Research Protocol

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Dentistry Section

Evaluation of Resistin and Fascin-1 Expression and their Correlation with Recurrence in the Patients of Oral Squamous Cell Carcinoma

FATEMA SAIFY[1], MINAL CHAUDHARY[2]

ABSTRACT

Introduction: Squamous cell carcinoma is the most common epithelial malignancy of the oral cavity. The five years survival rate is approximately 50% and is even lower i.e, 30% in the patients with recurrence of this disease. Since recurrence has a major influence on five years survival, it becomes imperative to identify the molecular elements responsible for recurrence of oral squamous cell carcinoma.

Aim: To evaluate the expression of resistin and fascin-1 and their correlation with recurrence in the patients of oral squamous cell carcinoma.

Materials and Methods: A cohort study will be conducted in the Department of Oral Pathology, Sharad Pawar Dental College and Hospital, Wardha, Maharashtra, India. The duration of the study will be 24 months. A 4 µm thick section from paraffin embedded blocks of formalin fixed biopsy tissues of 60 cases having primary oral squamous cell carcinoma will be processed and stained for mouse monoclonal resistin (clone C-10) and mouse monoclonal fascin-1 (clone 55k-2) antibodies.

Results: The results will be analysed by the correlation tests, Pearson and Spearman correlation coefficient.

Conclusion: Local recurrence is influenced by site of tumour, depth of invasion, resection margins and lymphovascular spread. As recurrence is directly related to the poor prognosis and survival rate in the patients of oral squamous cell carcinoma, so it is essential to identify molecular markers indicative of recurrence. Fascin and resistin markers could be used to identify a subset of oral squamous cell carcinoma patients which are prone to recurrence.

Keywords: Five years survival, Immunohistochemical staining, Monoclonal antibody, Semi-quantitative evaluation

INTRODUCTION

The most common oral malignancy in India is Oral Squamous Cell Carcinoma (OSCC). It is more prevalent among the males. Its morbidity and mortality is very high. In India, tobacco consumption is the most common cause of oral cancer [1,2]. However, the aetiology of OSCC is multifactorial and both intrinsic and extrinsic factors affects the progress of the disease [3]. Invasion into adjoining tissues and regional lymph nodes are two major problems leading to 90% of treatment failures. Clinically, the Tumour, Node and Metastasis (TNM) stage of the disease and the histopathologically grade of the tumour are the most important prognostic factors. In India, the five year survival rate is approximately 50% in the patients of oral squamous cell carcinoma. It is even lower (around 30%) in patients with recurrence. Local recurrence is influenced by tumour biology related to cell morphology and tumour microenvironment. Thus, it is imperative to identify the molecular elements indicative of recurrence in OSCC. This will also aid in protocol customisation [2-4].

Morphological changes with loss of adhesion, Extracellular Matrix (ECM) degradation and increase in cell migration is exhibited by metastatic and invasive tumour cells, which is the result of rearrangements of the cytoskeletal microfilaments [2]. Fascins constitute a group of 55 kDa proteins which are essential for maintaining the proper cytoskeleton of the cell. It is mostly found in cells forming protrusions of membrane and are mobile [5]. They are not expressed in normal epithelium but, often found in cancer cells. It help in invasion of tumour by the formation of invadopodia. Studies have shown that excessive presence of fascin leads to the metastasis and recurrence in epithelial cancers [2]. Hence, it may be of value as a significant prognostic marker in oral cancer.

Another important aspect in progression of oral squamous cell carcinoma is the role of intercellular signaling proteins called as cytokines [6,7]. Resistin (Retn) is a cysteine-rich 12.5 kD cytokine that is deposited at the site of inflammation. It is secreted by cells of defence system like neutrophils, monocytes, and macrophages in human [8]. It is an important regulatory cytokine triggering the release of other proinflammatory cytokines and the effects are regulated through the NFκB signaling pathway [9]. Tumour growth, metastasis, and chemoresistance are promoted by it through influencing cellular phenotypes as well as by modulating the tumour microenvironment acting through its different receptors [10]. From this point of view, resistin can be a biological indicator of prognosis in oral malignancy.

Tobacco causes persistent chemical and mechanical irritation to the oral mucosa and micro trauma that permits the diffusion of alkaloids into lamina propria eliciting an inflammatory response and release of cytokines such as resistin. The aggressive behaviour of the tumour arises from rearrangements of the cytoskeletal microfilaments which causes loss in the cell to cell contact and is responsible for the progression of tumour. The association of resistin and fascin-1 markers have been studied and is related to poor prognosis and survival rate in colorectal carcinoma but has not been studied in OSCC [11]. As recurrence is directly related to the poor prognosis and survival rate in the patients of OSCC, so, it is essential to identify molecular markers indicative of recurrence. This leads to the present research protocol question “Can the expression and correlation fascin-1 and resistin be a predictor of recurrence in patients of oral squamous cell carcinoma?” Thus, the aim of the study is to compare and correlate the expression of fascin-1 and resistin with recurrence and clinical parameters in oral squamous cell carcinoma.

