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Original Article

Mast Cells Density in Hyperkeratosis, Dysplastic Oral Mucosa and Oral Squamous Cell Carcinoma

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Abstract

Background: Mast cells normally present in small numbers in the connective tissue of all organs and release a variety of potent mediators like histamine, leukotrienes, cytokines, chymase, basic fibroblast growth factor (bFGF), tryptase and heparin through degranulation.

Objectives: The aim of the present study was the assessment of mast cell density in hyperkeratosis, dysplastic oral mucosa and oral squamous cell carcinoma (OSCC).

Methods: In this retrospective analytical study, paraffinized specimens from 15 cases of normal mucosa and 23 cases of well-differentiated OSCC, cases of hyperkeratosis and dysplasia were selected. Sections were stained with toluidine blue and then were counted at 400× magnification in hotspot areas under a light microscope. The results were analyzed using ANOVA. *P* values less than 0.05 were considered significant.

Results: Mast cells density (MCD) increased in hyperkeratosis, dysplasia, and OSCC. There was not any significant correlation between mast cell density and hyperkeratosis, dysplastic mucosa and normal mucosa. There were significant differences between mast cell counts in SCC and normal mucosa.

Conclusions: MCD is higher in hyperkeratosis and dysplastic oral mucosa than in normal mucosa. There were significant differences between mast cell counts in SCC and normal mucosa.

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Background

Mast cells normally present in small numbers in the connective tissue of all organs and more particularly, around blood vessels and nerves in the dermal layer of skin and surface of lung mucosa, and digestive system (1-3). Their sizes are ranging from 5 to 15 µm in diameter, and in histologic sections appear ovoid or spindle-shaped cells with cytoplasmic granules. They release a variety of potent mediators like histamine, leukotrienes, and cytokines, chymase, basic fibroblast growth factor (bFGF), tryptase, heparin through degranulation (1,4). It is considered that mast cells have conflicting dual roles from defending against tumors to causing tumor progression. Of course, some of their products induce angiogenesis in tumors (5-7). Oral squamous cell carcinoma (OSCC) remains a serious problem of oral health worldwide and has a complex biological behavior and despite the advances in the treatment modalities, the 5-year survival rates of the patients with OSCC have improved only slightly (8). Sharma et al showed that mast cells have a role in up-regulation of tumor angiogenesis in OSCC (9). The contribution of mast cells to angiogenesis during the progression from oral hyperkeratosis without dysplasia to oral hyperkeratosis with dysplasia or OSCC is not clear yet due to conflicting results within the literature (5).

Highlights

- Mast cells density increased in hyperkeratosis, dyspkasia, and OSCC.

Mohtasham et al found significant differences between the numbers of mast cells in oral dysplastic mucosa and OSCC (10).

Bivji showed an increase in the number of mast cells/unit microscopic field in oral leukoplakia compared to normal mucosa (11). Pazouki et al observed an increase in vascularization during the transformation from normal oral mucosa, through dysplasia, to in-situ and infiltrating carcinoma supporting the pivotal role of angiogenesis in malignancy progression (12). Flynn et al (13) demonstrated a direct correlation between sequential mast cell infiltration, activation and distinct stages of hyperkeratosis, dysplasia, carcinoma in-situ in the oral cavity and implicated the role of mast cells in configuring the angiogenic phenotype in premalignant lesions. Similarly, Iamaroon et al also observed a linear increase from normal oral mucosa, hyperkeratosis, premalignant dysplasia to squamous cell carcinoma suggesting the role of mast cells in upregulation of angiogenic process (14).

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Michailidou et al observed that the mast cell density and microvessel density did increase significantly in normal oral mucosa and oral leukoplakia without dysplasia and oral leukoplakia with mild, moderate or severe dysplasia (5). In contrast to the above-mentioned studies, Oliverira-Neto et al observed a decrease in mast cell numbers in premalignant and malignant oral lesions which was attributed to the failure of mast cell migration (6).

Our study showed an increase in mast cell density. Therefore, we studied the association of mast cells in oral hyperkeratosis, oral dysplastic mucosa and OSCC. For this purpose, toluidine blue staining for mast cells was used, and their relationship in normal oral mucosa, dysplastic, hyperkeratosis oral mucosa and OSCC (OSCC) were compared.

