Original Article

An Immunohistochemical Analysis for Evaluating the Diagnostic Role of Myofibroblasts in Oral Squamous Cell Carcinoma using α-Smooth Muscle Actin Antibody

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Abstract

Background: One of the most common types of malignancies affecting the head and neck region is oral squamous cell carcinoma (OSCC). Little less is known about the role of myofibroblasts in the pathogenetic process of OSCC. Hence, we assessed the involvement of myofibroblasts in the invasive process of OSCC using α-SMA (α-smooth muscle actin) antibody.

Materials and Methods: Four study groups in total were organized as follows: 40 cases each of well-differentiated OSCC (WDOSCC), moderately differentiated OSCC (MDOSCC), poorly differentiated OSCC (PDOSCC), and controls make up Group 1, Group 2, Group 3, and Group 4, respectively. The percentage of α-SMA immunopositive cells and staining intensity (A) multiplied together to determine the final staining score (B). The final staining index was produced by multiplying staining intensity (A) by the proportion of immunopositive cells that were stained with α-SMA (B) (FSI). Score Zero was graded as Index Zero by FSI while scores One and Two received an Index Low rating, scores Three and Four an Index Moderate rating, and scores Six and Nine an Index High rating.

Results: Significantly higher expression of myofibroblast was observed in OSCC group in comparison with the control group. However; no significant difference in myofibroblast expression was observed while comparing different grades of OSCC.

Conclusion: We recommend using myofibroblasts as a stromal marker to track the severity and development of OSCC.

Keywords: Carcinogenesis, carcinoma, myofibroblast, squamous cell

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Introduction

The most prevalent type of oral cavity cancer is oral squamous cell carcinoma (OSCC), which is also the 12th most common cancer worldwide. One of the main health issues in India and the countries of the Indian subcontinent is oral cancer. The primary etiological factor for oral carcinoma is tobacco use.[1] These nations utilize tobacco in a variety of ways, including betel quid, tobacco with lime, bidi, hookah, etc. Minor etiological factors for oral carcinoma include the human papillomavirus, nutritional deficits, and poor oral hygiene. Because lifestyle risk factors are more prevalent in lower socioeconomic societal strata, mouth cancer affects them more frequently.[2,3]

Like all cancers, oral carcinogenesis (OC) develops over time, with normal epithelium going through stages of dysplasia until becoming invasive morphologies. Squamous cell carcinoma is the most prevalent variety of OC, despite the fact that all types of carcinomas can be found in the oral cavity. In recent years, the molecular pathological picture of OC has been unveiled

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through the use of genomic and proteomic approaches. There is ongoing research to determine the role of genomic instability, epigenetic changes, and the generation of a gene expression profile in the development of oral cancer. Understanding these genetic alterations and the patterns of gene expression is essential for comprehending the molecular etiology of OC.[4,5]

The stroma was once thought of as a tissue that supported cancer cells, but the results of numerous scientific investigations showed that it can actually inhibit tumor invasion and metastasis. Myofibroblasts, a particular type of fibroblast, are one of the stromal responses. Due to the existence of contractile machinery, myofibroblasts resemble smooth muscles and were initially observed by "Gabbiani" in granulation tissue during wound healing using an electron microscope. These cells are essential for both pathological diseases, including reactive lesions, benign tumors, and locally aggressive tumors, and malignancies that impact the oral cavity as well as physiological processes like wound healing. Myofibroblasts are recognizable and referred to as juxtaparenchymal cells due to their position in normal skin tissues, pulmonary septa, and periodontal ligaments. It expresses α-smooth muscle actin (α-SMA), which results in the creation of collagen fibrils, tension fibrils, and a range of growth factors that cause the remodeling and traction of tissue throughout the healing and reparative processes.[5-7] To assess the involvement of myofibroblasts in the invasive process of oral squamous cell carcinoma, we, therefore, conducted this investigation.

