Original Article

Myofibroblasts as important diagnostic and prognostic indicators of oral squamous cell carcinoma: An immunohistochemical study using alpha-smooth muscle actin antibody

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity, with multifactorial etiopathogenesis. Data from the past literature suggest that myofibroblasts (MFs) can also contribute significantly to the pathogenesis of the disease. Hence, the present study was undertaken for assessing the expression of MF in well-differentiated OSCC (WDOSCC), moderately differentiated OSCC (MDOSCC), poorly differentiated OSCC (PDOSCC) and healthy controls by immunohistochemistry using alpha-smooth muscle actin (α-SMA) antibody.

Methodology: Forty cases each of WDOSCC, MDOSCC, PDOSCC and healthy controls were included. 4-μm thick sections from each tissue sample were stained with routine hematoxylin and eosin as well as immunohistochemically using α-SMA. Among different grades of OSCC, expression of MFs was compared. All the results were subjected to statistical analysis.

Results: While comparing the expression of MFs in between different grades of OSCC, nonsignificant results were obtained. While comparing the expression of MF in between OSCC cases and normal controls, significant results were obtained.

Conclusion: MFs are one of the vital pathogenetic components in OSCC cases in predicting their invasive behaviors. We advocate the use of MFs as a stromal marker for visualizing invasion and progression in OSCC patients.

Keywords: Alpha-smooth muscle actin, myofibroblast, oral squamous cell carcinoma

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Submitted: 22-Sep-2020, **Revised:** 02-Jan-2021, **Accepted:** 29-May-2021, **Published:** 28-Jun-2022

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.jomfp_389_20

How to cite this article: Gandhi P, Kaur M, Punia RS, Halappa TS, Singh HP. Myofibroblasts as important diagnostic and prognostic indicators of oral squamous cell carcinoma: An immunohistochemical study using alpha-smooth muscle actin antibody. J Oral Maxillofac Pathol 2022;26:156-60.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) contributes over 90% of all oropharyngeal carcinomas and is rapidly becoming a global health priority. Etiology relates toward multifactorial origin and pathogenesis involves molecular and histological multistage process featuring genetic and phenotypic markers. The main reason behind the increased morbidity and mortality associated with this disease is the lack of awareness among the population and delay in diagnosis, thus indicating the need of early diagnostic and prognostic markers.[1-3]

MFs were first described in the literature by Giulio Gabbiani. They were first recognised modified fibroblasts in granulation tissue. These cells are regarded as a specific type of fibroblasts which regulate fundamental body processes such as proliferation, apoptosis, cell motility, tissue repair, wound healing and immune response. It has alpha-smooth muscle actin (α-SMA) expression and leads to formation of tension fibrils, collagen fibrils and spectrum of growth factors leading to tissue traction and remodeling during healing and reparative process.[4,5]

It is generally accepted that fibroblast-MF differentiation represents a major event during the physiological rebuilding of connective tissue after injury. Their alterations in number and function have been implicated in pathologies with rich extracellular matrix deposition and resultant fibrosis. MFs are involved in the process called stromal reaction and promote cancer progression by creating an inspiring microenvironment for epithelial cancer cells. Stromal response in OSCC comprises liberation of numerous inflammatory mediators, chiefly the cytokines, which promote neoangiogenesis, the transdifferentiation of fibroblast into MFs, etc.[6] Hence, under the light of the above mentioned facts, the present study was undertaken for assessing the expression of MF in well-differentiated OSCC (WDOSCC), moderately differentiated OSCC (MDOSCC), poorly differentiated OSCC (PDOSCC) and healthy controls by immunohistochemistry (IHC) using α-SMA antibody a specific marker for MFs.

METHODOLOGY

The present study aimed at assessing the expression of MFs in WDOSCC, MDOSCC, PDOSCC and healthy controls by IHC using α-SMA antibody. Study sample included a total of forty histologically confirmed cases each of WDOSCC, MDOSCC, PDOSCC and forty tissue samples of histologically confirmed normal mucosa as a control group. All the tissue blocks were retrieved from archives as well as new cases reported to the Department of Oral Pathology and Microbiology. For normal mucosa as controls, dental follicle tissue obtained during therapeutic extraction for orthodontic purposes was included. Two 4-μm thick sections were obtained from each tissue block. One section was stained with routine hematoxylin and eosin (H&E), whereas another tissue section was subjected for immunohistochemical analysis using α-SMA marker (Leica Biosystems, New Delhi). H-&E-stained slides were used as reference slides for confirming and grading OSCC cases. After IHC staining, all the slides were evaluated using Etemad-Moghadam *et al*. criteria for assessment of α-SMA-positive cells.[7] According to this criteria, staining was scored by a product of staining intensity (SI) and percentage of immunopositive cells (as stained by α-SMA). SI was graded as follows:

