

## Quantitative analysis of mast cells in *oral submucous fibrosis*

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**Abstract:** *Back ground:* On the Indian subcontinent, the use of smokeless tobacco in various forms is very popular. Oral submucous fibrosis is a chronic condition of the oral mucosa, which results in a marked rigidity with progressive inability to open the mouth and has an increased risk of undergoing carcinomatous changes about four to eight percent. *Objectives:* The objective of this study was to evaluate whether early recognition and subsequent prevention of its transformation into an advanced stage would assist in early treatment of this condition. *Method:* This study was undertaken by retrieving the records and the paraffin blocks of the confirmed cases of 30 oral submucous fibrosis and 10 normal buccal mucosa as control. Two sets of sections were obtained. One set subjected to Toluidine Blue staining and the other set subjected to Hematoxylin & Eosin. *Result:* The mast cell response in oral sub mucous fibrosis was reported to be high in the early stages where the reaction of the tissue to the irritant is strong. As the disease advances tissue becomes less reactive, gets hyalinized and occasionally shows degeneration where the mast cells are reduced and are often even fewer than in the normal mucosa. *Conclusion:* Since this disease is known to be prevalent in India due to the habits, the study was conducted to observe different histopathological features and the occurrence of mast cells in various grades of Oral Sub Mucous fibrosis. This study also emphasizes the role of mast cells and its concentration in different grades of OSMF.

**Keywords:** Oral sub mucous fibrosis, mast cells.

### Introduction

Oral submucous fibrosis is a chronic condition of the oral mucosa. The condition is found amongst four in thousand adults in rural India and is known to be caused by areca nut additionally, it is estimated that as many as five million young Indians are suffering from this precancerous condition as a result of the increased popularity of the habit of chewing pan masala. It results in a marked rigidity with progressive inability to open the mouth and has an increased risk of undergoing carcinomatous changes about four to eight percent, which is known to be a more than leukoplakia [1-2].

The earliest symptom is inability to open the mouth accompanied by burning sensation, usually experienced while one is eating spicy food. Dryness of the mouth changes in taste sensation and hyper salivation may also be early complaints. The clinical examination reveals a great variability in the texture and colour of the oral mucosa depending upon the stage of the disease. Occasionally, it may be preceded by some vesicle formation [2].

The hallmark feature of oral submucous fibrosis is stiffening of the oral mucosa, which occurs due to the formation of fibrous bands widely spread in the oral cavity, reduced mobility of the tongue and soft palate, shrinkage of the uvula and the entire isthmus faucium, usually preceded by blanching of the mucosa. The mast cell response in oral sub mucous fibrosis was reported to be high in the early stages where the reaction of the tissue to the irritant is strong [3]. As the disease advances tissue becomes less reactive, gets hyalinized and occasionally shows degeneration where the mast cells are reduced and are often even fewer than in the normal mucosa. The vascular response in this condition was observed to be complex consisting of persistent dilatation of blood vessels especially in moderately advanced cases. These investigations opined that the mast cell and the vascular response in this condition are similar to those described in certain disease of autoimmune origin [3-4]. Mast cells are widely distributed throughout

the connective tissue of vertebrates, particularly around the small blood vessels, nerves and glandular ducts, as well as mucous, serous and cutaneous surfaces. They produce pharmacologically active substance like histamine heparin, proteolytic enzymes, platelet activating factor, leukotrienes, prostaglandin's etc, which are necessary for the physiological function of our body in response to various stimuli as and when it is required. So they are also known as the "Emergency Kit" [5].

A vascular response due to inflammation, apart from the connective tissue repair process has been very commonly found in oral submucous fibrosis. Normal, dilated and constricted blood vessels are also seen in combination. Apparent narrowing of the smaller vessels appears first in the upper mucosa and spreads gradually to the larger deeper vessels. Persistent dilatation has also been seen in many moderately advanced and advanced biopsies. Since this disease is known to be prevalent in India due to the habits, the study was conducted to observe different histopathological features and the occurrence of mast cells in various grades of Oral Sub Mucous fibrosis. This study also emphasizes the role of mast cells and its concentration in different grades of OSMF.

**Aims:**

1. To evaluate the number of mast cells in normal mucosa.
2. To evaluate the number of mast cells in different grades of oral submucous fibrosis.
3. To compare the number of mast cells in different grades of oral submucous fibrosis.
4. To compare the number of mast cells in normal oral mucosa and different grades of oral submucous fibrosis.

**Objective:** An earlier study on oral submucous fibrosis has shown that mast cell response in very early cases, where the reaction of the tissue to the irritant was the strongest, mast cell counts were highest. As the tissue gets converted to less reactive hyaline mass, there is a drop and even a reduction in the number of mast cells in normal mucosa. In clinically lower grade of oral submucous fibrosis, number of mast cells appears to be increased. The objective of this study was to evaluate whether early recognition and subsequent prevention of its transformation into an advanced stage would assist in early treatment of this condition.