Materials and Methods

With the intention of examining the expression of myofibroblasts in the three histopathological grades of OSCC—well-differentiated OSCC, moderately differentiated OSCC, and poorly differentiated OSCC—the current study was started at the Dasmesh Institute of Research and Dental Sciences in Faridkot. As controls, it was decided to use normal oral mucosa extracted following the therapeutic removal of impacted teeth. Four study groups in total were organized as follows: 40 cases each of well-differentiated OSCC, moderately differentiated OSCC, poorly differentiated OSCC, and controls make up Group 1, Group 2, Group 3, and Group 4, respectively. Based on how well, moderately, and badly the cancer cells differentiate, tumors were classified morphologically into three types of carcinomas. Well-differentiated, low-grade oral squamous cell carcinoma typically invades connective tissue, muscle, or bone before spreading to nearby lymph nodes. However, poorly differentiated, high-grade oral cancer was biologically more aggressive and had a propensity to spread early in the course of the disease to nearby lymph nodes.[8] Using α-SMA antibody and the immunohistochemistry (IHC) process, all specimens were stained. To confirm the diagnosis, a histopathological study of all tissue samples on H&E-stained slides was conducted. All of the study groups' paraffin-embedded tissue blocks were obtained from the departmental archives. Microtome was used to cut all the tissues into 3 m slices (Leica Biosystems, New Delhi, India). All of the samples were stained using IHC using the myofibroblast marker α-SMA antibody from Leica Biosystems in New Delhi, India. IHC-stained slides were evaluated using criteria set forth by Etemad-Moghadam *et al*.[9] This criterion is based on the assessment of cells that are α-SMA positive. According to these staining parameters, the percentage of α-SMA immunopositive cells and staining intensity (A) are multiplied together to determine the final staining score (B). Grading of staining intensity was done as 0% (absence of immunopositive cells), 1% (immunopositive staining observed at ×400 magnification only), 2% (immunopositive staining observed at ×400 and ×100, magnification only), and 3% (absence of immunopositive staining observed at ×400 and ×100 magnification only) (immunopositive staining observed at even magnification of ×40). The percentage of immunopositive cells was graded as Zero score (absence of immunopositive cells), One score (1%–25% immunopositive cells), Two score (26%–50% immunopositive cells), and Three score (51%–100% immunopositive cells) in cases of OSCC and subepithelial connective tissue, respectively. The final staining index was produced by multiplying staining intensity (A) by the proportion of immunopositive cells that were stained with α-SMA (B) (FSI). Score Zero was graded as Index Zero by FSI while Scores One and Two received an Index Low rating, Scores Three and Four an Index Moderate rating, and Scores Six, and Nine an Index High rating. To reduce intraobserver variability, every segment was examined twice. To ensure that the IHC staining was appropriate, blood vessels were used as a positive internal check. Assessment of all the findings was done by Statistical Package for Social Science 19 for all findings analysis (SPSS, version 20.0, IBM Analytics) followed by analysis of results using Mann–Whitney *U* test. *P* values lower than 0.05 were considered significant. Date of approval: 21/10/2019. Ethical approval number: Patho 689/19.

Results

As indicated in Table 1, the findings of the present investigation show that the mean staining intensity values for specimens from groups 1, 2, 3, and 4 were 2.82, 2.91, 2.98, and 0 correspondingly. In addition, it revealed that the specimens from groups 1, 2, 3, and 4 had mean immunopositive cell percentage scores of 2.88, 2.95, 2.97, and 0 accordingly, as shown in Table 1. The mean final staining index for samples from groups 1, 2, 3, and 4 was, correspondingly, 8.12, 8.58, 8.85, and 0. When group 1, group 2, and group 3 specimens' mean final staining indices were compared, the results were nonsignificant. Significant findings were achieved when comparing group 1 and group 4, group 2 and group 4, and group 3 and group 4 as shown in Table 2. This demonstrates that the mean final staining index was considerably greater in the OSCC groups with well, moderate, and poor differentiation compared with healthy controls. Significant results were found when comparing the final staining index between the total OSCC group (Groups 1, 2, and 3) and the controls (Group 4) as shown in Table 3. Figures 1-4 demonstrate the expression

Table 1: Final staining index score

Group	*n*	Mean percentage of myofibroblast score	Mean staining intensity score	Final staining index score=Mean percentage of myofibroblast score × Mean staining intensity score
Group 1	40	2.82	2.88	8.12
Group 2	40	2.91	2.95	8.58
Group 3	40	2.98	2.97	8.85
Group 4	40	0	0	0

