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Histochemical Mapping of Mast Cells in Oral Dysplasia: A Window into Carcinogenesis in Rats

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ABSTRACT

Background: The most common cause of death is cancer. Oral squamous cell carcinoma is the initial phase of more than 95% of oral cavity carcinomas. The earliest histological indication of cancer is dysplasia, which occur before squamous cell carcinoma (SCC) forms. The risk of transformation to cancer is greater in dysplasia than in normal oral epithelium. Objectives: To demonstrate the correlation between the mast cell count and oral dysplastic lesions severity. Methods and Materials: the study involve 30 rats who randomly allocated into two groups: control and experimental groups. Formaldehyde and 7,12-Dimethylbenz[a]anthracene (DMBA) were applied topically to the rats' buccal mucosa in order to cause carcinogenesis. Mast cell detection using toluidine blue stain. Results: the mast cell count (MCC) was more significant in oral epithelial dysplasia (OED) (high- risk and low- risk dysplasia) than in control and most significantly expressed was in high-risk epithelial dysplasia. Conclusion: As OED severity increased, there was also a significant increase in the number of infiltrating mast cells (MCs). By reducing their tumor-promoting properties, MCs may be a viable target for the treatment of oral premalignant and malignant cancers.

Key words: Mast cells, Oral dysplasia, Carcinogenesis, Histochemical mapping, Buccal mucosa, T.B. staining (Toluidine Blue), and Oral epithelial dysplasia (OED).

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INTRODUCTION

Oral epithelial dysplasia (OED) is characterized by abnormal cell growth with varying degrees of severity (1). The exact causes of OED remain unclear, risk factors such as tobacco use (smoking and chewing), excessive alcohol consumption, and exposure to certain viruses are implicated (2). Tobacco use significantly elevates the risk of developing premalignant oral lesions like leukoplakia, erythroplakia, and oral submucous fibrosis (3). Recognizing and managing dysplastic lesions are crucial to prevent their progression into malignancy (4).

Oral epithelial dysplasia (OED) is a histological diagnosis characterized by architectural and cytological changes, assessed microscopically using hematoxylin and eosin (H&E) staining (5). Despite its importance, grading OED is subjective, leading to variability among observers (6). To improve consistency, the WHO classification (2005) was applied to a binary system (2006), which categorizes OED into low-risk (fewer changes) and high-risk (greater changes) groups (7).

The World Health Organization (WHO) classification (2005), which assesses architectural and cytological alterations, has been used to validate a binary system (2006) for grading oral epithelial dysplasia (OED), categorizing lesions as low-risk (fewer architectural and cytological changes) or high-risk (more extensive changes) (7). Mast cells (MCs), granular white blood cells abundant in the oral mucosa, play a vital role in immune responses and tissue homeostasis (8). MCs release mediators like histamine, VEGF, and FGF-2 through secretory processes such as degranulation during allergic reactions, supporting epithelial and fibroblast proliferation, thereby maintaining tissue integrity and promoting repair (9).

Hegde and Marla's study found that OED and OSCC had higher MCC and microvascular density (MVD) than normal mucosa. However, only OED showed a statistically significant positive association. These findings most likely suggest that MCs play a part in the "angiogenic switch." These MC-secreted angiogenic substances either directly stimulate MC migration and/or proliferation or indirectly promote angiogenesis by degrading extracellular matrix (10). Anuradha et al. showed that asignificant increase in the MCC was observed in microscopic sections of OSCC when compared to normal mucosa suggesting their contributing role in tumor growth and progression as the infiltrating MCs degranulate and activate dermal fibroblasts which intensify angiogenesis (11).

Zaidi and Mallick in their study of SCC of buccal/labial mucosa showed that MCC was higher in OSCC when compared with controls. There may be significant clinical ramifications and direct clinical significance to the role of MCs in malignancies. They said that blocking mast cell function may stop tumor growth and that MCs may be a new therapeutic target for the treatment of cancer (12). This research aims to map mast cell count and distribution in a rat model of oral epithelial dysplasia, assessing their role in cancer development.