MATERIALS AND METHODS

This cohort study will be conducted in the Department of Oral Pathology, Sharad Pawar Dental College and Hospital, Wardha, Maharashtra, India. The duration of the study will be 24 months. The Institutional Ethical Clearance was obtained for the study (590/2021).

Clinical parameters such as age, gender, habits, size of tumour, site of tumor, lymph node status will be obtained from the records of the patients. The resection margins and lymphovascular spread will be noted whether they are positive or not. A 4 µm thick section from paraffin embedded blocks of formalin fixed biopsy tissues of 60 cases having primary oral squamous cell carcinoma will be processed and stained for mouse monoclonal resistin (clone C-10) and mouse monoclonal fascin-1 (clone 55k-2) antibodies. The cases will be divided into group A and group B according to the respective antibodies mentioned above. The cytoplasmic expression of resistin and fascin-1 will be analysed as per the criteria mentioned by Wang CQ et al., and Lee TK et al., respectively [11,12]. The tumours with high levels of both resistin and fascin-1 will be correlated with patients whether the recurrence occurred or not within the span of two years.

Inclusion and Exclusion criteria: All patients with primary disease as oral squamous cell carcinoma will be included in the study. Patients with past history of malignancy of other site or recurrent oral squamous cell carcinoma and patients with metabolic or immune disorders will be excluded from the study.

Sample size calculation: The following formula will be used for calculation of sample size:

$$n=[2*\{Z_{(1-\alpha/2)}+Z_{(1-\beta)}\}^2*\sigma^2]/d^2$$
$$=[2*(1.96+1.64)^2 1*1]^2/*0.69*0.69$$
$$=54.4$$

Where,

- Z value for 5% level of significance ($Z_{(1-\alpha/2)}$)=1.96
- Z value for 95% power ($Z_{(1-\beta)}$)=1.64
- Expected Standard deviation (σ)=1
- d (Effect size)=0.69 (Effect size calculated on basis of Immunohistochemical expression scores of fascin in well differentiated and poorly differentiated OSCC)

$$s_p = \sqrt{\frac{(n_1-1)s_1^2+(n_2-1)s_2^2}{(n_1+n_2-2)}} = 1.30$$

$$d=(\mu_1.\mu_2)/S_p$$
$$=0.9/1.30=0.69$$

- S1=Standard Deviation for group 1
- S2=Standard Deviation for group 2
- n1=Sample size for group 1
- n2=Sample size for group 2

Procedure

Immunohistochemical staining will be done on 4 µm thick sections, taken from paraffin embedded blocks of formalin fixed biopsy tissues. The Streptavidin-Biotin method will be used. Sections will be prepared. After treating by alcohol and xylene, the slides are cleaned by phosphate-buffered saline and then the antigens are fixed by placing slides in microwave for 30 minutes. A 3% hydrogen peroxidase will be used to inhibit internal oxidation. The sections will be then incubated with antiresistin (clone C-10) and monoclonal antibody against fascin-1 (clone 55k-2) for half an hour. The slides will be washed and will be treated with streptavidin followed by diaminobenzidine hydrochloride exposure as a chromogenic reagent. Haematoxylin will be used for counter staining. Normal epithelium can be taken as the negative control.

Method of slide interpretation: The semi-quantitative evaluation of entire tissue section under light microscopy will be done and scored separately by two pathologists who will be unaware of the clinical details.

Fascin-1 cellular localisation: cytoplasmic

The staining will be seen in both stromal and epithelial cells. According to Lee TK et al., the cytoplasm of epithelial tumour cells should be stained. Expression of fascin will be studied as follows [12]:

Percentage Score (PS): On the basis of cytoplasmic staining, the stained cells will be counted under a light microscope, which will be then divided into four groups.

Score 1: Percentage of positive cells <10%

Score 2: 11-50%

Score 3: 51-80%

Score 4: <81% stained cells

Intensity Score (IS):

Score 1: No staining

Score 2: Stain is seen but with some difficulty (poor)

Score 3: Medium: pale brown (oak)

Score 4: Extreme: dark brown

Percentage score and intensity score will be added to get the final score [1,3]:

Score 1: Negative

Score 2: Positive

Resistin: Cytoplasmic

Score 0: Nil

Score 1: Weak

Score 2: Moderate

Score 3: Strong

Percentage of Staining will be calculated as:

Score 0: 0%

Score 1: 1%-25%

Score 2: 26%-50%

Score 3: 51%-75%

Score 4: 76%-100%

The staining will be seen in both stromal and epithelial cells. According to Wang CQ et al., staining intensity will be scored; tumour cells are considered positive for resistin if the sum of more than three for intensity and more than 5% of cells show cytoplasmic staining. The sum of more than six of both the scores will be considered to be strongly positive for resistin [11]. The tumours with high levels of resistin will be correlated with patients whether the recurrence has occurred or not within the span of two years.