- Intensity 0%: Absence of immunostaining
- Intensity 1%: Immunostaining positive and observed at ×400 magnification only
- Intensity 2%: Immunostaining positive and observed at ×400 and ×100 magnification only and not at ×40
- Intensity 3%: Immunostaining positive and observed even at ×40.

The percentage of immunopositive cells stromal cells (nonendothelial and noninflammatory) at the tumor invasive front of OSCC was calculated in five high power fields (HPFs). Afterwards, average percentage per HPF was calculated and recorded as follows:

- 0% = Absence of immunopositive cells
- 1% =1%–25% immunopositive cells
- 2% =26%–50% immunopositive cells
- 3% =51%–100% immunopositive cells.

Final staining index (I) was obtained by multiplication of the percentage and SI of each specimen. Final indexing of immunopositive staining was as follows:

- Index zero: Score 0
- Index low = Score 1 and 2
- Index moderate = Score 3 and 4
- Index high = Score 6–9.

All sections were counted twice to avoid intraobserver variability. All the results were recorded in Microsoft Excel sheet and were analyzed by SPSS software. Mann–Whitney *U*-test and Chi-square test were used for evaluation of level of significance.

RESULTS

The current study was performed in three different grades of OSCC and included forty cases each of WDOSCC, MDOSCC, PDOSCC and normal mucosa as controls. After evaluating the specimens immunohistochemically using α-SMA marker, results revealed a mean final staining index score of 8.41 in WDOSCC cases [Figure 1], 8.26 in MDOSCC cases [Figure 2] and 7.67 in PDOSCC cases [Figure 3]. However, negative expression was seen in controls [Figure 4], as shown in Graph 1.

Intergroup comparison of final staining index score [Table 1] among different grades of OSCC showed no statistical significance ($P \leq 0.05$) in the results and also expression of MFs in between different grades of OSCC showed nonsignificant results. On the other hand, a comparison of final staining index score [Table 2] between OSCC and normal controls and the expression of MF between OSCC cases ($n = 120$) and normal controls showed high statistical significance ($P \geq 0.05$).

DISCUSSION

Oral cancer comprises a spectrum of malignant neoplasms affecting the lips, oral cavity, oropharynx, nasopharynx, hypopharynx and other intraoral sites.[8,9] Process of invasion and progression in epithelial malignancies have been regarded as a stepwise accretion of genetic alterations within the target epithelium. Data from the past literature consist of significant proportion of studies demonstrating such molecular progression involving oral cavity. In the head-and-neck region, appearance of premalignant pathologies (dysplasia of varying grades), followed by invasion and formation of carcinomas, is paralleled by increases in tissue's genetic alterations.[5,7,8]

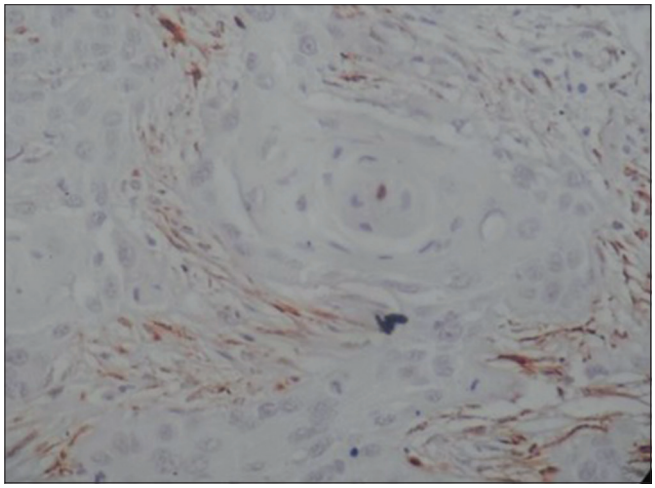


Figure 1: Expression of myofibroblast in well-differentiated oral squamous cell carcinoma