Methods

In this retrospective analytical study, formalin-fixed, paraffin-embedded specimens of 15 clinically normal oral mucosal tissue (Normal mucus specimens were prepared from the margins of the lesions or gingival samples obtained from crown lengthening surgery or latent wisdom tooth surgery), and 23 cases of OSCC, 19 cases of hyperkeratosis and 17 cases with dysplastic mucosa were obtained from the Department of Oral and Maxillofacial Pathology, School of Dentistry in Kerman University of Medical Sciences. Two 4 µm thick sections from each paraffin block were prepared and placed on a slide. The solution used to stain mast cells contained toluidine blue, which was 0.2 g in 100 mL of distilled water and 2 mL of acetic acid. Deparaffinization was done by immersing in xylene and descending grades of ethanol, then, the sections were rinsed in distilled water. The sections were stained by toluidine blue for 10 minutes, and then were rinsed again in distilled water, dehydrated, and mounted. The number of mast cells in normal mucosa and oral SCC, hyperkeratosis and dysplastic mucosa in four fields under a light microscope (Olympus BX41TF, Tokyo, Japan) at a magnification of 400× on an ocular grid were counted, and the average count in each case was recorded. Statistical analysis was performed using SPSS version 21.0 by ANOVA test at significance level of $P < 0.05$.

Results

In this study, 55.2% of the lesions were observed in men and 44.8% in women with a mean age of 57.41±14.69 years. Based on lesion location, the highest number of mast cells was observed in the lower lip, with no positive correlation (Figure 1). There was not any significant difference between sex and mast cells density (MCD).

The average number of mast cells in all lesions was 8.03±4.46. The mean numbers of mast cells in hyperkeratosis and dysplastic mucosa were 6.97±3.85 and 5.66±4.65, respectively. The highest number of mast cells was seen in squamous cell carcinoma (10.51±3.19), which is shown in Table 1 and Figure 2.

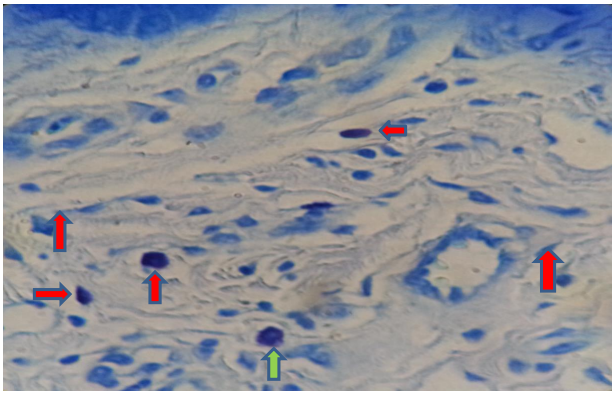

Figure 1. Mast Cells Stained (Red arrows) and Mast Cell Degranulated in Dysplastic Mucosa (Green arrow).

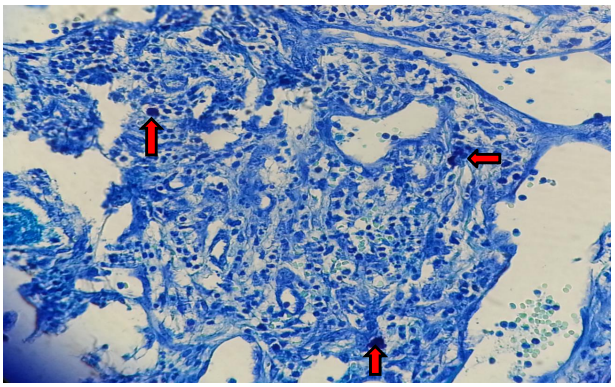

Figure 2. Mast Cells Stained by Toluidine Blue in SCC (arrows).

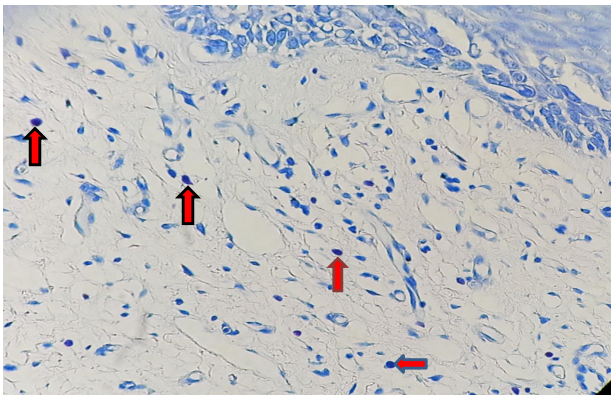

Figure 3. Mast Cells Stained by Toluidine Blue in Hyperkeratosis Mucosa (arrows).

