Myofibroblasts as important diagnostic and prognostic indicators of oral squamous cell carcinoma

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Abstract

Background: The most prevalent oral cancer, oral squamous cell carcinoma (OSCC), has a complex etiopathogenesis. Myofibroblasts (MFs) may have a significant contribution to the aetiology of the disease, according to data from earlier research. As a result, the current investigation was conducted to evaluate the expression of MF in well-differentiated OSCC (WDOSCC), moderately differentiated OSCC (MDOSCC), poorly differentiated OSCC (PDOSCC), and healthy controls using immunohistochemistry and an alpha-smooth muscle actin (α-SMA) antibody. **Methodology**: There were 100 cases of WDOSCC, MDOSCC, PDOSCC, and healthy controls total. Each tissue sample was cut into 4-m thick sections, which were then both conventionally stained with hematoxylin and eosin and immunohistochemically stained with α-SMA. The expression of MFs was compared among OSCC grades. Statistics were applied to all of the outcomes. **Results**: The current study was performed in three different grades of OSCC and included 100 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 9.67 in WDOSCC cases, 9.23 in MDOSCC cases and 8.12 in PDOSCC cases. **Conclusion**: It was concluded that MFs are one of the essential pathogenetic elements in OSCCs based on the facts of the current investigation, and that evaluating them might assist anticipate their invasive behaviour. Therefore, we support the use of MFs as a stromal marker for OSCC patients to visualise invasion and progression.

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

Introduction:

One of the commonest forms of cancer is head and neck cancer.[1] Its prevalence is different in various parts of the world; in unindustrialized countries, like India, it is the cancer most commonly diagnosed in male patients whereas in the Western world, it is responsible for 1–4% of all cancers.[2] Lip, oral cavity, and oropharynx combined were responsible for about 4,47,751 new cancer cases with an estimated 2,28,389 deaths in 2018, which accounts for 2.4% of all cancer deaths.[3] Among other cancers, head and neck cancer is fourteenth in terms of incidence but thirteenth in terms of mortality. The Asian continent has the highest incidence and mortality rates of oral cavity and oropharynx cancers among all other countries.[4] More than 90% of cancer cases in head and neck region are OSCCs.[5] OSCC develops in the oral cavity and oropharynx and can occur due to many etiological factors, but smoking and alcohol remain the most common risk factors especially in the Western world.[6] In South Asian countries, consumption of smokeless tobacco and areca

nut products are the main etiological factors associated with OSCC.[7] Gene mutations may also cause cancer development in the pharynx and oral cavity; however, no specific gene has been identified in OSCCs.[8] Activation of proto-oncogenes (ras, myc, EGFR) or inhibition of tumor suppressor genes (TB53, pRb, p16) by environmental factors such as smoking, irradiation, and viral infection may increase the risk of oral and oropharynx OSCC.[9] Most of the oral and oropharynx OSCC cases occur in elderly male patients, with tonsils and tongue being the most commonly affected sites.[10]

Hence, the current study was undertaken to assess the role of myofibroblasts as important diagnostic and prognostic indicators of oral squamous cell carcinoma.

Material and methods:

The current investigation used IHC with a SMA antibody to evaluate the expression of MFs in WDOSCC, MDOSCC, PDOSCC, and healthy controls. A total of forty cases with WDOSCC, MDOSCC, and PDOSCC with histological confirmation were included in the study sample, along with forty tissue samples from normal mucosa with the same confirmation. As well as new cases submitted to the Department of Oral Pathology and Microbiology, all tissue blocks were collected from archives. Dental follicular tissue removed therapeutically for orthodontic purposes was used as controls for normal mucosa. Each tissue block yielded two 4 m thick slices. A tissue section was exposed to immunohistochemical examination using the SMA marker (Leica Biosystems, New Delhi) while another tissue segment was stained with standard hematoxylin and eosin (H&E). For verifying and grading OSCC cases, H &E stained slides were used as reference slides.

Results:

The current study was performed in three different grades of OSCC and included 100 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 9.67 in WDOSCC cases, 9.23 in MDOSCC cases and 8.12 in PDOSCC cases. However, negative expression was seen in controls. Intergroup comparison of final staining index score 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 between OSCC and normal controls and the expression of MF between OSCC cases and normal controls showed high statistical significance ($P \geq 0.05$).