MATERIALS AND METHODS

For this study, 30 adult male Egyptian albino rats—obtained from Cairo University's Faculty of Medicine in Egypt—were kept in the animal house as an inbred colony. Rats weighing between 100 and 200 grams and ranging in age from 3 to 4 months were chosen to participate in this experiment. The rats were split equally into two groups: the experimental group, which included 24 rats given Formaldehyde and 7,12-Dimethylbenz[a]anthracene (DMBA) and formaldehyde, and the control group, which included 6 rats not receiving any treatment. Twelve rats are included in each group based on the date of sacrifice, which is six weeks (B1) or nine weeks (B2) after the painting begins (13).

The animals were kept in a controlled setting with 12 hours of darkness and light cycles at a temperature of 25 ± 2°. During the course of the study, all rats were

kept on a basic diet consisting of distilled water and ordinary rat chow, which is designed to satisfy the nutritional requirements of rodents. Four rats per stainless steel cage were kept in identical humidity and temperature conditions. Mammals that breed need bedding, extra nesting materials, and a sturdy floor in a nest box at the very least. The Random Sequence Generator application (random.org) was used to disperse the rats at random. Rats were sacrificed six weeks after the painting began for low-risk OED and control, and nine weeks for high-risk OED and control; twelve rats were sacrificed for each interval. An overdose of the anesthetic drugs (1 milliliter per 100 grams) resulted in the rats' sacrifice (The 2014 IACUC guidelines) (13).

Hematoxylin and Eosin (H&E) stain: To find evidence of dysplasia caused in the rat's buccal mucosa, sections of paraffin blocks, each 5 microns thick, were cut out and placed on glass slides for standard H&E staining and subsequent observation under a standard light microscope.

To detect mast cells, slices ranging from four to five microns were cut, placed on ordinary glass slides, and then subjected to T.B. histochemical staining, which was acquired from Novus, SNF Medical in Egypt. Both groups' data were gathered, collated, statistically examined, and presented in tables and figures. Means and standard deviations were used to summarize the data. A statistical program called SPSS was used to evaluate the collected data.

To compare the low-risk and high-risk dysplasia groups with the control group and with each other, a student t-test was utilized. At $P < 0.05$, the results were deemed significant. The twenty four rats of experimental group were anesthetized by intraperitoneal ketamine 80-100 mg/kg and xylazine 10-12.5 mg/kg according to The INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE guidelines (Application protocol 2012). The rats had their buccal mucosa painted (topically) with DMBA and formaldehyde; 0.5% DMBA in acetone 3 days/week and after 9 days 10% formaldehyde/water was used side by side with DMBA throughout the study period using number.3 camel hair brush.

RESULTS

Histopathological examination of H & E-stained sections of control group (normal rat's buccal mucosae) showed normal keratinized stratified squamous epithelium overlying normal connective tissue after both intervals 6 weeks and 9 weeks fig. (1). surface hyperkeratosis and acanthosis of the prickle cell layer in low-risk dysplasia fig 2). Similar criteria of the low risk group except that signs of dysplasia were seen in the lower half and extended to more than the lower half of the epithelial thickness fig. (3). The allocation was implemented as follows: Numbers from 1 to 48 were written on folded papers that were placed in opaque sealed envelopes, matching of threats with the numbers was done blindly through the technician in charge at the animal house, each rat was attached to its number till the end, then the numbers were opened and threats were allocated in their groups according to the program's recommendation.

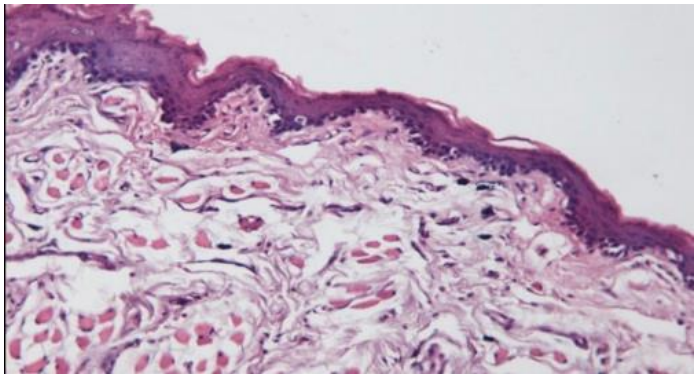

Figure 1: A photomicrograph of a rat's buccal mucosa in the control group shows normal connective tissue on the outermost keratinized stratified squamous epithelium (H&E, x200).