**Material and Methods**

A study was designed retrospectively to review 30 histologically confirmed cases of oral submucous fibrosis and 10 normal cases. Constant effort was taken to focus on the concentration of mast cells together and to correlate it with vascularity. This study was undertaken by retrieving the records and the paraffin blocks of the confirmed cases of oral submucous fibrosis and normal buccal mucosa from the Department of Oral Pathology and Microbiology, Bharati Vidyapeeth Dental College & Hospital, Pune (20 Cases) and Department Of Oral Pathology, K.L.E.'S Institute of Dental Science, Belgaum (10 Cases). 10 volunteers were selected, without any habit of tobacco in any form. Biopsies were taken from the buccal mucosa after a written 6 consent was obtained. Procedures were done to process the tissue and sections were obtained as given by Bancroft J. D. A total of 40 cases were included in this study.

**Study Samples:** Two sets of sections were obtained. One set subjected to Toluidine Blue staining and the other set subjected to Hematoxylin & Eosin. (Toluidine Blue labeled as T.B. -1 and Hematoxylin and Eosin as H & E -1 for patient no-1.) The sections were scanned and studied under 5x, 10x and 40x magnification. The grading of oral submucous fibrosis was done according to the method followed by Sirsat and Pindborg (1967) and Bhatt & Dholakia (1971) [2].

1. *Very early – (grade - I):* Collagen bundles finely fibrillar, dispersed with the presence of marked oedema, Fibroblastic response with plump young cells with abundant cytoplasm was seen. Blood vessels sometimes normal, often dilated and congested, inflammatory cells, mainly polymorph nuclear leucocytes with occasional eosinophils may also be seen.

2. *Early – (grade - II):* Juxta - epithelial area shows early hyalinization. Collagen fibers still seen as separate bundles, which are thickened (this term is used to denote early coalescence within the bundle, which is still recognizable as a separate wavy unit). Plump young fibroblasts are present in moderate numbers. The blood vessels are often dilated and congested. The inflammatory cells are mostly

mononuclear lymphocytes, eosinophil and an occasional plasma cell.

3. *Moderately advanced - (grade - III):* Collagen is moderately hyalinized, the amorphous change starting from the juxta epithelial membrane. Occasionally thickened collagen bundles are still seen separated by slight residual oedema. The fibroblastic response is much less. The cells present being mostly adult fibrocytes with elongated spindle shaped nuclei and scanty cytoplasm. Blood vessels are either normal or constricted due to increased surrounding fibrous tissue. The inflammatory exudates consist mainly of lymphocytes and plasma cells though an occasional eosinophil is seen.

4. *Advanced - (grade - IV):* Collagen is completely hyalinized, and is seen as a smooth sheet with no separate bundles discernible. Oedema is absent, the hyalinized areas are devoid of fibroblasts though a thin elongated cell or vestigial nucleus is seen at rare intervals along the fiber bundles. [6]

*Staining procedure for Toluidine Blue (S.T.B.) method [7]:*

1. The sections were deparafinised in two changes of xylene, hydrated through two changes of alcohols, 5 minutes in each solution.
2. Then the sections were kept in water for 5 minutes.
3. The sections were then placed in a coupling jar containing Toluidine Blue stains for 30 minutes then the sections were blotted carefully.
4. After this the sections were placed in absolute isopropanol for one minute Later the section was cleared in xylene and mounted on the slide using Dibutyl Phthalate Xylene (DPX).

The granules of mast cells were stained purple and the rest of the section was stained blue. The mast cells were counted throughout the connective tissue under 40x (high power magnification). The counting of mast cells was done by three different observers who were blinded to the study to minimize the subjectivity. The results were approximately the same. The other set of section was stained in the routine manner with Hematoxylin & Eosin stain as put

forth by Bancroft & Stevens. The mast cell count was carried out on each section under 40x microscopic field. The number of metachromatic cells was counted in 10 grid fields. Great care was always taken to maintain uniformity in counting, starting from the right upper edge of the section in every case. The mast cells were counted under 40x magnification using oculometer grid, results were expressed as average number of mast cells per grid field. The number of mast cells per sq. mm was calculated by using the formula.

$$\text{Number of mast cells} = \frac{\text{Average no. of mast cells per grid field sq mm}}{\text{per sq mm}} \quad 0.0128$$

All the slides were assessed by three observers each blinded to the patient's clinical history as well as to others observations.

### Results and Discussion

The present study comprised of total 40 cases, which were divided into two categories, 10 as control subjects, which were age matched and 30 as oral submucous fibrosis patients of the age group of 15-50 years. Histological grading for oral submucous fibrosis was done. The mast cells were counted throughout the connective tissue irrespective of the fields. H & E stained section of OSMF were graded as Grade I, II, III & IV using Pindborg's criterion of classification [6, 8]. All Toluidine Blue stained sections were examined under high power (40x) objective lens.

