WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 5, 171-179. Research Article ISSN 2277– 7105

EMPHASIZING MYOFIBROBLASTS THROUGH ALPHA SMOOTH MUSCLE ACTIN IN ORAL SQUAMOUS CELL CARCINOMA

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Article Received on
06 March 2016,
Revised on 27 March 2016,
Accepted on 17 April 2016
DOI: 10.20959/wjpr20165-6069

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ABSTRACT

Objective: To highlight the role of Alpha smooth muscle actin by immunohistochemistry in oral squamous cell carcinoma (OSCC). **Materials and methods:** In this cross-sectional investigation of 80 oral squamous cell carcinoma cases we studied myofibroblasts by means of immunohistochemical evaluation using alpha smooth muscle actin as a biomarker with histological grading constraints. The statistics was exposed to descriptive data and chi-square using SPSS version 16. **Results:** The study includes 80 patients in which 50 were males (62.5%) and 30 were females (37.5%) age range from 18-78 (mean 45 ± 14.1). A significant association between the extraction of ASMA and histological grading of OSCC has been related with poor prognosis was determined. (*P*-value: 0.000). **Conclusion:** This analysis shows that high illustration of alpha smooth muscle actin is valuable for determining an aggressive presentation of oral squamous cell carcinoma.

KEYWORDS: Oral squamous cell carcinoma, Stromal myofibroblasts, Alpha smooth muscle actin.

INTRODUCTION

In the head and neck region the most common cancer; originating from oral keratinocytes is the oral squamous cell carcinoma (OSCC).[1] OSCC in general is altering grades of occurrence and death around the world with high proportions strikingly in south East Asia

and in Eastern Europe.[2] It has been considered to infer for the massive disease burden worldwide.[2] The main risk factor worldwide is chiefly Tobacco, though betel nut is being used by 20% of world's population.[1] OSCC patient has poor prognosis and has a low five year survival rates worldwide.[1] Similarly the second most common malignancy in Pakistan is oral carcinoma in both genders.[3] According to (WHO, 2005) Pakistan is a developing country, with low income resources.[4] Bhurgri Y et[4] al from Pakistan have stated yearly incidence rate of oral cancers 4.1 per 100,000/ year in males and 4 per 100,000/year in females.

The key role in gross and aggressive behavior of diverse pathological grades of OSCC is tumoralstroma.[5] Epithelial cancers undergo an unique process during development of connective tissue cells and extracellular matrix formation; this process has been called the stromal reaction.[6] Stromal reactions in cancer diagnosis and prognosis has a major significance.[5] In carcinogenesis it has a pivotal role in supporting tumor invasive behavior.[5] Stromal cells bear the proficiency to actively contribute in tumor development through secretion of proteolytic enzymes, which allow invasion and metastasis.[6]

The Alpha Smooth Muscle Actin (ASMA), similar in feature to smooth muscle cells (SMC) and found in majority at vascular smooth muscles.[7] Regarding normal body cell presence of ASMA, it is present in myoepithelialcells and stromal cells in variety of vital body organs.[8] The invasive malignancies expresses high volume of ASMA, probably due to dense population of myofibroblasts in their stromal compartment.[9] As an additional favor ASMA in the form of antitumor therapy may be successfully utilized in opposition to myofibroblast at some stage in fibrotic and malignant conditions in regulation to manage tumor progression.[10]

The importance of the study is to understand the function of tumoralstroma in whole and aggressive behavior of different grades of OSCC. Thus the purpose of our investigation is to evaluate the expression of alpha smooth muscle actin (SMA) in oral squamous cell carcinoma (OSCC) to confirm their diagnostic and prognostic significance and to reveal the process of myofibroblast development in OSCC.

MATERIAL AND METHODS

Tissue samples

This cross-sectional study was approved by the local ethics committee. 80 samples were taken from patients presenting with oral squamous cell carcinomas. All the specimens obtained will be studied using routine Hematoxylin & Eosin staining for histological parameters and Immunohistochemistry for Alpha smooth muscle actin.

Histological grading of OSCC

The histological grade was determined according to the degree of differentiation of the tumor (broder's classification).

