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**Research article**

# Studying Tryptase-Positive Mast Cells in Squamous Cell Carcinoma and Normal Oral Mucosa by Immunohistochemistry

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## ABSTRACT

**Introduction:** squamous cell carcinoma (cancer) of oral mucosa is the most common oral cavity cancer, and although it is less common than other cancers, it is more important,because oral cavity considered as a strategic cavity. Mast cells are complex cells and have multiple functions,different results have been reported about the role of these cells in development and spread of tumors. The aim of this study was to evaluate tryptase-positive mast cells in squamous cell carcinoma normal oral mucosa by immunohistochemistry.

**Materials and methods:**In this descriptive analytical study, 15 samples from blocks related to squamous cell carcinoma and 15 samples of normal mucosa were selected from archives of Hamadan dental school, and were evaluated byimmunohistochemistry using stainedtryptase marker. The number of stained cells in each samplewerecounted by a pathologist, and were analyzed by using independent t tests, chi-square and Fisher's exact test. The significant level of <0.05 was considered.

**Findings:** The average number of tryptase-positive mast cells in squamous cell carcinoma was 17.89 ± 6.83 and in normal oral mucosa was 10.81 ± 5.59. The average number of tryptase-positive mast cells in oral squamous cell carcinoma was significantly higher than normal oral mucosa (P = 0.004).

**Conclusion:** Because in the present study, the average number of mast cells in oral squamous cell carcinoma was higher thannormal oral mucosa, this can be indicative of the possible role of mast cells in development and spread of tumor.

**Keywords:**Mast cell, Tryptase, Oral mucosa, Squamous cell carcinoma

## INTRODUCTION:

Oral squamous cell carcinoma (OSCC) is the eleventh most common cancer in the world and includes 94% of all oral cancers. Females show a lower annual incidence rate in each age group compare to males, and the overall ratio of male to female is 3 to 1. Like many of carcinomas, the risk of oral cancer increases with increasing of the age (especially in males). Scc is a multifactorial disease.No single causative agent or factor has been clearly defined or accepted. in any case, both foreign and domestic factors may be involved. Internal factors are malnutrition, anemia, iron deficiency, and external factors are tobacco, alcohol, syphilis and sun's rays in carcinomas of the vermilion of the lower lip. Oncogenes are likely effective on onset and development of oral

squamous cell carcinoma. On the other hand, mutation or inactivation of tumor suppressor genes also indirectly causes tumor. Until now, abnormalities of Ras, Myc, 1C-erb Boncogens and P16, E-cadherin, PRB and P53 tumor suppressor genes have been detected in oral carcinoma. Despite advances in therapy and molecular pathogenesis of oral cancer, the survival rate has not significantly increased in recent decades, and it is remained in the range of 50-59%. Therefore, early detection and prevention is necessaryto improve the patient outcome (1, 2). Mast cells are cells that their role in innate immune responses as well as allergies are known. Mast cells are single-cell spherical cells from granulocytes family, thatoriginate from bone marrow cells. Mast cell granules include heparin, hestamine, and enzymes like tryptase. These cells can cause tumor growth by expanding blood supply (angio genesis) through the extracellular matrix, and they are capable of triggering angiogenic cytokines such as VEGF, 2FGF, tryptase and chymase serines, TNF, TGF-β, 8IL- and MGF, and on the other hand they are capable of extracellular matrixdegradation. One way to evaluate the mast cells in a tissue is detection of enzymes secreted by these cells, which are one of the main enzymes of tryptase enzyme. This enzyme is of serine protease family and is coded by the gene on the chromosome 16 arm. Most stored mediator is in mast cell granules, and its release from secreting cells is indicative of mast cell degranulation, it is the gold standard for determining mast cell phenotypes in different tissue components (3-5).

Mast cells are complicated cells and have multiple functions,different results have been reported about the role of these cells in development and spread of tumors. In the study by DastPak et al. it was shown that the number of mast cells in SCC has been decreased compare to normal oral mucosa (6). Anurdah et al. (2014) in their study, evaluated the incidence rate of mast cells in SCC, the results showed a marked increase in the number of mast cells in tissue sections of oral squamous cell carcinoma, which was indicative of the role of mast cells in development and growth of tumors compare to normal mucosa (5).

