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Evaluation of Mast Cell Density in Adenoid Cystic Carcinoma and Polymorphous Low-grade Adenocarcinoma.

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ABSTRACT

Mast cells are involved in tumor progression, and prognosis. Moreover, despite dissimilarities in the prognosis and biologic behavior of Adenoid cystic Carcinoma (ACC) and Polymorphous Low-grade adenocarcinoma (PLGA), these two tumors have histological similarity. This research, compares mast cell densities in these two tumors for the purpose of their differential diagnosis. In this experimental study, 30 paraffin blocks of ACC and 10 paraffin blocks of PLGA with acceptable inclusion criteria were selected and stained with Toluidine blue. The numbers of mast cells were counted in 5 random microscopic fields at 400x magnification. The mean of mast cell counts in both groups were compared using Student's t-test with a significance level of 0.05. The means of mast cell counts in ACC and PLGA were 8.24 ± 5.70 and 6.53 ± 3.28, respectively, demonstrating a statistically significant difference ($p \leq 0.05$). Based on our results, it is possible to use mast cell counts to confirm differential diagnosis of ACC and PLGA. In addition, the more invasive behavior of ACC compared with that of PLGA could probably be attributed to its larger number of mast cells.

Keywords: Adenoid Cystic Carcinoma, Polymorphous Low-grade Adenocarcinoma, toluidine blue, mast cell.

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INTRODUCTION

Salivary gland tumors constitute a major part of mouth, jaw, and face lesions. Although these tumors are uncommon, they are of significance because of their specificity to this region (1). Malignancy risk increases in all salivary gland tumors with increasing tumor size, and this confirms the importance of early diagnosis of such lesions (2). One of the most common malignant salivary gland tumors with invasive behavior and a strong tendency for distant metastasis and local relapse is ACC, which is treated by excisional surgery, complementary radiotherapy, and chemotherapy, and which has a poor prognosis. PLGA is also a minor salivary gland tumor first introduced in 1983. Before its identification as a distinct tumor, instances of this tumor were categorized as pleomorphic adenoma (PA) or ACC. However, it was realized that it exhibits a distinct biologic behavior and is one of the most common minor salivary gland malignancies. The best treatment for PLGA is wide excisional surgery; yet, instances of relapse and metastasis are very infrequent to rare. This tumor displays great similarity with ACC from clinical and histopathological perspectives, which could lead to the administration of an invasive, detrimental treatment because it is misdiagnosed as ACC (3).

Mast cells (mastocytes) are connective tissue-resident cells in the immune system involved in protecting the body (4). They are round to oval-shaped, 8-20 micrometer in diameter, and originate from bone marrow stem cells and enter peripheral blood (5). They contain cytoplasmic granules with major mediators of allergic reactions, lipid mediators, and some cytokines that are involved in inflammation (6).

Mast cells can be observed in different types of cancer such as skin, liver, breast, gastric, melanoma and colorectal adenocarcinoma. These cells form aggregates in response to absorption of chemicals in tumor growth locations, and their infiltration is an indicator of tumor growth. Mast cells play a dual role in that they promote or inhibit tumor growth depending on the condition of the stroma surrounding the tumor (7, 8).

Different methods such as immunohistochemical methods and Giemsa, safranin, toluidine blue, and Alcain blue stains are used to stain mast cells.

This study intended to compare mast cell density in two malignant salivary glands (ACC and PLGA) using the toluidine blue staining method.

In this cross-sectional study, 30 paraffin blocks of ACC and 10 paraffin blocks of PLGA with acceptable fixation and tissue volume without much inflammation or bleeding were selected. 4-micron slices of these blocks were prepared and spread on a slide for toluidine blue staining. The slices were deparaffinized with xylene, placed in various percentages of alcohol (absolute ethanol, ethanol 96%, 80%, and 70%) for 2 hours, and subsequently rinsed with water. They were then placed in toluidine blue stain (prepared by dissolving 2 g of toluidine blue powder made by the German company Merck in 100 cc of water with a pH value of 2.3 for 1 minute), and were rinsed subsequently.

Two observers using an OLYMPUS optical microscope at× 400 magnification counted the mast cells in five microscopic fields of each specimen's stroma blindly.