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

Groups	P value
Well differentiated oral squamous cell carcinoma V/s Moderately differentiated oral squamous cell carcinoma	0.931
Well differentiated oral squamous cell carcinoma v/s poorly differentiated oral squamous cell carcinoma	0.204
Moderately differentiated oral squamous cell carcinomav/s poorly differentiated oral squamous cell carcinoma	0.269

Discussion:

Oral squamous cell carcinoma (OSCC) is a malignancy with high mortality and morbidity. Early diagnosis and treatment of OSCC and other potentially malignant lesions of the oral mucosa is the clinician's best weapon to improve prognosis, since it greatly worsens as the disease becomes more advanced. In Western countries, oral cancer represents a rather uncommon malignancy, with oral squamous cell

carcinoma (OSCC) being most frequent.[11] OSCC has high mortality and morbidity,[12] which significantly increases with diagnostic delay.[13] As the most common risk factors for OSCC are well known and are for the most part behaviors that can be eliminated, primary prevention consists in educating the population against these behaviors.[14] Once the cancer is present, early diagnosis is the single most important element in improving prognosis, since clinical and pathological staging is the most important factors that influence survival rates.[15]

Concurrent with the conversion of nondiseased epithelial tissue to precancerous epithelium to carcinoma, the stroma also changes from normal to "primed" to "activated or tumor associated." Remodeling of the extracellular matrix (ECM) or "stromagenesis" is initiated by tumor cells, while stromal cells are responsible for the organization of this process. Fibroblasts are considered as one of the most important mesenchymal cells involved in tumorprogression.Myofibroblasts are a unique group of cells phenotypically intermediate between smooth muscle cells and fibroblast.[16] In addition to their normal role in tissue homeostasis and repair, altered number and function of myofibroblasts have been implicated in diseases with increased ECM deposition and resultant fibrosis, and now, researchers have started understanding their role in cancers. They modulate the tumor stroma through secretion of a myriad of factors such as chemokines, growth factors, and matrix-degrading enzymes like MMPs. MF are prominent feature of tumor stroma of many but not all OSCCs.[17]

In this study, theresults revealed a mean final staining index score of 9.67 in WDOSCC cases, 9.23 in MDOSCC cases and 8.12 in PDOSCC cases. However, negative expression was seen in controls. Intergroup comparison of final staining index score 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 between OSCC and normal controls and the expression of MF between OSCC cases and normal controls showed high statistical significance ($P \geq 0.05$).

MVShete et al[18]evaluated, compared, and correlated the presence of myofibroblasts in normal oral mucosa, oral epithelial dysplasia, and OSCC and to observe different patterns of myofibroblast arrangement using alpha-smooth muscle actin (α-SMA) as a marker, Thus assisting in early diagnosis, treatment, and prognosis of oral carcinomas.Thirty-six cases including 12 cases of OSCC, 12 cases of epithelial dysplasia, and 12 cases of normal oral mucosa were stained with hematoxylin and eosin to confirm the diagnosis and immunohistochemically using α-SMA antibody. The slides were evaluated for positivity and intensity of staining. The result was subjected to statistical analysis using Fisher's exact test.α-SMA expression in the stroma of squamous cell carcinoma was greater than its expression in epithelial dysplasia and normal oral mucosa.

Adegboyega et al.[19] in 2002 used α-SMA and vimentin IHC staining on myofibroblasts, for normal colon mucosa, hyperplastic polyps, and colorectal adenomatous in their research. α-SMA-negative fibroblasts and vimentin-positive ones were observed in the colon mucosa, whereas α-SMA- and vimentin-positive fibroblasts were observed in hyperplastic and neoplastic polyps. They concluded that in neoplastic cases, intercellular fibroblasts differentiate into myofibroblasts in the stroma of SCC. They also studied its relationship with the tumor stage and reported that there was a relationship between the expression of α-SMA and tumor stage.

Conclusion:

It was concluded that MFs are one of the essential pathogenetic elements in OSCCs based on the facts of the current investigation, and that evaluating them might assist anticipate their invasive behaviour. Therefore, we support the use of MFs as a stromal marker for OSCC patients to visualise invasion and progression.

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