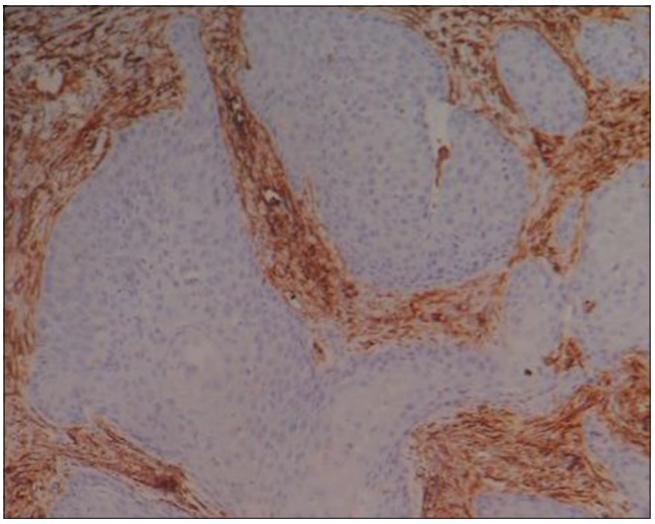


Figure 2: Expression of myofibroblast in moderately differentiated oral squamous cell carcinoma

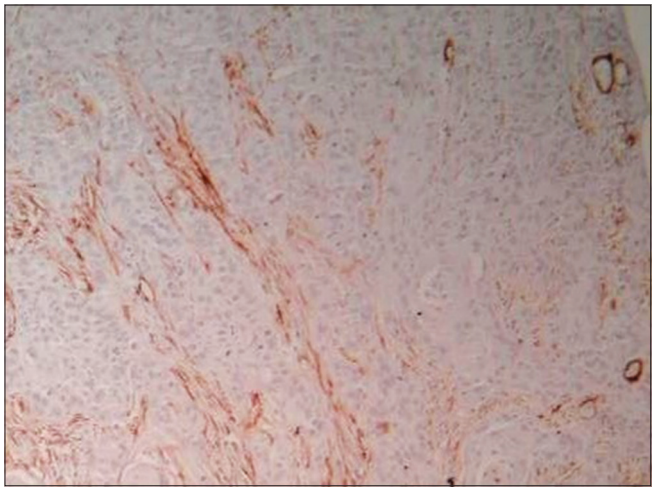


Figure 3: Expression of myofibroblast in poorly differentiated oral squamous cell carcinoma

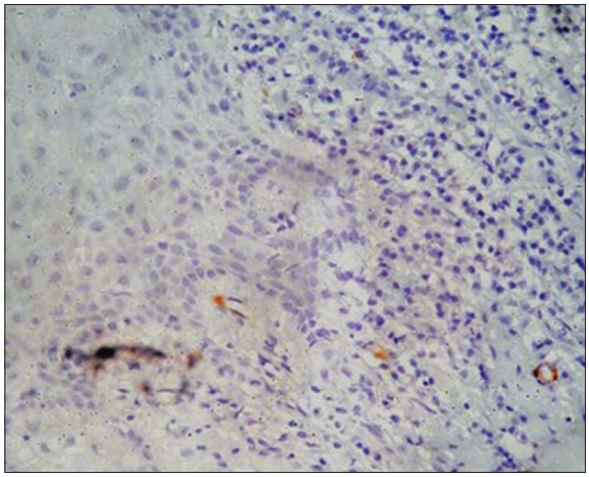


Figure 4: Expression of myofibroblast in normal controls

Table 1: Comparison of final staining index score between different grades of oral squamous cell carcinoma

Groups	*P*
WDOSCC (*n*=40) versus MDOSCC (*n*=40)	0.850
WDOSCC (*n*=40) versus PDOSCC (*n*=40)	0.148
MDOSCC (*n*=40) versus PDOSCC (*n*=40)	0.338

OSCC: Oral squamous cell carcinoma, WDOSCC: Well-differentiated OSCC, MDOSCC: Moderately differentiated OSCC, PDOSCC: Poorly differentiated OSCC