The metachromatic mast cells were counted in 10 microscopic grid fields for a methodical and systematic investigation of mast cells using a 40x objective and 10x eyepiece giving a total magnification of 400x. Cells containing met achromatic granules of variable size and shape in toluidine blue stained sections were counted in normal mucosa and Oral Submucous Fibrosis. The mast cells were counted from the area just below the tips of retepegs along the entire length of the section. Total number of mast cells was divided by number of microscopic field. The result thus obtained by the above method were subjected to statistical analysis for obtaining P value by the "T" test Out of the 10 control cases 5 subjects showed mild response and 5 subjects

showed moderate inflammatory response. This was in agreement to that of Sirsat and Pindborg (1967). The normal mucosa is constantly subjected to the external stress, both physical and chemical hence the biopsies may show a mild to moderate sub clinical inflammation.

This could lead to moderate inflammatory reaction. The mast cell mean count was 14.9 from biopsies taken from the buccal mucosa, which were apparently free from any other disease. This range has been considered normal for the purpose of comparison and is in accordance with Sirsat and Pindborg (1967). 10 Out of the 30 cases were grade I oral submucous fibrosis. The mean number of mast cells in these subjects was 12.6 as shown in table no.1 ( $P>0.05$ ), which was in accordance to that as stated by Sirsat and Pindborg (1967) [2].

Here the concentration of mast cells was more or less similar to normal mucosa as the disease was in its very early stage. The difference between number of mast cells of normal mucosa and OSMF grade I does not hold any particular statistical importance. On histopathological basis, 8 patients were diagnosed as grade II OSMF which according to the classification is considered to be the early stage of oral submucous fibrosis. The mean number of mast cells was 9.6( $P<0.01$ ), which was lower than that shown by grade I subjects as shown in table no. 2 and graph no. 1& 2 All the 8 cases of grade II showed increase inflammatory cell response which was moderate. As the disease progresses, the blood vessels are dilated due to the histamine, which is released from the mast cells and acts as a vasodilator [8]. As the mast cells extravasate from the blood vessels there is an increase in the inflammatory cell response [9].

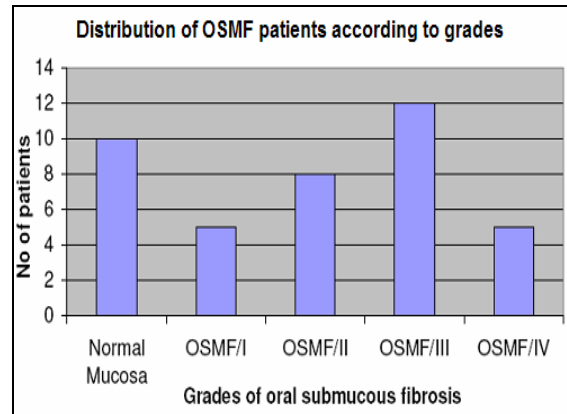
**Table-1:** Distribution of Oral Sub Mucous Fibrosis patients according to the Age

Different Age groups of patients	No. of patients	%age of patients
15-25	8	20%
26-35	28	70%
36-45	3	7.5%
>45	1	2.5%

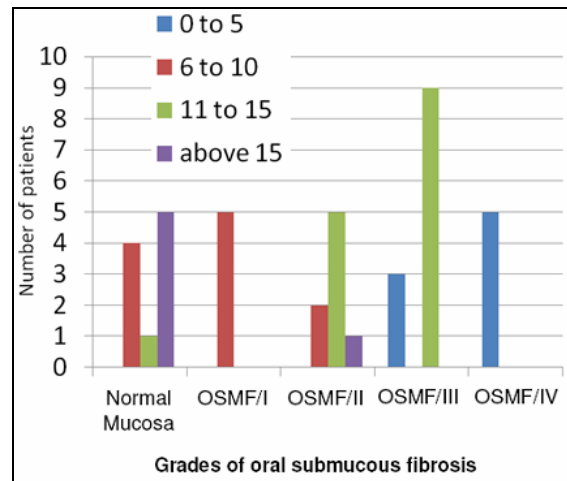
**Table-2:** Shows no. of cases according to mean no. of mast cells in control & different grades of OSMF

Grades of OSMF & Control	Total No. of patients	Cells & SD
Normal Buccal Mucosa	10	14.9± 3.87
Grade I OSMF	05	12.6±1.34
Grade II OSMF	08	9.6±2.66
Grade III OSMF	12	5.8 ±1.52
Grade IV OSMF	05	2.4± 0.89

**Graph-1:** Mast cells showing degranulation (100X)



**Graph-2:**



As both above graph & table shows, in the present study maximum number of patient were in 25-35 age group (70%).