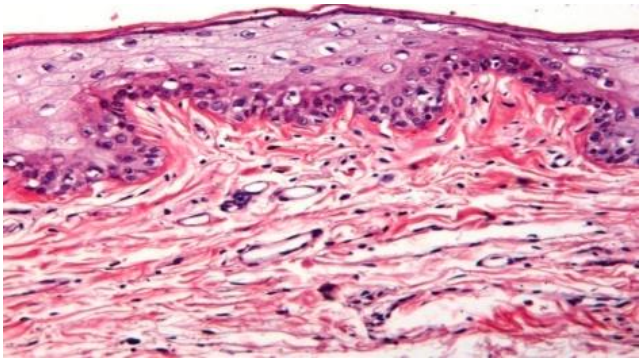

Figure 2: Photomicrograph of low risk group (B1) showing slight acanthosis, slight surface hyperkeratosis. Nuclear hyperchromatism and pleomorphism are prominent in basal and suprabasal layers (H&E X 200).

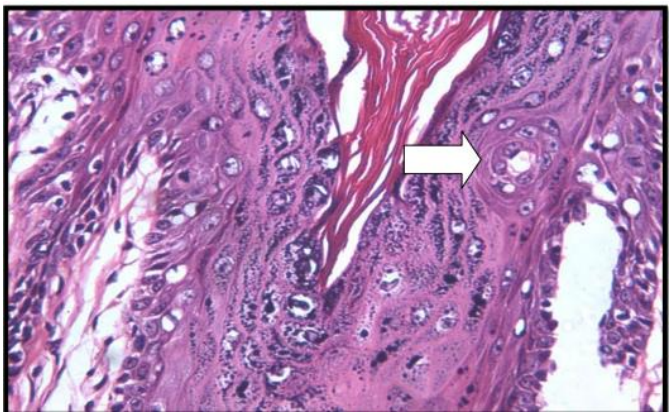

Figure 3: Higher magnification of the high risk group showed cell nest formation in the upper layers of the epithelium (white arrow) (H&E X 400).

In stained sections, histopathological findings of T.B revealed various amounts of mast cells, which were large, rounded cells that infiltrated connective tissue. In some of them, purple cytoplasmic granules were obvious. In control group MCs were scarcely detected in the connective tissue fig. (4). In the experimental groups, high risk dysplasia showed most significant than low risk dysplasia and normal buccal mucosa risk group figures (5 & 6) and tables (1, 2 & 3).

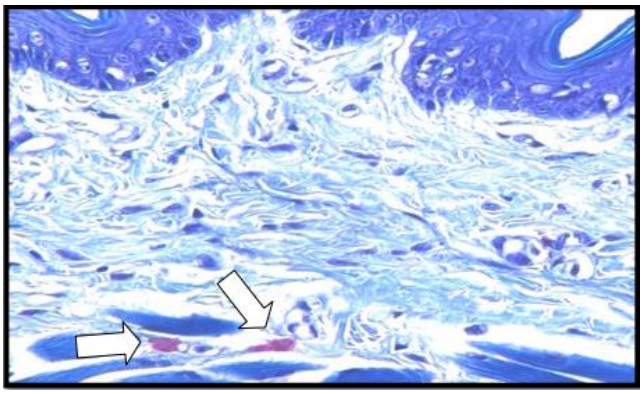

Figure 4: Higher magnification of the control group showing two MCs deep in the connective tissue (arrows) (TBx400).

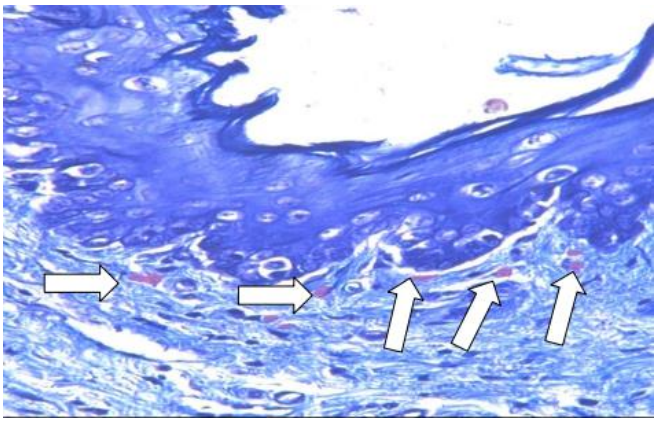

Figure 5: Higher magnification of the low-risk group showing nine rounded large MCs with cytoplasmic red granules (arrows) haphazardly scattered in the subepithelial connective tissue (TB X400).