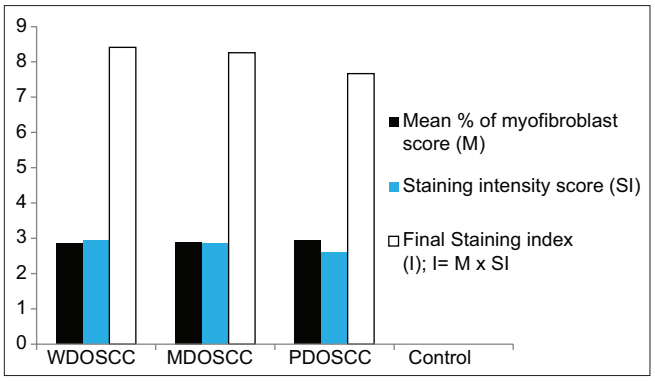
Table 2: Comparison of final staining index score between oral squamous cell carcinoma and normal controls

Groups	*P*
Controls (*n*=40) versus WDOSCC (*n*=40)	0.023 (S)
Controls (*n*=40) versus MDOSCC (*n*=40)	0.030 (S)
Controls (*n*=40) versus PDOSCC (*n*=40)	0.018 (S)
Controls (*n*=40) versus OSCC (*n*=120)	0.010 (S)

S: Significant, OSCC: Oral squamous cell carcinoma, WDOSCC: Well-differentiated OSCC, MDOSCC: Moderately differentiated OSCC, PDOSCC: Poorly differentiated OSCC

Facts from various clinical trials on epithelial–mesenchymal interactions in malignancies of head-and-neck region have demonstrated that both keratinocytes along fibroblastic factors are vital for epithelial morphogenesis and differentiation. In OSCC patients, MFs are the activated stromal cells that are responsible for the advancement and metastasis of the tumor. These MFs have smooth muscle properties and are said to secrete various degrees of inflammatory mediators and growth factors that facilitate tumor progression. Statistics from recent studies have shown that these MFs are present in significant proportion in the stroma of head-and-neck cancer cases.[9-11]

In the present study, intergroup comparison of final staining index score and expression of MFs among different grades of OSCC revealed nonsignificant results. These results were in accordance with the results published by Gandhi and Prasad and Prasad *et al.* They also reported similar findings in their respective studies.[3,12] Our results were also in concordance with the results obtained by Etemad-Moghadam *et al.* and Kellermann *et al.* They also observed any significant association between the expressions of MF in different histopathologic grades of OSCC. These results support the hypothesis that MF differentiation occurs only during the invasive process of OSCC and further increase in severity and advancing histopathologic grade does not alter their expression.[7,13] In previous literature, results have demonstrated a significant correlation between tumor invasions and differentiation of MF. These studies also showed that the formation of MF in OSCC cases is dependent upon transforming growth factor-beta and other growth factors.[12-15]



Graph 1: Percentage of myofibroblast score, staining intensity score and final staining index score among all the study groups

In the present study, while comparing the expression of MF among different grades of OSCC cases (n = 120) and normal controls, significant results were obtained. In another study conducted by Lewis *et al.*, the authors showed the presence of MF at the tumor invasive front of OSCC cases but did not observe any MF expression in mucosal polyps. Vered *et al.*, in their study, demonstrated the presence of the MF in the stroma of most human OSCC cases.[11,14] However, some of the studies have associated MFs with tumor prognosis. Majority of these studies have demonstrated the increased expression of MFs to be associated with poor prognosis. In their previous study comprising tongue carcinoma cases, the authors also reported a significant difference in 5-year survival rate in patients with differential MF expressions. They concluded an 82% 5-year survival rate in patients with negative or weak MF expression in comparison to 38% 5-year survival rate in patients with positive MF expression.[11,14,15]

Another study conducted by Mashhadiabbas *et al.* on odontogenic lesions suggested that the expression of MFs was an indicator of invasive behavior, suggesting that treatment therapy targeting MF could be beneficial as an auxiliary method for treating more invasive lesions.[16] Significantly higher expression of MF in OSCC cases in comparison to premalignant pathologies has also been demonstrated in the past literature. The authors also demonstrated the negative expression of MF in controls, followed by higher expression of MF in oral submucous fibrosis cases (premalignant condition) and further significantly higher expression in OSCC cases, highlighting their role in the pathogenesis of the disease.[3]

CONCLUSION

Based on the facts of the current study, we conclude that MFs are one of the vital pathogenetic components in OSCCs and their evaluation helps in predicting their

invasive behavior. Thus, we advocate the use of MFs as a stromal marker for visualizing invasion and progression in OSCC patients. However, further studies involving larger samples are recommended for additional facts to these findings.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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