Original Article

MAST CELL DENSITY IN DIFFERENT STAGES OF ORAL SUBMUCOUS FIBROSIS

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ABSTRACT

Objective: Oral submucous fibrosis is a premalignant condition of the oral cavity affecting pharynx also. Pathogenesis for this was always questionable.Mast cells which are present in this lesion were always seen to be associated with a variety of inflammation and fibrotic conditions.These mast cell mediators show varied variations with the change in the microenvironment but still only a little is known about their role in Oral submucous fibrosis.Thus making this study interesting.

Study design: Correlation between mast cells in oral submucous fibrosis, oral squamous cell carcinoma (OSCC) and normal buccal mucosa was studied using toluidine blue staining.

Results: Density of mast cell was significantly higher in OSMF when compared to normal buccal mucosa.There was no significant difference between mild, moderate and severe oral submucous fibrosis and oral submucous fibrosis when compared to OSCC.This study failed to demonstrate any definite association between oral squamous cell carcinoma and Mast cell count density(MCD)

Conclusion: This study shows MCD of different groups (mild, moderate and severe OSF and OSSC) on comparison with MCD of controls showed statistically significant variation. Intergroup comparison between mild, moderate and severe OSF and OSF with OSCC showed no statistical significance.

KEYWORDS: premalignant lesion, Premalignant condition, oral Submucous fibrosis, Squamous cell carcinoma

INTRODUCTION

OSMF is a premalignant condition of the oral cavity which occasionally may extend into pharynx and esophagus.[1,2] The pathogenesis of OSMF is still not established. Many etiologies have been put forward but arecanut is considered the most common etiology. Mast cells are the most commonly seen constituent of connective tissue in various inflammatory conditions but their role in pathogenesis of OSMF is still unknown. Previous studies reveal great amount of mast cells in the early stages of OSMF.It is reported earlier that the mast cell density increases with the increase in the severity of OSMF [3,7].It is also seen that the microvessels and mast cell count increase in OSCC in comparison to normal buccal mucosa and in cases with

hyperkeratosis[8].This suggests that mast cell density increases as the lesion progresses from normal oral buccal mucosa to malignancy.Our study aimed to determine the density of mast cell in various stages of OSMF. The rate of its transformation to oral squamous cell carcinoma is approximately 13% .Mast cells (MCs) are seen adjacent to connective tissue stroma. It is both pro-inflammatory and immunoamplifying in action and it produces mitogenic cytokines.The degranulation products of mast cell transforms mesenchymal pleuripotent cells into the synthetic fibroblasts.

Aim and objectives:

1. To evaluate mast cell density in oral submucous fibrosis and its comparison with normal mucosa
2. To correlate density of mast cells both clinically and histopathologicaly

METHODOLOGY

Study was performed on selected patients by subdividing them into two groups. All cases attending outpatient department of the same institute during the 10 month study period having clinically diagnosed OSF and willing for biopsy. Oral squammous cell carcinoma patients attending outpatient Department of Oral Medicine, Indra gandhi Govt. Dental College & hospital.Jammu over a period of 10 months. From January 2016 to October 2016). The Control group consisted of 10 age and sex matched subjects, with clinically normal-appearing mucosa which were undergoing extraction for orthodontic treatment or disampaction. It also included 5 persons with chewing habits and 5 persons without any deleterious habits.

The inclusion criteria for the study were the patients who are clinically diagnosed with OSF and OSCC associated with oral submucous fibrosis . Patients with any history of any previous treatment for cancer.Patients with systemic ailments and unwilling for biopsy.

After clinical examination, subjects were divided into four groups:

- mild OSF
- moderate OSF
- severe OSF
- OSF with OSCC

Two sections were made from each block one was stained with H&E and other with 1% toluidine blue stain. This stains the mast cell granules metachromatically by reacting with sulphated mucopolysaccharides.Mast cells were then counted using an occulometer grid under x100. Mast cell count is expressed as the number of mast cells per grid field and the number of mast cells per square millimetre.We grouped our lesions into (i) early (which included very early and early cases)fig 1,2 (ii) late (which included moderately advanced and advanced cases)fig 3.Biopsies of 10 samples from normal buccal mucosa were also selected as control group. Squammous cell carcinoma (n=10) was also used for mast cell demonstration.OSCC fig .[8] Mast cell counts for oral submucous fibrosis and oral squammous cell carcinoma were analyzed.p value < 0.05 was considered statistically significant.Annova test was applied.Modified toludiene blue stain was used.[9] Mast cells are spindle to oval shaped cells which show staining characteristics similar to the fibroblastswhen stained with hematoxylin and eosin staining thus making it easy to differentiate from fibroblast cells. Mast cell granules are purplish red and the nuclei of mast cells appear sky blue in colour. The MCs were counted using an oculometer grid fields under a magnification of x100. Mast cell count was expressed as the number of mast cells per square millimetre[10] Table 1 shows significance.