The above results show that there is a statistically significant difference between

number of mast cells of normal mucosa and OSMF grade II. Another 12 patients were histopathologically diagnosed as grade III OSMF. Here a decrease in the mean of mast cells count was noted. Mean number of mast cells was 5.8 as shown in table no.2 ( $P < 0.01$ ). When compared to normal and grade I the difference was not significant, but with grade II it was found to be very significant. The inflammatory cell response shown by the majority of the subjects was mild. This would be due to increase in hyalinization leading to decrease in vascularity.

This could also be due to decrease in nutrition to the cellular elements. Thus the difference between 11 number of mast cells of normal mucosa and OSMF grade III shows statistical significance. In grade IV, 5 subjects were studied and they showed the mean number of mast cell count 2.4 as shown in table no.2 ( $p < 0.01$ ) which is less than the normal as well as the other grades of OSMF. A very mild inflammatory cell response was noted which is in agreement with the above authors. This grade showed a significantly decreased number of mast cells as compared to grade I & II.

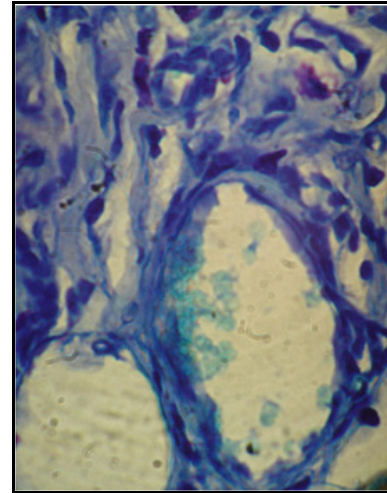
**Table-3:** No. of mast cells range in controls and different grades of Oral Sub Mucous Fibrosis

	0-5	5-10	10-15	>15
Control	0	1	4	5
Grade I	0	0	5	0
Grade II	0	5	2	1
Grade III	3	9	0	0
Grade IV	5	0	0	0
Total	8	15	11	6

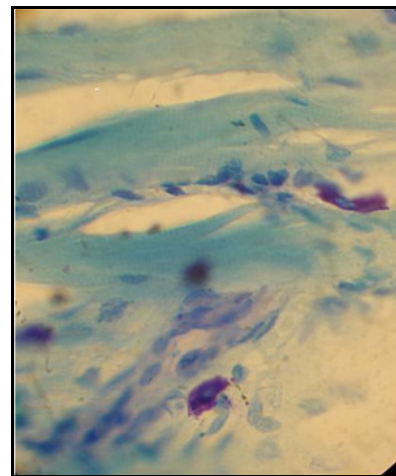
There is a marked difference between number of mast cells of normal mucosa and OSMF grade IV, which shows a continuous decrease and is of immense significance. The difference in number of mast cells is represented in table no.3 and graph no. 2 which shows OSMF I and II, OSMF I and III and OSMF I and IV. There is statistically significant difference between number of mast cells as shown in table no.3 and graph no.2 shows (a) OSMF grade II & III (b) OSMF grade II & IV (c) OSMF grade III & IV.

*Mast Cells Surrounding the Blood Vessels in Oral Submucous Fibrosis:*

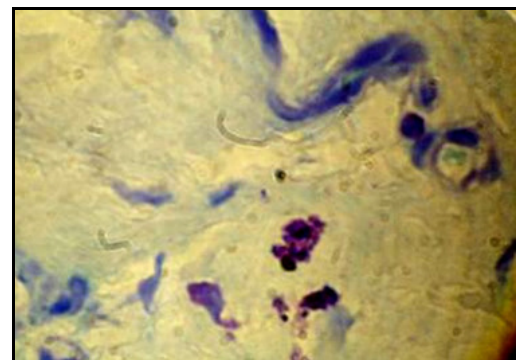
**Figure-1:** 10X Magnification



**Figure-2:** 40 X Magnifications



**Figure-3:** Mast cells showing degranulation 100X magnification



From the above results a specific conclusion, which can be drawn, is that, there is a significant difference between number of mast

cells of normal mucosa and different grades of OSMF. Active degranulation of the mast cells (Figure 3) with continuous histamine release in the early stages might also account for the paucity of mast cells in the more advanced stage of the disease. Vascular dilatation and persistent oedema are predominant features of early submucous fibrosis. (Figure 1)

Vascular response in OSMF is a complex process consisting of persistent dilatation of blood vessels especially in moderately advanced and advanced cases (Figure 2). Here sometimes mast cells

disappear [8-10]. The inflammatory cell concentration increases and later decreases which could be a reason for the decreased mast cell count. The disease process progresses as inflammatory cells extravasate from the blood vessels present in the tissue. Thus the concentration of inflammatory cells in tissue is the reflection of vascularity [6].

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