

ORIGINAL ARTICLE

Evaluation of Myofibroblasts in Oral Squamous Carcinoma: An Immunohistochemical Study

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ABSTRACT:

Background: The presence of cancer is followed by some changes that happen in epithelium & the normal stroma and normal stroma becomes a reactive one. Myofibroblasts secrete cytokines and matrix metalloproteases which in turn contribute to the destruction of extracellular matrix and cause tumor growth. Hence; we planned the present study to assess the expression of myofibroblasts in oral squamous cell carcinoma (OSCC) cases. **Methods:** The present study included immunohistochemical assessment of expression of alpha smooth muscle actin (α -SMA) in OSCC cases and 10 normal control were included in the present study. N Immunohistochemical staining of all the OSCC sections and normal controls (NC) was done. Immunostaining was assessed by the evaluation of the staining intensity and percentage of α -SMA-positive cells. Staining index for each group was calculated and compared. All the results were analyzed by SPSS software. **Results:** Staining index of myofibroblasts was positive in all the sections of OSCC and was significantly higher (p - value <0.05) as compared to normal control group. **Conclusions:** Myofibroblasts do play a significant role in epithelial malignant transformation. **Key words:** Alpha-smooth muscle actin, myofibroblast, oral squamous cell carcinoma.

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This article may be cited as: Dar MS, Rehan AD, George J. Evaluation of Myofibroblasts in Oral Squamous Carcinoma: An Immunohistochemical Study. J Adv Med Dent Scie Res 2017;5(12):46-48.

Access this article online	
<p>Quick Response Code</p> 	<p>Website: www.jamdsr.com</p>
	<p>DOI: 10.21276/jamdsr.2017.5.12.14</p>

INTRODUCTION

Squamous cell carcinoma is defined as “a malignant epithelial neoplasm exhibiting squamous differentiation as characterized by the formation of keratin and/or intercellular bridges”.

The presence of cancer is followed by some changes that happen in epithelium & the normal stroma and normal stroma becomes a reactive one.¹⁻⁴ The formation of reactive stroma is associated with the secretion of cytokines such as $\text{tg}\beta$ -1 from cancerous cells that promotes differentiation of fibroblasts into myofibroblasts, increases the number of blood vessels, increases the inflammatory cells, causes desmoplasia, decreases the expression of epithelial markers (cadherins), and increases the expression of mesenchymal markers such as vimentin. Myofibroblasts in turn secrete cytokines and matrix metalloproteases which in turn contribute to the destruction of extracellular matrix and cause tumor growth.⁵⁻⁸ Hence; we planned the present study to assess the expression of myofibroblasts in oral squamous cell carcinoma (OSCC) cases.

MATERIALS AND METHODS

The present study was conducted in the Lucknow in 2013 and included immunohistochemical assessment of expression of alpha smooth muscle actin (α -SMA) in OSCC cases. A total of 10 OSCC cases and 10 normal control were included in the present study. Normal control included tissues taken while performing frenectomy procedure. H and E staining was done for confirming the diagnosis of OSCCC. Immunohistochemical staining of all the OSCC sections and normal controls (NC) was done. Immunostaining was assessed by the evaluation of the staining intensity and percentage of α -sma-positive cells, according to the method used by etemad-moghadam et al.⁵ The percentage of immuno-positive cells in the non-inflammatory and non-endothelial stromal cells in the subepithelial connective tissue of osmf and non – endothelial and non – inflammatory cells at the tumour invasive front of oscc in 10 high power fields (hpf) and average percentage per hpf was calculated and recorded as : 0% = no positive cells, 1% = 1–25% positive cells, 2% = 26–50% positive cells, and 3% = 51–100% positive cells. Staining intensity (s.i) was evaluated as: 0% = when there was no staining; 1% = in parts where positivity was observed only at a

magnification of x 400; 2% = in cases where the staining was obvious at x 100, but not at x 40; and 3% = in fields where immuno-positive cells were seen even at x 40. Multiplication of the percentage and intensity scores comprised the staining index (i) of each specimen. This index was classified as: zero = 0, low = 1, 2, moderate = 3, 4 and high = 6–9. Staining index for each group was calculated and compared. All the results were analyzed by SPSS software. Chi- square test and student t test were used for assessment of level of significance. P- value of less than 0.05 was taken a significant.

RESULTS

In the present study, we observed that staining index of myofibroblasts was positive in all the sections of OSCC and was significantly higher (p – value <0.05) as compared to normal control group.

DISCUSSION

Myofibroblasts may be defined morphologically and immunologically through identification of expressed cytoskeletal proteins. The simplest definition of a myofibroblast is that they are smooth-muscle-like fibroblasts. Myofibroblasts may well represent an intermediate state between fibroblasts and smooth muscle cells.^{9- 12} OSCC, like other epithelial malignancies is characterized by abnormal cellular division, invasion of malignant epithelial cells in the connective tissue and specifically, aberrant keratinization in the form of keratin pearls. Keratin production by the cells is the symbol of their functional differentiation.^{13- 15} Hence; we planned the present study to assess the expression of myofibroblasts in oral squamous cell carcinoma (OSCC) cases.

