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Comparative Evaluation of Mast Cell Tryptase Activity in Oral Squamous Cell Carcinoma (OSCC), Oral Precancerous Pathologies (OPP) and Normal Oral Mucosa (NOM)

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ABSTRACT

Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity. Many times it is preceded by oral precancerous pathologies (OPP) namely leukoplakia, erythroplakia, and oral submucous fibrosis. The rate of malignant transformation is variable for these premalignant pathologies. Mast cells have a predominant role in tumor progression and metastasis. Mast cells contain proteolytic enzymes tryptase and chymase which is responsible for degradation of extracellular matrix. In the current research project, we have studied the comparative expression of mast cell tryptase activity in OSCC, OPP & NOM using immunohistochemistry method.

Keywords: *Mast cell tryptase, mmunohistochemistry, oral squamous cell carcinoma, oral precancerous pathologies, Normal oral mucosa.*

1. INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the sixth most common malignancy worldwide. It is the most common malignancy in Indian males and third most common malignancy in Indian females. In the oral cavity, many times OSCC is preceded by oral precancerous pathologies (OPP). Oral precancerous pathologies include leukoplakia, erythroplakia, and oral submucous fibrosis. Most of these OPP's are associated with tobacco consumption in some or other form. Malignant transformation of OPP is variable. Many factors have been proposed for tumor formation, tumor progression and tumor metastasis in OPP and OSCC. Mast cells play a major role in tumor progression and tumor metastasis in OSCC and OPP. These mast cells contain proteolytic enzymes namely tryptase and chymase. Tryptase and chymases induce proteolysis of the extracellular matrix. Thus, degradation of the extracellular matrix leads to tumor progression and tumor metastasis in OSCC and OPP[1,2,3].

In the current research project, we have evaluated immunohistochemical expression of mast cell tryptase in OSCC, OPP, and NOM. Mast cell density was evaluated by using Anti-mast cell tryptase antibody.

2. MATERIALS & METHODS

2.1 Collection of Samples

After obtaining permission from the institutional ethics committee, 150 paraffin-embedded tissue blocks were retrieved from the departmental achieves. Histopathologically proved 50 cases of OSCC, 50 cases of OPP and 50 cases of NOM were considered for the study. Each paraffin-embedded tissue block was subjected to two 3-5µ thick sections. One section was taken on the normal slide and another section was taken on silane coated slide. A section taken on the normal slide was subjected to H& E staining and the section taken on silane coated slide was used for immunohistochemistry.

2.3 Histopathology & Light Microscopy

All H& E stained sections were observed under a light compound microscope to determine the degree of differentiation in OSCC and degree of dysplasia in OPP. We analyzed 28 cases of well-differentiated OSCC and 22 cases of moderately differentiated OSCC. Among OPP, 34 cases showed dysplastic features and 16 cases were non-dysplastic.