RESULTS

- Majority of patients (25 cases) complained of burning sensation
- 18 patients chewed betel quid
- 6 chewed both betel quid and commercial arecanut
- Only 4 chewed commercial arecanut only

After toluidine blue staining average mast cell count per grid field was determined, the mast cell density (MCD) i.e. mast cell number per square millimeter was calculated using the following formula:

MCD=No. of mast cells in x 400 grid field / Area of ocular grid (0.0625 mm^2)

1.Highest mean MCD was seen in mild OSF and density of MCD decreased in moderate and severe OSF

2.Mean MCD in OSCC was raised when compared to controls but lower than in OSF

3. MCD in OSCC showed great variation ranging from 1.44 to 124.16

4. In OSCC, MCD was not influenced by clinical severity of OSF

Group 1	Group 2	p – value
Control	Mild OSF	0.000
	Moderate OSF	0.011
	Severe OSF	0.034
	OSF with OSCC	0.015

TABLE 1: Shows significant p – values

DISCUSSION

OSMF is described as a chronic disease which affects the oral cavity many a times leading to trismus and inability to eat.[11] Areca nut is

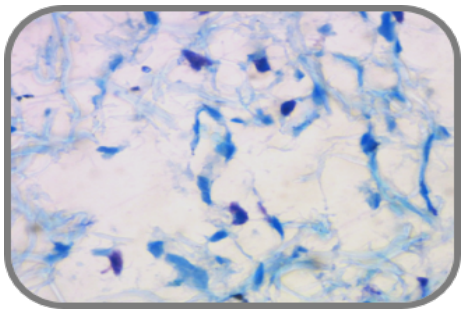

Fig 1: Mast cell count in mild Oral Submucous Fibrosis

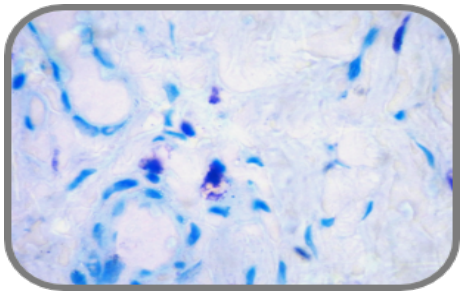

Fig 2: Mast cell count in moderate Oral Submucous Fibrosis

considered the most important etiological factors for OSMF. The areca nut produces alkaloids mostly arecadine and arecoline have been proposed to cause fibrosis.[2] Only few studies have been done on mast cell which are seen associated with various chronic inflammatory disorders. [9] In the present study we compared our cases with control group constituted of normal buccal mucosa.We concluded an increased mast cell density in both early and late OSMF case.

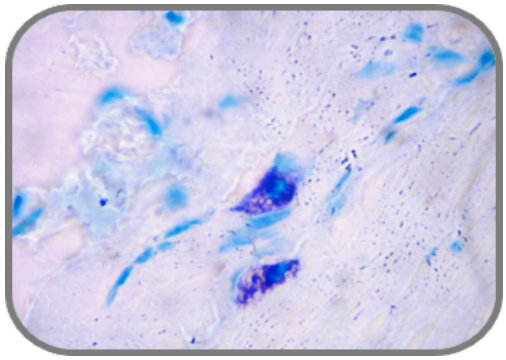

Fig 3: Mast cell count in Severe Oral Submucous Fibrosis

Chronic irritants in the form of areca nut causes continuous mast cell activation and its degranulation.After degranulation mediators of mast cell gets deposited in the extracellular environment. Mast cells subsequently produce and secrete additional mediators which are present in the form of granules.[9]These mediators lead to initial inflammatory signs of burning sensation, stomatitis ,glossitis in oral submucous fibrosis.Occasional vesicle formation is also seen. Previous studies concluded that mast cell count decreased with the increase in the severity of the disease.The previous studies concluded that the release of mast cell granules initiate change in the ground substance of connective tissue by changing the intracellular fluid. [12] It is thought that in early cases of OSF, when tissue reaction to irritant is strongest, mast cell counts are highest and as tissue gets converted to less reactive hyaline, mast cell count decreases. No particular association was found between MCD and occurrence of OSCC in OSF.OSCC was seen to arise in different specimens with numbers of MCs varying from higher than in mild OSF to same as control specimens. Many investigators studied density of MCs in different stages of OSF but association of MCD, if any, to development of OSCC has not been made.Studies evaluating MCD in OSCC have shown both increase and decrease of the same. This study failed to demonstrate any definite association between OSCC and MCD. It probably suggests that MCs have no active role in pathogenesis of OSCC in

OSF and their decrease or increase is most likely a chance finding. There was significant increase of MCD and it showed steady decline from mild OSF to OSF with OSCC. But intergroup comparison between mild, moderate and severe OSF and OSF with OSCC showed no statistical significance. Literature search has failed to reveal studies comparing mast cell densities in relation to clinical severity of OSF while comparisons with histopathologic grading are many.Earlier studies mark the presence of highest number of mast cells in early OSF and as the severity of OSF increases, number of mast cell decreases. Immunohistochemistry report similar findings.Mast cell count when studied with biochemical ,histological tests revealed close relationship between mast cell count and fibroblasts in the pathogenesis of OSMF which goes in accordance with our findings.[13]We suggest mast cell count to be the gatekeepers which become the destructors leading to the initiation and progression of oral submucous fibrosis. Mast cell stabilizers can be used for the treatment of oral submucous fibrosis.Further studies are needed to study the possible role of MC in the pathogenesis of OSMF.[12]

CONCLUSION

In this study, mast cell density count of different groups (mild, moderate and severe OSF and OSSC) on comparison with mast cell density count MCD of controls showed statistically significant variation.Intergroup comparison between mild, moderate and severe OSF and OSF with OSCC showed no statistical significance

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