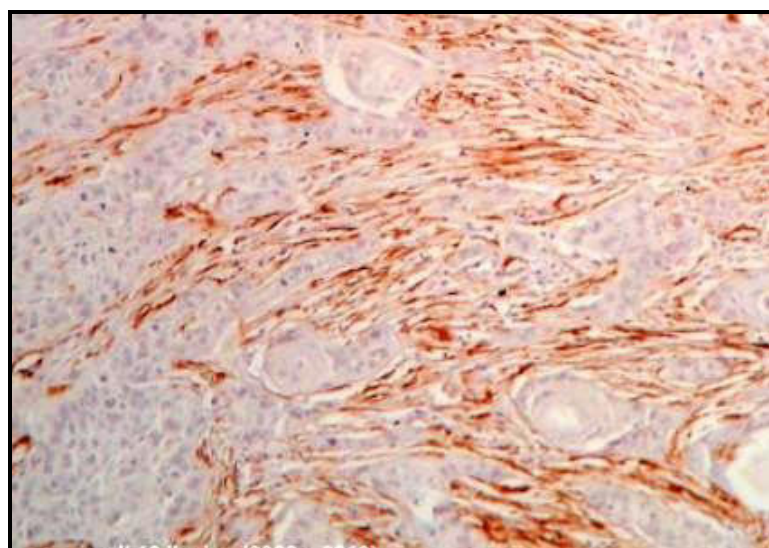
In the present study, we observed significant difference in the staining intensity of α -SMA in OSCC specimens in comparison to NC specimens. These findings may suggest that the formation and differentiation of myofibroblasts is induced somewhere in the invasive stage of OSCC, and further increase in severity and differentiation would not affect the number of these cells. Our results were in correlation with the results obtained by previous authors. Gandhi P et al evaluated and compared the presence of myofibroblasts in normal mucosa, different grades of OSMF, and oral squamous cell carcinoma (OSCC). The present in vitro cross-sectional descriptive study sample consisted of three groups, including 40 OSCCs, 40 OSMF, and 10 sections of normal oral epithelium taken as control group. Alpha-smooth muscle actin was used to identify myofibroblasts using immunohistochemical technique. P < 0.05 was taken as statistically significant. The presence of myofibroblasts was significantly higher in OSMF cases when compared with normal epithelium specimens. The presence of myofibroblasts was significantly higher in OSCC compared to OSMF cases. A significant difference was not observed between the different grades of OSCC. These findings favored the possibility that OSMF actually represents an abnormal healing process in response irritation caused by areca nut. A significant increase in myofibroblasts in OSCC as compared to OSMF also highlights the possible role it may play in the malignant transformation of OSMF.⁴ Gupta K et al evaluated and compared the distribution of MFs using alpha smooth muscle actin (α -SMA) in OL, OSMF, and various histopathological grades OSCC.

Table 1: Comparison of staining intensity between OSCC and normal control

Groups	No. of specimens	Percentage of myofibroblasts score (a)		Staining intensity score (b)		Staining index score (i) i = a x b	
		T - value	P – value	T - value	P - value	T – value	P - value
OSCC vs NC	20	7.558	< 0.05 (s)	10.225	< 0.05 (s)	11.245	< 0.05 (s)

S = significant

Figure 1: Photograph showing positive staining of alpha SMA in OSCC sections.



Sixty formalin-fixed paraffin-embedded tissue blocks consisting of histopathologically diagnosed cases of normal mucosa (n = 10), OL (n = 14) hyperkeratosis with various grades of dysplasia, OSMF (n = 11), and OSCC (n = 25) were subjected to immunohistochemistry using α -SMA antibody for detection of MFs. MFs were not detected in normal oral mucosa. On comparison of frequency of mean scores in OL, OSMF, and OSCC the values were 0.6 ± 0.2 (0-2), 1.2 ± 0.68 (1-2), and 2.6 ± 1.34 (0-4), respectively. The results were statistically significant ($P < 0.001$). These findings were suggestive of role of MFs with the creation of a permissive environment for tumor invasion in OSCC.¹⁶ de-Assis EM et al evaluated the presence of stromal myofibroblasts in OL and OSCC. Differences in the presence of myofibroblasts among OL with distinct grades of epithelial dysplasia as well as between histologically high- and low-invasive OSCC were also assessed. A total of 30 OL and 41 OSCC from archival formalin-fixed, paraffin-embedded specimens were evaluated. 10 samples of normal oral mucosa were used as a control. Myofibroblasts were identified by immunohistochemical detection of alpha smooth muscle actin and its presence was classified as negative, scanty or abundant. Myofibroblasts were not detected in normal oral mucosa and OL, whatever its histological grade. In OSCC, the presence of stromal myofibroblasts was classified as negative in 11 (26.8%), scanty in 15 (36.6%), and abundant in 15 samples (36.6%). The presence of stromal myofibroblasts was statistically higher in high-invasive OSCC than in low-invasive OSCC ($p < 0.05$). These findings suggested that myofibroblasts are associated with the creation of a permissive environment for tumor invasion in OSCC.¹⁷

CONCLUSION

From the above results, the authors concluded that myofibroblasts do play a significant role in epithelial malignant transformation. However; future studies are recommended.

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Source of support: Nil

Conflict of interest: None declared

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