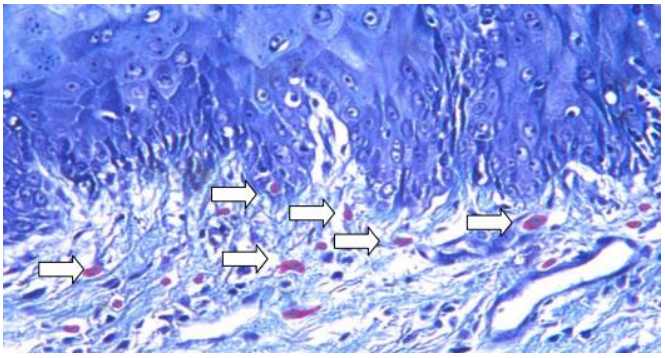

Figure 6: Higher magnification of the high risk group showing many of rounded and elongated large MCs with cytoplasmic red granules (arrows) haphazardly scattered in the subepithelial connective tissue (TB X400).

Table (1): Mean MCC in control group and low risk groups Student-T-test

	Group	Mean	SD	P value
MCC	control	4.33	0.51	***P*** <0.0001*
	low risk	13.5	1.87	

* Significant

Table (2): Mean MCC in control group and low risk groups Student-T-test

	Group	Mean	SD	P value
MCC	control	4.33	0.51	***P*** <0.0001*
	High risk	33.33	1.87	

Table (3): Mean MCC low risk group and high-risk groups Student-T-test.

	Group	Mean	SD	P value
MCC	Low risk	13.5	1.87	***P*** <0.0001*
	High risk	33.33	3.55	

* Significant

DISCUSSION

White blood cells of the granular kind make up the MC. It has sizable cytoplasmic granules that store a range of mediators, including heparin and histamine.(14) By activating pro-tumor mediators like the "angiogenic switch," the MC contributes to the growth of tumors. These MC-secreted angiogenic substances may directly stimulate MC migration and/or proliferation or indirectly promote angiogenesis by degrading the extracellular matrix (10). As invasion-causing MCs degranulate and stimulate dermal fibroblasts, which enhance angiogenesis, MCs aid in the formation and evolution of tumors. These even activate progelatinase B, a matrix metalloproteinase family member that regulates angiogenesis and extracellular remodeling (11).

In this regard, the present research has made an effort to emphasize MCs in both normal and dysplastic oral buccal mucosal tissue sections using the T.B. For both the high and low risk OED groups, the number of MCs was computed and compared to the control group (15). The current study's findings demonstrated that there were not many MCs in the connective tissue of the typical oral buccal mucosa. This result is in line with Telagi et al.'s study, which found that normal oral mucosa had a small number of MCs in comparison to OED (16).

MCs were observed invading the connective tissue beneath the dysplastic epithelium in both the low and high risk OED groups in this experiment. This result is consistent with the findings of Coussens et al. (1999), who showed that MCs in dysplasia are closely positioned in relation to the basement membranes and epithelium. According to the author, these are the locations of active extracellular matrix remodeling, and MCs aid in the breakdown and remodeling of extracellular matrix by releasing progelatinase B, two MC-specific serine proteases, a tryptase, and a chymase (17).

Additionally, this study found that MCs in high-risk OED were significantly higher than those in low-risk OED and normal buccal mucosa. This is consistent with study which demonstrated an increase in MCC from squamous cell carcinoma and mild dysplastic leukoplakia to severe dysplastic leukoplakia. The author suggested that chemo attractants that are induced by tumor cells or normal connective tissue cells as a response to the tumor can be a reason for gradual increasing in mast cell count from to OED and OSCC (18). It can be therefore use the MCs as an indicator for the prognosis of lesions and therapy target because of this notable difference in MCC values.

CONCLUSION

The severity of oral epithelial dysplasia is significantly correlated with mast cell infiltration, according to the current study. Mast cells are increased when OED

becomes more severe, particularly in high-risk dysplastic lesions. These results demonstrated the potential of mast cells as a therapeutic target and a diagnostic marker in the treatment of oral cancer.

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