Scope: Molecular studies particularly targeted at causes of recurrence are very few and since recurrence has a major influence on five years survival, it is essential to identify the molecular elements responsible for recurrence in oral squamous cell carcinoma.

DISCUSSION

Oral squamous cell carcinoma is the eleventh most commonly diagnosed cancer per year worldwide. Owing to the late diagnosis and frequent development of the loco-regional recurrences and second primary tumours, mortality rates are 50% over five years and have remain unchanged over the recent decades [4].

Recurrence is an important prognostic factor in patients with oral squamous cell carcinoma. According to the previous studies, the recurrence rate was found to be approximately 33% and the recurrence time ranged from 2-96 months, with a median of 14 months. Tumour size, degree of differentiation and lymph node involvement was considered independent factors of recurrence [13]. Molecular studies particularly targeted at causes of recurrence are very few and it is essential to identify molecular markers indicative of

recurrence. In the current study, authors have used a combination of fascin and resistin as molecular markers to identify a subset of patients who are prone to recurrence. The present study attempts to find a significant prognostic marker and assess its association with recurrence in oral squamous cell carcinoma.

Cell motility is an important factor in the recurrence and metastasis of cancers. Recently, fascin has been linked to tumour progression by induction of cell motility [12]. It supports cellular structures like filopodia, invadopodia and other protrusions underneath the plasma membrane which are essential for cell migration and cell matrix adhesions [1]. Increased expression of fascin was associated with recurrence in colorectal, gastric, breast and lung carcinomas [11]. Previous studies carried out in oral squamous cell carcinoma correlated fascin with the degree of dysplasias, grade of carcinoma, lymphatic metastasis and poor survival rate. In the present study, authors will be correlating the expression of fascin with recurrence of oral squamous cell carcinoma by semi-quantitative analysis.

The association between inflammation and carcinogenesis is well established. Resistin is a proinflammatory cytokine which causes upregulation of several other cytokines such as Tumor Necrosis Factor Alpha (TNF-α), Interleukin-6 (IL-6), Vascular Endothelial Growth Factor (VEGF). These cytokines plays an important role in immune response, cellular proliferation, differentiation and angiogenesis in physiological process and carcinogenesis. Previously, role of resistin was studied in other diseases like rheumatoid arthritis, systemic lupus erythematosus, obesity and periodontitis [7-9]. Increased expression of resistin was associated with poor prognosis in colorectal, gastric, breast and lung carcinomas [10]. A study conducted in the past included resistin gene profiling in oral squamous cell carcinoma tissues and correlated it with tumour size [14]. In the present study, authors will be using resistin in oral squamous cell carcinoma tissue samples and its expression will be correlated with the recurrence. It will be analysed semi-quantitatively, which is not been conducted earlier.

Previous studies found that epidermal growth factor induced the expression of fascin-1 via activation of the p44/p42 Mitogen-Activated Protein Kinase (MAPK) Extracellular-Regulated Kinase (ERK1/2) pathway which subsequently promoted breast cancer cell migration and invasion while other reports have shown that resistin also promotes angiogenesis and the proliferation of cells through the p44/p42 MAPK (ERK1/2) pathway [9,11]. However, whether any association exists between resistin and fascin-1 expression had been unclear. It is hypothesised that fascin-1 utilises the resistin-MAPK signaling pathway to play functionally.

In a study, by Wang CQ et al., done in colorectal carcinoma patients they concluded that survival was worse in colorectal carcinoma patients with high levels of both resistin and fascin-1 than in those with high levels of only one protein alone [11]. The clinical significance of resistin expression in oral squamous cell carcinoma is unclear and also there is no study of any correlation between resistin and fascin-1 expression together with the recurrence in oral squamous cell carcinoma. The present study will try to explore the association of these above mentioned molecular markers with recurrence in oral squamous cell carcinoma for protocol customisation.

Limitation(s)

Gene profiling cannot be done.

Implications: Fascin and resistin may be used to identify a subset of patients of oral squamous cell carcinoma which are prone to recurrence for protocol customisation.

Expected outcome: This study might help in improving the survival and quality of life in patients with oral squamous cell carcinoma by recognising the recurrence potential so that these patients could be treated appropriately for better prognosis.

CONCLUSION(S)

Since, recurrence has a major influence on five years survival in oral squamous cell carcinoma, so it is essential to identify the molecular elements responsible for recurrence in it. Fascin-1 and resistin can be used as significant prognostic marker to identify a subset of oral squamous cell carcinoma patients which are prone to recurrence.

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PARTICULARS OF CONTRIBUTORS:
1. Reader, Department of Oral Pathology, Government Dental College, Raipur, Chattisgarh, India.
2. Professor and Director, Department of Oral Pathology, Datta Meghe Institute of Medical Science, Wardha, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Fatema Saify,
Reader, Department of Oral Pathology, Government Dental College,
Raipur, Chattisgarh, India.
E-mail: saify5152@rediffmail.com

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