Thus in this study, we decided to evaluate the amount of mast cells in squamous cell carcinoma and normal oral mucosa, so in the case of incidence of them in tumors, we would be able to prevent spread of tumors in the future by inhibiting them, and use this a new strategy in treatment.

**MATERIALS AND METHODS:**

The present study was a descriptive – analytical study that was conducted as a cross-sectional method on two groups of squamous cell carcinoma and normal oral mucosa. By exploring the archives of pathology of Hamedan School of Dentistry, from 1383 to 1395, related injuries based on the content of record and pathology reports of patients and then confirming it by microscopic reviewing based on the sample size determined for the study, 15 cases of squamous cell carcinoma and 15 samples of normal oral mucosa were selected, and the incidence of tryptase in these two types oftissues were examined and compared to each other.

Samples that met the above criteria were selected and then their slides of H & E were delivered. After matching the number of slides with medical record of patients and their pathology reports, slides were examined usingoptical microscope by a pathologistfor reaffirming histopathologic diagnosis, and histopathological characteristics defined forsquamous carcinomas in the book ofNeville, and normal oral mucosa in the book of ten Kate were considered.

The criteria of breaking down the slides were sufficient slides, integration of pieces and optimal fixation.

Stained slides were studied under Olympus41 BS optical microscope made by America with magnification of 40 and 100 times, and we assured ofthe accuracy of stain by comparing with positive control sample (human tonsil tissue for tryptase).

First, at 40X magnification, the five areas with the highest number of stained mast cells (using

tryptase marker) are selected (7), and from this areaa,photos will be prepared using DP12 digital camera model U-TV/5 XC-3 mounted on Olympus BX 41 microscope, and then by using Analysis LS Starter software, the number of stained cells in this areas are counted by pathologists and the average of them was obtained. We discussed the observations using measures of central tendency and dispersion. The groups were compared according to the result of Shapiro-Wilk test (P=0.20) and distribution of data normality, using independent t-test, chi-square test and Fisher's exact test. Finally, SPSS ver.19 software was used to analyze the data and a significant of 0.05 was considered.

**FINDINGS:**

A total of 15 patients with squamous cell carcinoma consists of 10 males (66.7%) and 5 females (33.3%), aged between 28 to 91 years old, with the mean and standard deviation of 60.07 ± 17.36 years, and 15 samples of normal mucosa consist of 5 males (33.3%) and 10 females (66.7%), aged between 11 to 70 years old, with the mean and standard deviation of 46.17 ± 40.42 years, were examined.

According to the data presented in table 1, there was a significant difference between the gender of patients in two groups (p=0.041).

**Table 1.**Frequency distribution of gender in both study groups

| chi-square test | Gender | | Frequency | Group |
|---|---|---|---|---|
| | **Female** | **Male** | | |
| $X^2 = 33.3$ | 5 (33.3%) | 10 (66.7%) | 15 | Squamous cell carcinoma |
| P = 0.068 | 10 (66.7%) | 5 (33.3%) | 15 | Normal mucosa |

According to Table 2, there was no significant difference between the location of the injury in both groups (p = 0.106)

**Table 2.**Clinical information about the location of the injury in both study groups

| Variable | Variable levels | group | |
|---|---|---|---|
| | | Normal flora | Cell carcinoma Squamous |
| Buccal mucosa | Number | 4 | 5 |
| | Percent | 26/7 | 33/3 |
| Floor of the mouth | Number | 0 | 1 |
| | Percent | 0 | 6/7 |
| Language | Number | 0 | 4 |
| | Percent | 0 | 26/7 |
| Palate | Number | 2 | 2 |
| | Percent | 13/3 | 13/3 |
| Alveolar ridge | Number | 8 | 3 |
| | Percent | 53/3 | 20 |
| Lip | Number | 1 | 0 |
| | Percent | 6/7 | 0 |
| Total | Number | 15 | 15 |
| | Percent | 100 | 100 |
| Fisher's exact test | p=0/106 | | |

In the present study, all samples of squamous cell carcinoma and normal oral mucosa weretryptase-positive marker in terms of staining. The average number of tryptase-positive mast cells in squamous cell carcinoma was 17.89 ± 6.83 and in normal oral mucosa was 10.81 ± 5.59. A significant difference was observed in relation to the average number of mast cells in both groups (P = 0.004), in which the average incidence of the number of tryptase-positive mast cells in the

group of squamous cell carcinoma was significantly higher than normal mucosa.