Table 2: Comparison of final staining index score

Groups comparison		*P*	Significance
Group 1 (*n*=40)	Group 2 (*n*=40)	0.087	Nonsignificant
Group 1 (*n*=40)	Group 3 (*n*=40)	0.096	Nonsignificant
Group 1 (*n*=40)	Group 4 (*n*=40)	0.018	Significant
Group 2 (*n*=40)	Group 3 (*n*=40)	0.280	Nonsignificant
Group 2 (*n*=40)	Group 4 (*n*=40)	0.012	Significant
Group 3 (*n*=40)	Group 4 (*n*=40)	0.001	Significant

Table 3: Comparison of final staining index score between OSCC and controls

Groups comparison	*P*	Significance
Group 1+ Group 2+ Group 3 (*n*=120)		
Group 4 (*n*=40)	0.0001	Significant

of myofibroblast in Group 1, Group 2, Group 3, and Group 4, respectively.

Discussion

The sixth most prevalent cancer worldwide is oral squamous cell carcinoma. Up to 80,000 new cases of oral cancer are recorded each year in India, according to the National Cancer Registry Program of the Indian Council of Medical Research. The use of a pan to chew tobacco, smoking, and alcohol were common risk factors for oral squamous cell cancer.[8-10] The most prevalent oral subsite in India was buccal mucosa squamous cell carcinoma, which was physiologically distinct from the other oral subsites, was aggressive in nature, and required multimodality treatment.[10-12] In oral squamous cell carcinoma (OSCC), the tumor stroma is linked to the release of several cytokines from cancerous cells, including transforming growth factor β-1, which encourages the differentiation of fibroblasts into myofibroblasts, neo-angiogenesis, increases the number of inflammatory cells, and boosts the expression of mesenchymal markers like vimentin. By destroying the ECM, myofibroblasts in turn promote the formation of tumors. Myofibroblasts have been found in the stroma of head and neck squamous cell carcinomas, according to recent investigations. According to recent studies, several tumor forms have a poor prognosis and an increase in myofibroblasts in the neoplastic stroma.[13-16]

In the present study, significant findings were found in the current study when comparing the expression of myofibroblast

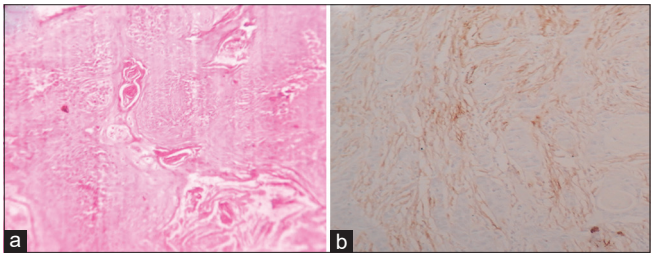

Figure 1: (a): H&E-stained section of Group 1 (WDOSCC group); (b): IHC staining of Group 1 (WDOSCC group) showing positive staining for myofibroblast in the stroma

in patients with OSCC and healthy controls. Our findings agreed with those of Gandhi *et al*. (2017), who similarly showed significantly increased myofibroblast expression in OSCC specimens. Gandhi *et al*. (2022) also showed considerably increased myofibroblast expression in patients with OSCC in comparison with healthy controls in their later extended research among patients with carcinoma in the Malwa region of Punjab.[13,16] Recent therapeutic trials examining the use of stromal fibroblasts in the treatment of cancer have yielded encouraging results. In addition, stromal cells have the benefit of being genetically more stable than tumor cells and may share characteristics with other tumor types. Perhaps therapy regimens designed to target stromal cell-mediated protumorigenic processes could enhance the efficacy of regimens that target the tumor cells directly. These statistics support the idea that using stromal tumor cells as a therapeutic therapy for solid neoplasms originating from epithelial tissue is an effective strategy.[5]

In the current research, results from the intergroup comparison of the final staining index score and the expression of MFs among the various grades of OSCC in the current investigation were nonsignificant. These findings were consistent with those reported by Gandhi *et al*. They each reported identical results from their research.[13] In addition, our findings agreed with those of Etemad-Moghadam *et al*.[9] and Kellermann *et al*.[14] In addition, they looked for any associations that were statistically significant between MF expressions in various OSCC histopathological grades. These findings support the idea that MF differentiation only takes place during the invasive phase of OSCC and that further escalation of severity and rising histopathological grade do not change the expression of these molecules. Results from earlier literature have shown a substantial relationship between tumor invasions and MF differentiation. These investigations also demonstrated