Immunohistochemistry

Immunohistochemistry was performed with a sensitive peroxidase-streptavidin method using antibody against A-SMA.

- Histogrip (appendix 1V) coated slides were used for mounting 3-4 µm thick tissue sections embedded in paraffin, which were then dried at 56° C for 30 minutes.
- Xylene was used to deparaffinize the tissue specimen which was then rehydrated in a serial gradation (100%, 90%, 70% and 50%) using water- ethanol solutions and deionized water was used for rinsing.
- Target retrieval solution (DAKO, Denmark) was used for retrieval of antigen.
- Adequate amount of target retrieval solution was filled in the Couplin jar and positioned in water bath. The water bath was heated to 95-99℃.
- Then the tissue section is submerged in to a preheated target retrieval solution in water bath and incubated for 20-40 minutes.
- The whole container was after that removed and container with slide from water bath permitted to cool for twenty minutes at room temperature.
- Once antigen recovery endogenous peroxidase activity was prevented by immersion of slides in peroxidase block solution (3% hydrogen peroxide containing sodium Azide for ten minutes.
- Following cleaning by means of TBST (Tris buffer saline with Tween 20) (appendix 4) tissue slides were incubated with principal antibody of preference. Principal antibody will be incubated for one hour in optimized dilution at room temperature.

- Tissue sections were cleaned to eliminate extra buffer and incubated with peroxidase labeled polymer complex (provided in the Envision system) for 35 minutes. (appendix V)
- The tissue sections were cleaned with 1X TVST buffer solution and after that with distil water. Antigen antibody color advance was conceded out by applying substrate-chromogen solution. (appendix)
- Sections were developed with DAB (3, 4, 3', 4'- tetra amino biphenyl hydrochloride) for five minutes at 37℃, cleaned meticulously with distil water and counter stained with Harris Haematoxylin.
- The tissue sections were irrigated with running water for ten minutes, decolorized in 1% acid alcohol and wiped again with water for five minutes.
- Specimens were dried out in 70% alcohol, 80% alcohol, 95% and 100% alcohol for two minutes.
- Specimens were cleaned in a solution of xylene: phenol (1:1), 2 shifts of xylene for two minutes individually and finally fixed in DPX.

Immunohistochemical evaluation[11, 12, 13]

ASMA immuno-staining was considered positive when the cytoplasmic staining in the stromal cells was stained. Quantitative scoring method was performed by counting both staining intensity and number of positive cells. Less than 10% positive cells were considered as negative and characterized as 0, 10% - 25% positive cells were assessed as 1, 25% - 50% positive cells as 2, 50% - 75% positive cells as 3 and more than 75% positive cells as 4.Immunohistochemical assessment was independently investigated by 2 researchers who were blinded to the follow-up. Consensus was obtained by discussing the cases which had different scores.

STATISTICAL ANALYSIS

The statistical analysis was carried out with SPSS version 16.0 software. Descriptive statistics were conceded out as means ± standard deviations of a given observations. The statistically significant relationship of the distribution of (ASMA expression), Chi-square test was used as appropriate. Pearson correlation coefficient (r) and the significance for it (p) were calculated between p values. P values less than or equal to 0.05 were considered statistically significant.

RESULTS

In this study 50(62.5%) were males and 30(37.5%) were females out of 80 cases of OSCC, exhibiting male preponderance. The mean age of patients was 45 ± 14.1. Buccal mucosa was the most common site of the tumor. There were 36 out of 80 cases of buccal mucosa as shown in figure 3. On histological assessment, the tumor was well differentiated in 27(33.8%) of patients, moderately differentiated in 38(47.5%), poorly differentiated in 5(18.8%) as shown in (**Figure 1)**. Moderately differentiated squamous cell carcinoma stayed most frequently identified in both genders **(Table1)**.

Immunostaining

The frequency of positive cells and intensity of staining of alpha smooth muscle actin.

The histological grading of OSCC in relation to Alpha smooth muscle actin is shown in **(Table 2)**. All 80 specimens were effectively evaluated by immunochemistry staining.

ASMA staining was high in 38 specimens (47%), medium in 36(45%) specimens and low in 6(7.5%) specimens.

