

Assessment of Expression of Myofibroblast in Oral Submucous Fibrosis

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ABSTRACT

Background: Oral Submucous fibrosis (OSMF) is an insidious, chronic disease affecting any part of the oral cavity and sometimes the pharynx. After tissue injury, fibroblasts differentiate into contractile and secretory myofibroblasts that contribute to tissue repair during wound healing, but that can severely impair organ function when contraction and extracellular matrix (ECM) protein secretion become excessive. Hence, we assessed the expression of alpha smooth muscle actin (a-SMA), which is a marker of myofibroblasts, in OSMF.

Materials & methods: The present study included assessment of 10 cases of oral submucous fibrosis from the archives. Another set of 10 oral mucosal specimens were taken as normal control. These cases of OSMF along with normal oral mucosa were subjected to immunohistochemistry for a-SMA for detection of myofibroblasts. Stromal spindle cells positive for a-SMA were regarded as myofibroblasts. Immunostaining was assessed by the evaluation of the staining intensity and percentage of a-SMA-positive cells, according to the method used by Etemad-Moghadam et al. All the results were analyzed by SPSS software.

Results: None of the OSMF cases showed zero staining index while 6 and 4 cases showed moderate and high staining index

respectively. In the control group, out of 10 cases, all them showed absence of staining with zero staining index score. Significant results were obtained while comparing the staining index score in between the two study groups.

Conclusion: Myofibroblasts increase significantly in OSMF patients.

Key words: Myofibroblasts, Oral Submucous fibrosis.

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INTRODUCTION

Oral Submucous fibrosis (OSMF) is an insidious, chronic disease affecting any part of the oral cavity and sometimes the pharynx. The hallmark of the disease is submucosal fibrosis that affects most parts of the oral cavity, progressive trismus due to rigid lips, cheeks, pharynx and upper third of the esophagus leading to dysphagia. ¹ The disease is mainly seen in Asian countries and the prevalence is more in India.

OSMF was first reported by Schwartz in 1952 while examining five Indian women from Kenya, which he called as "atrophica idiopathica (tropica) mucosae oris". Later in 1953, Joshi from Mumbai re-designated the condition as OSMF, implying predominantly its histological nature. ^{2, 3} After tissue injury, fibroblasts differentiate into contractile and secretory myofibroblasts that contribute to tissue repair during wound healing, but that can severely impair organ function when contraction and extracellular matrix (ECM) protein secretion become excessive, such as in hypertrophic scars, scleroderma, and Dupuytren's disease as well as in heart and kidney fibrosis. ⁴⁻⁶

Hence, we assessed the expression of alpha smooth muscle actin (a-SMA), which is a marker of myofibroblasts, in OSMF.

MATERIALS & METHODS

The present study was conducted in the department of oral pathology and included assessment of 10 cases of oral submucous fibrosis from the archives. Another set of 10 oral mucosal specimens were taken as normal control which were taken from the patient during frenectomy, or dental extractions. These cases of OSMF along with normal oral mucosa were subjected to immunohistochemistry for a-SMA for detection of myofibroblasts. Stromal spindle cells positive for a-SMA were regarded as myofibroblasts. Immunostaining was assessed by the evaluation of the staining intensity and percentage of a-SMA-positive cells, according to the method used by Etemad-Moghadam et al. ⁷ All the results were analyzed by SPSS software. Chi-square test and student t test were used for the assessment of level of significance.

RESULTS

None of the OSMF cases showed zero staining index while 6 and 4 cases showed moderate and high staining index respectively (Table 1). In the control group, out of 10 cases, all them showed

absence of staining with zero staining index score. Significant results were obtained while comparing the staining index score in between the two study groups.

Table 1: Immunohistochemical index for myofibroblasts in OSMF and normal control

Groups	A- SMA index				p-value
	Zero	Low	Moderate	High	
OSMF (n = 10)	0	0	6	4	0.01*
Control (n = 10)	10	0	0	0	

*Significant

DISCUSSION

OSMF is multifactorial but the exact pathogenesis is not well-established.⁸ The mechanisms responsible for the pathogenesis are increased collagen accumulation, increased expression of fibrogenic cytokines, genetic polymorphisms and autoimmunity. The increased collagen accumulation results from increased collagen production and stabilization or decreased breakdown of collagen.⁹ Fibroblasts are changed into different phenotypes under the influence of areca nut alkaloids, which secrete more amount of collagen. Increased fibrosis is also thought to be due to increased cross-linking of collagen through up-regulation of lysyl oxidase (present in copper which is present in betel nut) activity in OSMF fibroblasts.¹⁰ Thus, OSMF is now considered a collagen metabolic disorder. Stabilization of collagen structure is produced by catechin and tannins from the areca nut.¹¹ Hence, we assessed the expression of alpha smooth muscle actin (a- SMA), which is a marker of myofibroblasts, in OSMF.

In the present study, we observed that expression of a-SMA was found to be significantly in the OSMF group in comparison with the control group. Ganesan et al evaluated and compared the presence of myofibroblasts in normal mucosa, early invasive carcinoma and different grades of OSCC. The study included the archival tissues of 18 OSCC of well, moderate and poorly differentiated grades, three early invasive carcinomas and five normal mucosa. Myofibroblasts were identified by immunohistochemical detection of h1 calponin. The percentage and intensity of h1 calponin were examined and positive immunostaining was observed in the myofibroblasts of all SCCs and early invasive carcinomas; however, these cells did not stain in the normal epithelium specimens. The presence of myofibroblasts was significantly higher in invasive pattern of OSCCs compared to normal mucosa cases ($P < 0.070$). A significant difference was not observed between the different grades of OSCC ($P \leq 0.812$). These findings show the presence of myofibroblasts in OSCC but not in normal mucosa, suggesting that the genetically altered epithelium (carcinomatous epithelium) may have an inductive effect on the adjacent stroma to produce myofibroblasts. Also transdifferentiation of myofibroblasts is induced somewhere in the invasive stage of SCC irrespective of the epithelial cell differentiation. From the above results, the authors concluded that myofibroblasts increase significantly in OSMF patients. However, future studies are recommended.¹²

De-Assis et al evaluated the presence of stromal myofibroblasts in OL and OSCC. Differences in the presence of myofibroblasts among oral leukoplakia (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. Differences in the presence of myofibroblasts among OL with distinct grades of epithelial dysplasia as well as between high- and low-invasive OSCC were analyzed using the Mann-Whitney test. 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 ($p < 0.05$). Stromal myofibroblasts were not detected in OL, indicating that these cells are not important during oral carcinogenesis. Nevertheless, stromal myofibroblasts were heterogeneously detected in OSCC and its presence was higher in tumors with a more diffuse histological pattern of invasion. These findings suggested that myofibroblasts are associated with the creation of a permissive environment for tumor invasion in OSCC.¹³

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