Table 1. Mast Cells Density According to Type of Lesions

Lesion	No	Mast Cell Density	
		Mean	SD
Hyperkeratosis	19	6.97	3.85
Dysplasia	17	5.66	4.65
Squamous cell carcinoma	23	10.51	3.19
Normal mucosa	15	1.28	0.52

Table 2. Correlation Between Mast Cell Density and Normal Mucosa, Hyperkeratosis Lesions, Dysplasia Lesions and Squamous Cell Carcinoma

Type of Lesion		Mean differences	Standard Error	*P* value
Normal mucosa	Hyperkeratosis	-5.69143	2.24023	0.109
Normal mucosa	Mild dysplasia	-5.05333	2.31671	0.215
Normal mucosa	Moderate dysplasia	-3.72000	2.56651	0.602
Normal mucosa	Squamous cell carcinoma	-9.23667	2.03650	0.001*

*$P<0.05$ is significant.

There was no significant difference between the mean number of mast cells and the type of lesions and sex. However, there was a significant difference between the means of mast cells in normal mucosa and squamous cell carcinoma ($P=0.001$) (Table 2 and Figure 3).

Discussion

In the present study, we used toluidine blue staining to identify and count mast cells. MCD was found to be significantly higher in the OSCC group compared with the normal mucosal group. These findings were similar to those reported by many previous studies on various tumors (1,4,5,9,10,15-18).

Flynn et al (13) demonstrated a direct correlation between sequential mast cell infiltration, activation and distinct stages of hyperkeratosis, dysplasia, carcinoma in-situ in the oral cavity and implicated the role of mast cells in configuring the angiogenic phenotype in premalignant lesions. Similarly, Iamaroon et al observed a linear increase from normal oral mucosa, hyperkeratosis, premalignant dysplasia to squamous cell carcinoma suggesting the role of mast cells in upregulation of angiogenic process (14). Michailidou et al observed that the mast cell density and microvessel density did increase significantly in normal oral mucosa, oral leukoplakia without dysplasia and oral leukoplakia with mild, moderate or severe dysplasia (5).

Shojaei et al (19) showed that mast cells are present in all the investigated inflammatory periapical lesions and might play a role in the pathogenesis of these lesions. Gholizadeh et al (20) showed that Ki-67 is extensively accepted as an important biomarker in diagnosis, prognosis and treatment of cancerous and precancerous lesions, a high degree of presence of this biomarker was observed in chronic precancerous lesions, such as erosive oral lichen planus.

It is shown that mast cells play a major role in promoting tumor angiogenesis (18). However, Kalra et al found mast cells make up only a part of the complex process of angiogenesis along with other factors secreted by SCC (21).

However, Oliveira-Neto et al (6) found that the MCD is lower in OSCC and premalignant lesions compared to normal controls. They attributed this to the migration failure of mast cells, which possibly reflect a modification in the microenvironment during tumor initiation and progression. On the other hand, researchers have shown antitumor functions of mast cells, including natural cytotoxicity and release of antitumor compounds (9).

In the present study, there was not significant difference between mast cells densities in dysplastic mucosa and SCC. These findings were similar to Costa et al who reported that the number of mast cells was higher in lip SCC and actinic cheilosis than in normal mucosa, but there was no significant difference between them (7). Researchers showed a direct correlation between mast cells activity and different phases of hyperkeratosis, dysplasia-carcinoma in-situ and oral carcinoma (22).

In the present study, MCD was higher in SCC and dysplastic and hyperkeratotic mucosa than in normal mucosa, but there were no significant differences between them. It is similar to the results of the study done by Michailidou et al who showed that a higher MCD was observed in SCC (5). Sathyakumar et al reported the increase of MCD in leukoplakia in comparison with normal mucosa (23).

Conclusion

Overall, our results showed a significant difference between the mean values of MCD in normal mucosa and OSCC. In addition, no positive correlation was observed between the values of MCD and hyperkeratotic, dysplastic mucosa and normal mucosa.

Authors' Contribution

MT designed the study. MAH helped in study design and preparation of the manuscript. JH edited the manuscript. ME performed the study.

Ethical Statement

The study was designed as a cross-sectional study with the ethical code IR.kmu.ac.1392.920165. The study was approved by the Institutional Human Research and Ethics Committee of Kerman University of Medical Sciences, Kerman, Iran.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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