**DISCUSSION AND CONCLUSION:**

The aim of this study was to examine the number of mast cells in squamous cell carcinoma and normal oral mucosa, and compare them with each other. Oral squamous cell carcinoma is the most common cancer of oral cavity, which has remained as a serious problem all around the world (6). OSCC has complex biological behavior, which despite advances in the treatment and molecular pathogenesis, its survival rate has not significantly increased in recent decades (6).

Various studies have shown that inflammation is a crucial part for tumor progression (8). Immune cells and inflammatory infiltration including macrophages, lymphocytes, eosinophils, mast cells and etc. in tumor stromal, can be effective in progression and metastasis of it. The number and the type of immune cells, that are infiltrated into tumor,are dependent on the production of chemokines and other chemotactic factors, which are produced by tumoral and stromal cells (9). Mast cells play a specific role in the pathophysiology of many diseases. The release of their mediators in tissue damage, inflammation and immune responsesplay a role (8).Recent studies have demonstrated the presence of mast cells in invasive cancers such as melanoma, breast carcinoma adenocarcinoma of the colon, stomach, esophagus, oral cavity and the lung (6, 10). In the present study, mast cells were present in all samples of squamous cell carcinoma and normal oral mucosa, but the average number of mast cells in OSCC were significantly higher than normal mucosa.The results of present study are consistent with the results of the study by lamaroon, Zaidi, mohtasham, Anuradha et al. In their study, the average number of mast cells were also significantly higher than normal oral mucosa (5, 8, 10, 11).In another study by Ranieri et al. on SCC and non-dysplastic oral leukoplakia, it was determined that there is a significant relationship between the number of mast cells and small blood vessels in the lesions compare to normal oral mucosa, which regarding the number of mast cells is consistent with our study (12). Other studies have examined the presence of mast cells in other lesions; Zou et al. in a study on lung cancer concluded that the number of mast cells and blood vessels in this lesion is significantly higher than normal mucosa, and there was a significant relationship between the number of mast cells and blood vessels in cancerous lesions (13). In the study by Ibaraki et al. on adenocarcinoma, Scc and normal lung tissue, it was revealed that the number of tryptase-positive mast cells in the periphery of these lesions was higher than normal mucosa. There was a significant relationship between the number of tryptase-positive cells and blood vessels (14). In this study, similarly, the number of mast cells in cancers have increased compare to normal mucosa. Numerous studies have reported that peptides derived from tumoral cells and monocyte chemotactic protein can play a role in colonization of mast cells into tumor (8, 9), which this could be due to the presence of mast cells in in different cancers. Mast cells can contribute to progress, invasion and metastasis of tumors (8). Since in our study, the number of mast cells in squamous cell carcinoma was higher than normal oral mucosa, we can say that maybe one of the factors in the development, progression and metastasis of this lesion are mast cells.

DastPak et al. in a study concluded that the average number of mast cells in OSCC decreasescompare to normal oral mucosa, which they attributed this to the toxicity activity of mast cells in the tumor. They reported that these cells though toxicity activity causethe suppression of tumor activity (6). Jahanshahi et al. also concluded that there is no positive relationship between the number of mast cells and angiogenesis in SCC, and this relationship in some cases is even reverse (3). The difference between the results of the study with other studies is probably due to the difference in the location of tumor, type of the stain and the marker used. For example, in the study by Dastpak and Jahanshahi, toluidine blue was used to evaluate the mast cells, while in the present study, tryptase and immunohistochemistry were used for this

evaluation. Immunohistochemistry has higher precision compare to toluidine blue staining. Therefore, in this regards, the present study is superior thantwo studies cited. Conflicting results regarding the role of mast cells in different cancers can be due to following reasons; first, these cells have cytotoxic activity when infiltrated into the tumor, and induce suppression of tumor activity, but subsequently, tumoral cells cause facilitation of angiogenesis and reduce their cytotoxic activity through increasing of angiogenesis activity of mast cells (6). In the present study, since the average number of mast cells in OSCC has increased compare to normal oral mucosa, this can be indicative of important changes in the location of this lesion and presence of chemotactic factors of mast cells, and in many ways these cells can contribute to progression, invasion and metastasis of tumor (15). In general, since in this study the average number of mast cells in oral squamous cell carcinoma was higher than normal oral mucosa, this could indicate a possible role of mast cells in progression and spread of tumor.

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