2.4 Immunohistochemistry Study

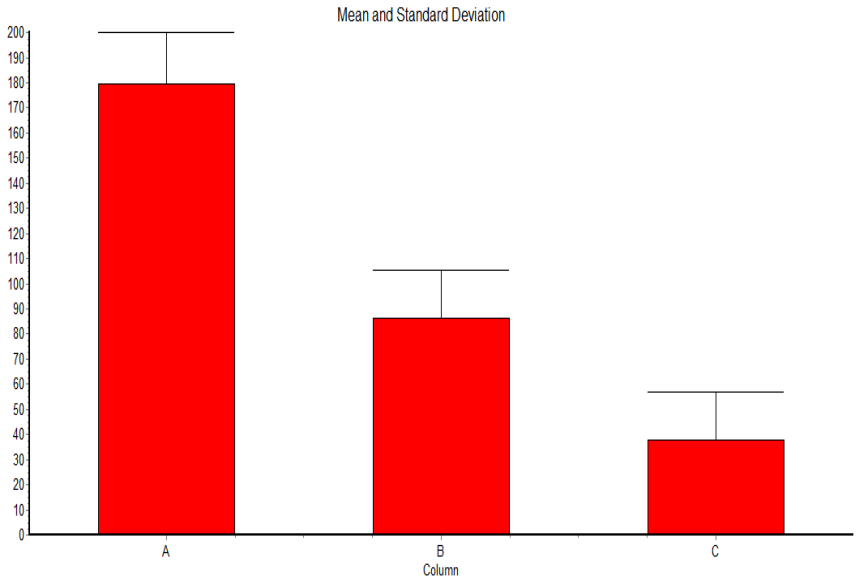
Immunohistochemistry was performed on sections taken on silane coated microscope slides. Anti-mast cell tryptase antibody from Dako (clone name AA1) was used. Sections were subjected to series of xylene, absolute alcohol, and water. Sections were thoroughly washed under running water. Antigen retrieval was carried out using citrate buffer at pH 6.2. Heat mediated antigen retrieval technique using the microwave at 800 watts for 10 minutes, 420 watts for 10 minute and 360 watts for 5 minutes was used. Sections were washed in Tris buffer at pH 7.4. Sections were transferred to 3% hydrogen peroxide in methanol for 5 minutes. Sections were then incubated with serum from same species in which primary antibody was raised. Sections were washed in Tris buffer at pH 7.4 for 5 minutes. Sections were incubated in humidifying chamber at room temperature with anti-mast cell tryptase antibody for 45 minutes. Sections were washed in tris buffer solution for 5 minutes. Sections were subjected to poly-HRP super sensitive secondary detection antibody kit from Biogenex. Sections were washed in tris buffer. Sections were incubated with substrate DAB. Sections were washed in tris buffer. Sections were transferred to increasing grades of alcohol and xylene. Sections were mounted using DPX. All the sections were observed under a compound microscope. Brown stained mast cells were calculated in ten high power fields. Mast cells were expressed as per square mm.

3. RESULTS

The study was conducted on paraffin-embedded tissue blocks of 50 OSCC, 50 OPP, and 50 NOM cases. Among OSCC, 28 cases were well differentiated OSCC and 22 were moderately differentiated OSCC. Among OPPs, 34 were dysplastic and 16 were non-dysplastic. Mean density of mast cell tryptase-positive cells was 179.34 in OSCC, 86.48 in OPP and 37.56 in NOM. The statistical test employed was ANOVA. The p-value <0.0001 was considered highly significant. Table 1 shows statistical comparison among the groups.

Table-1: Comparison of Mean Value among the Groups

Comparison	Mean Difference	q	p-value
OSCC vs OPP	92.60	33.285	P<0.001
OSCC vs NOM	141.78	50.819	P<0.001
OPP vs NOM	48.920	17.535	P<0.001



