stromal reaction. One of the cellular components of stromal reaction is the appearance of specialized fibroblasts called myofibroblasts.[5] Tumor-derived cytokines and growth factors are responsible for transdifferentiation of fibroblasts to myofibroblasts.[9]

The results of our study showed that the mean number of MF was significantly higher in OSCC compared to epithelial dysplasias and normal mucosa which were devoid of MF [Figure 1] which was similar to the previous study conducted by Moghadam *et al.*,[10] chaudary *et al.*[11] and Gupta[12] *et al.* who compared the presence of myofibroblasts in normal mucosa, oral epithelial dysplasia and different grades of OSCC and reported that the presence of myofibroblasts was significantly higher in OSCC [Figures 2 and 3]compared to epithelial dysplasia and normal mucosa. This indicates that invasion of epithelium into the underlying connective tissue beyond the basement membrane is necessary to evoke a myofibroblastic response,

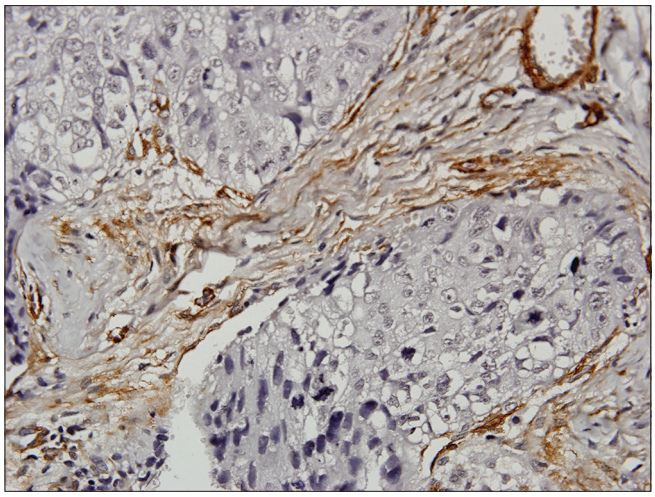

Figure 5: Network and spindle arrangement of myofibroblasts

and these invaded tumor cells secrete numerous growth factors and cytokines that help in the transdifferentiation of fibroblasts to myofibroblasts. In 2010, Seifi *et al.*[13] evaluated for the presence of myofibroblasts in OSCC, epithelial dysplasia and hyperkeratosis and reported that 67% of OSCC, 22% of oral epithelial dysplasia and 1 case of hyperkeratosis showed the presence of myofibroblasts and concluded that the number of myofibroblasts would increase in the process of carcinogenesis.

In 2008, Kellerman *et al.*[8] studied for the presence of myofibroblasts in 83 cases of tongue squamous cell carcinoma, 8 cases of normal oral mucosa and 16 cases of dysplasia and found that myofibroblasts are present in 60% of cases of OSCC and their presence was associated with poor prognosis. Kellerman's study results are in accordance with the results of our study. However, we could not associate the presence of myofibroblasts with the prognosis of the lesion as there was no follow-up of the study cases.

In 2009, Vered *et al.*[14] investigated the concomitant expression of epithelial membrane antigen and α-SMA in the cells at tumor connective tissue interface in human tongue carcinoma and reported that myofibroblasts were almost exclusively associated with carcinomas and not in premalignant lesions which was in accordance with our study. Vered *et al.* study revealed that 41% of the cells were positive both for epithelial membrane antigen and α-SMA can be a manifestation of epithelial mesenchymal transition. Mahajan *et al.*[15] in 2020 have evaluated expression of α-SMA and vimentin in normal mucosa, epithelial dysplasia and OSCC and reported that statistically significant index was observed between α-SMA and vimentin in epithelial dysplasia and OSCC and postulated that MF may play a role in initial tumorigenesis.

In our study, there was no difference in the mean number of MF between different grades of SQCC which was in agreement to the study conducted by Kellerman *et al.*[8] and Moghadam *et al.*[10] who did not find any difference between the observation of myofibroblasts and different grades of squamous cell carcinoma indicating that there is transdifferentiation of MF when the epithelium invades beyond the basement membrane and further increase in grade does not affect the number of myofibroblasts. In contrast to our study, Bhtacharjee[16] who have compared MF in epithelial dysplasia and OSCC reported that number of MF was higher in poorly differentiated OSCC and may be associated with tumor invasiveness.

In our study, poorly differentiated cases of OSCC have network and spindle-shaped arrangement whereas well-differentiated cases showed focal arrangement and these findings were n concordance to the study conducted by Khalid *et al.*[17] who showed that invasive cases of OSCC showed network pattern of arrangement and was associated with poor prognosis.

Numerous *in vitro* invasion essays revealed that tumor cells cultured on the top of preparation containing type I collagen invade the collagen gel only when myofibroblasts were present, indicating that these cells have paracrine role in invasion of OSCC cells.[18,19] These studies also revealed that tumor cells secrete TGF-β that helps in the transdifferentiation of fibroblasts to myofibroblasts.

Thus, to conclude, the presence of myofibroblasts in the stroma of OSCC and their absence in normal oral mucosa and epithelial dysplasia may be due to the growth factors secreted by the tumor cells which helps the existing fibroblasts to transdifferentiate into myofibroblasts. These myofibroblasts were inturn thought to secrete numerous growth factors that help in further invasion and progression of the tumor.

CONCLUSION

Further studies, using larger sample size, follow-up together with more sophisticated techniques is required to clarify the role of these myofibroblasts in the epithelial and connective tissue compartments. If our findings are confirmed with these future studies, treatment strategies targeting the myofibroblasts and their byproducts may be beneficial in OSCC patients.

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Conflicts of interest
There are no conflicts of interest.

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