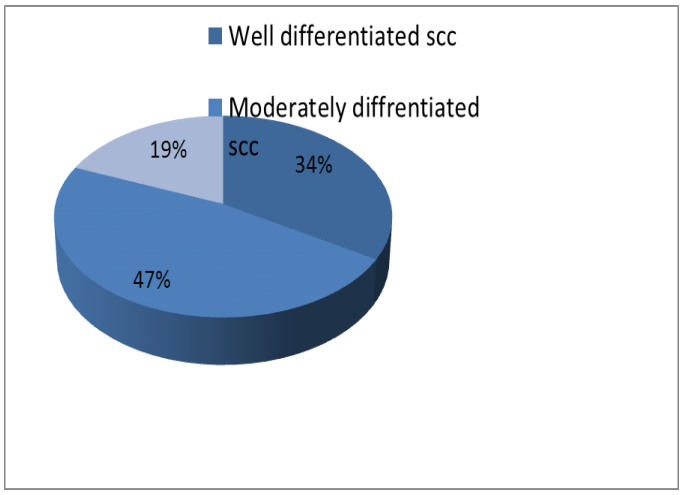

Fig 1: Grading Frequency of OSCC cases.

Table 1: Grading in relation to gender distribution in OSCC case.

Grading	Male	Female	Total
Well differentiated	16	11	27
Moderately differentiated	26	12	38
Poorly differentiated	8	7	15
Total	50	30	80

Table 2: ASMA and Histological Grading of OSCC cross tabulation for association.

ASMA Positive cells	Well differentiated	Moderately differentiated	Poorly differentiated	Total
Low	6	0	0	6
Medium	14	22	0	36
High	7	16	15	38
Total	27	38	15	80

Chi-square; p-value <0.01.

DISCUSSION

The regional accumulation of connective tissue cells and ground substance in tumors is well documented in the literature by the name of stromal response.[14, 15] The myofibroblasts are an imperative part of the tumor connective tissue stroma.[14] The myofibrobasts interrelate chemokines and cytokines through epithelial and connective cells to cause angiogenesis and local tumor incursion.[14-15] Considering these abilities myofibroblast may be used as an important target, during antitumor therapy.[16]

The total number of cases in the current study was 80, out of which (62.5%) 50 were males and (37.5%) 30 females, with mean age of 45 years ± 14.1 SD. The most common site of oral cancer in our study was the buccal mucosa (45%). Buccal mucosa is more prevalent in our population may be because of frequent betel quid, arecanut and tobacco use.[17]

On histological examination, most excisional biopsies showed moderately differentiated squamous cell carcinoma 38 (48%) cases in our study.

Safora Seifiet al[18] in 2010 in their study reported that alpha smooth muscle actin demonstration in the stroma of OSCC was significant (p= 0.000), the finding was exactly similar to the present study, we found a (p = 0.000) value for ASMA with histological grading. Although the figure of cases in their research were only 54, but with a similar mean age of 45 years ± 16 SD to our study.

Kellerman et al[19] in 2008 investigated the manifestation of myofibroblasts by means of immunohistochemical staining with alpha smooth muscle Actin protein in 83 samples of tongue SCC and 34 cases as the control group (8 samples of normal oral mucosa and 16 samples of dysplasia) and stated that the connective tissue of the oral mucosa and epithelial dysplasia did not demonstrate any alpha smooth muscle actin cells with the exclusion of the tumor vessel endothelium. They found myofibroblasts in sixty percent of cases. These

findings were, in a manner, in agreement with our investigation. However, the percentage of alpha smooth muscle actin positive myofibroblasts was 48% in our study.

The present study strongly indicates that immunohistochemical expression of ASMA might be of interest to select tumor with increased malignant potential. To obtain more profound knowledge within this area, the detection of a high risk group of patients may be helpful so that these patients can be given adjuvant treatment or a targeted and specific therapy with long-term follow-up is required in the near future.

CONCLUSION

Our findings, all together with the facts available in the literature, present convincing data for the prognostic effect of expression. Therefore it can be concluded that:

- An increase in the amount of αSMA-positive myofibroblasts suggests higher invasive characteristics and weaker prediction of oral squamous cell carcinoma.

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