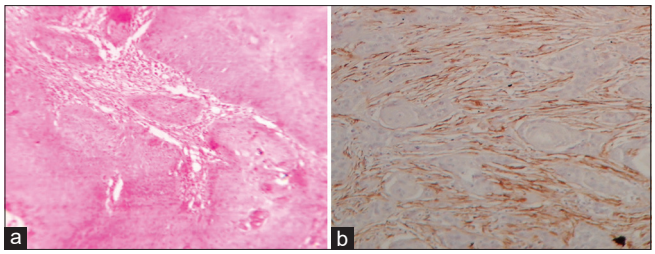

Figure 2: (a): H&E-stained section of Group 2 (MDOSCC group); (b): IHC staining of Group 2 (MDOSCC group) showing positive staining for myofibroblast in the stroma

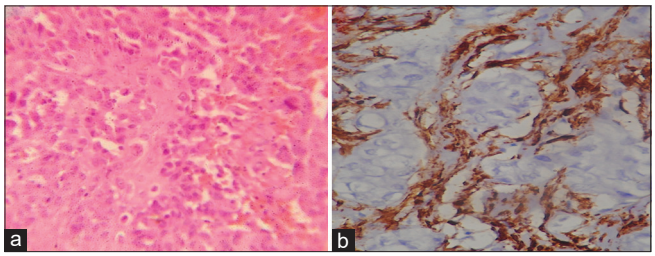

Figure 3: (a): H&E-stained section of Group 3 (PDOSCC group); (b): IHC staining of Group 3 (PDOSCC group) showing positive staining for myofibroblast in the stroma

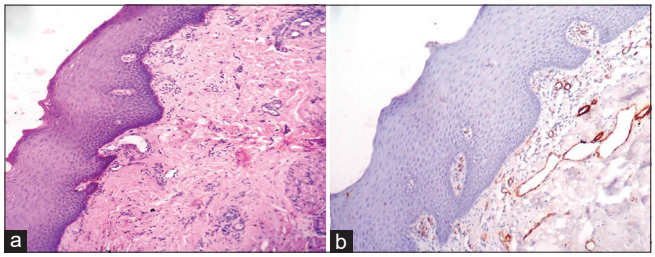

Figure 4: (a): H&E-stained section of Group 4 (Healthy controls); (b): IHC staining of Group 4 (healthy controls) showing positivity around blood vessels only (Internal control) as depicted by black arrowhead and negative expression in the stroma

that transforming growth factor-β and other growth factors are necessary for the development of MF in cases with OSCC.[9,14,17,18]

Myofibroblasts may also serve as possible targets in the therapy of cancer, according to research. These tactics, which include the regulation of the signaling pathways involved in myofibroblast production, differentiation, and direct eradication, attempt to prevent interactions between myofibroblasts and malignant cells.[19] Numerous studies have demonstrated that the microenvironment of neoplastic tissues actively contributes to tumor progression. In addition, the stroma would change to an activated state where fibroblasts, one of this compartment's most significant constituents, are transdifferentiated into active myofibroblasts, resembling a critical step in human carcinogenesis, in addition to the conversion of normal epithelial tissue into dysplastic epithelium and carcinoma. Transforming growth factor-β (TGF-β), in particular, appears to be responsible for this shift in myofibroblasts' condition, whereas myofibroblasts themselves release a variety of growth factors and inflammatory agents that promote the proliferation of epithelial neoplastic cells. Since myofibroblasts are cancer-induced host cells, numerous studies have demonstrated the significance of stromal components in tumor genesis, growth, invasion, and aggressiveness.[20-22] In a similar study carried out by Shete *et al*.,[23] authors have reported significantly higher expression of myofibroblasts in patients with carcinoma in comparison with healthy controls. They concluded that genetically altered epithelium (malignant epithelium) might have an inductive impact on the adjacent stroma to produce myofibroblasts. Pinisetti *et al*.,[24] in another study, reported that myofibroblasts may play a role in cancer cell invasion; so, the treatment strategies targeting the myofibroblasts might be beneficial in patients with OSCC. Similar findings were also reported by Khalid *et al*. who also reported a significant positive role of myofibroblasts in invasive pathogenic process of OSCC.[25]

Conclusion

As a result, we recommend using myofibroblasts as a stromal marker to track the severity and development of OSCC. But more investigation can be done to determine the precise pathways by which myofibroblasts contribute to the development and behavior of OSCC.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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