Graph-1 Mean and Standard Deviation among Groups
A: OSCC. B: OPP. C: NOM

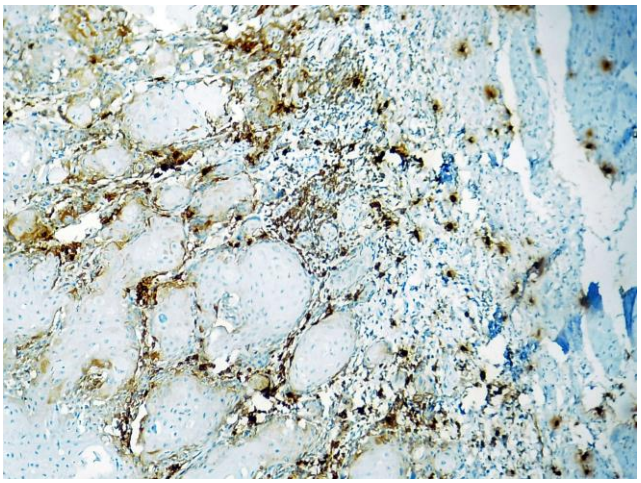


Fig. 1 Immunohistochemical Expression of Anti-mast Cell Tryptase in OSCC

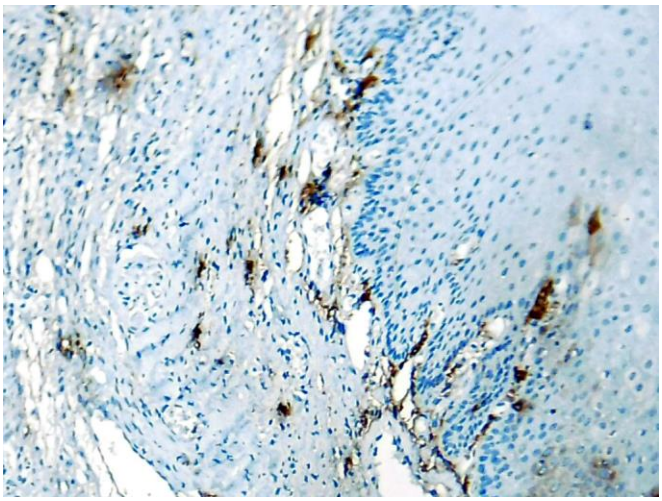


Fig. 2 Immunohistochemical Expression of Anti-mast Cell tryptase in OPP

4. DISCUSSION

Mast cells are a multipotent hematopoietic progenitor cell. They are multipotent in nature. From hematopoietic region, they migrate to peripheral tissue through vascular channels. Mast cells participate in immune responses [4,5].

Mast cells contain proteolytic enzymes like tryptase and chymase. They also release heparin, histamine, basic fibroblast growth factor, matrix metalloproteinase, interleukins, and chemokines. Proteolytic enzymes particularly tryptase and chymase degrade the extracellular matrix [6, 7,8,9,10].

Mast cell granules are rich in tryptase and serine proteinase.Tryptase contributes to inflammation, tissue remodeling, and extracellular matrix destruction and it is measured to be an essential angiogenic factor [10, 12, 13, 14].

Angiogenesis and neovascularization are responsible for the various biological processes. Angiogenesis aid in progression and metastasis of different malignant tumors including oral cavity. Mast Cells encourage neovascularization by the release of angiogenic factors, such as VEGF, or different substances with angiogenic properties, such as tryptase, IL- 8, tumor necrosis factor (TNF), basic fibroblast growth factor (bFGF), heparin, and histamine. Heparin present in the mast cells induces vessel proliferation as well as increases half-life of angiogenic substance like basic fibroblast growth factor. Thus they promote tumor angiogenesis and facilitate the local invasion and interleukins are responsible for epithelial proliferation. Degradation of extracellular matrix through the proteolytic activity of proteases like tryptase and chymase stimulate angiogenesis and aid in invasion and metastasis through extracellular matrix remodeling [15].

In the current project, we used anti-mast cell tryptase antibody to locate the activity of mast cell tryptase in OSCC, OPP, and NOM. Mean density of mast cell tryptase-positive cells was 179.34 in OSCC, 86.48 in OPP and 37.56 in NOM which is statistically highly significant. The findings of our study are in comparison to the studies mentioned in the literature [16, 17]. The activity of mast cell tryptase was evident at the advancing front of the lesion and around the blood vessels. Thus, angiogenesis and degradation of extracellular matrix by mast cell tryptase help in tumor progression in OPP and OSCC and tumor metastasis in OSCC.

5. CONCLUSION

Mast cells contain proteolytic enzymes like tryptase and chymase. These proteolytic enzymes cause degradation of extracellular matrix. This event is associated with angiogenesis, tumor formation, tumor progression and tumor metastasis in OSCC. Such proteolytic activities of proteolytic enzymes could be responsible for the transformation of OPP into OSCC. The proteolytic enzymes in mast cells can be used as therapeutic targets to prevent tumor progression and tumor metastasis in both OPP and OSCC.

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