The mean mast cell count for each tumor (ACC and PLGA) was calculated, and T-test was used to compare mast cell density in both tumors. The P value ≤0/05 was regarded significant.

FINDINGS

Data obtained by the two observers on 30 paraffin blocks of ACC and 10 paraffin blocks of PLGA was analyzed. Results showed no significant difference between the data obtained by the two observers at the 0.01 probability level. The mean mast cell density of ACC and PLGA was separately calculated (Table and Diagram 1). Student's t-test demonstrated a significantly higher mast cell density in ACC than PLGA with a confidence level of about 95% (p = 0.05).

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Table 1: Descriptive characteristics of mast cell densities in ACC and PLGA

Total	N		Mean	SD	SEM
Mast cells	ACC	30	5.70	8.24	0.75
	PLGA	10	3.28	6.53	1.03

400 magnification)×Figure 1: Mast cells in the ACC tumor (

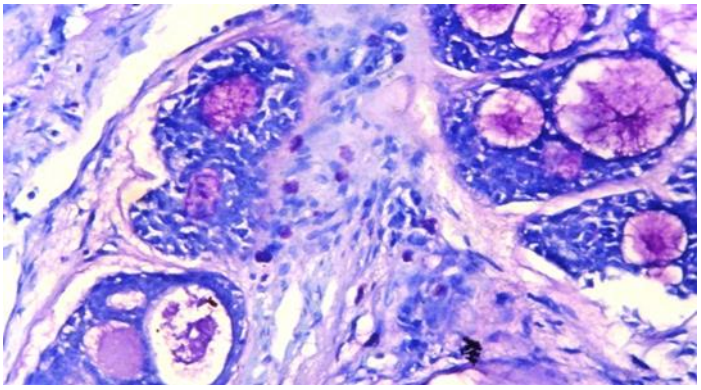
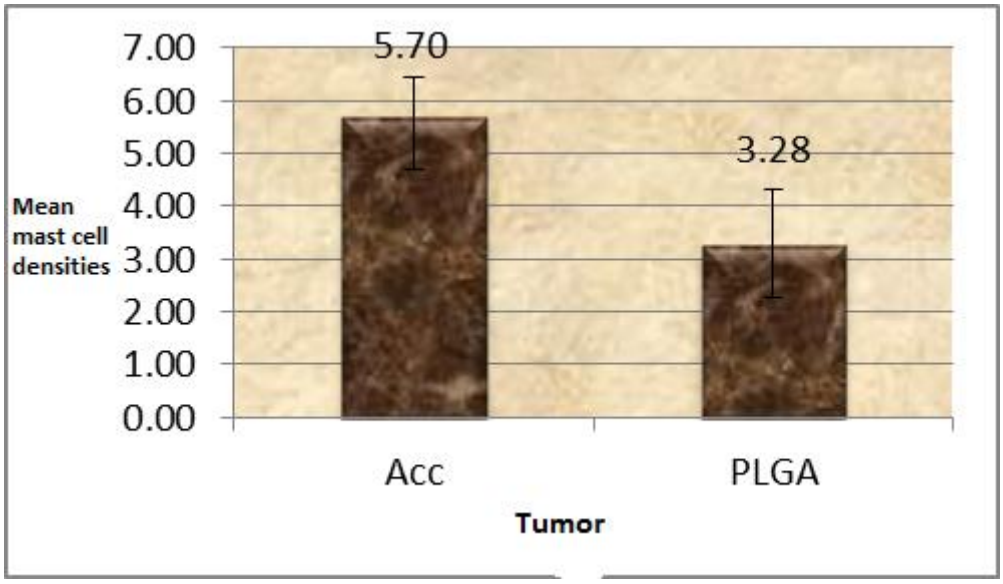


Diagram 1: Mean mast cell density in the two tumors



DISCUSSION AND CONCLUSION

Results of this study, which was conducted to compare mast cell densities in ACC and PLGA salivary gland tumors, revealed that the number of mast cells in ACC was significantly greater than that of PLGA. This conforms to the biologic behavior of this tumor since, as mentioned previously, mast cells are involved in the tumorigenesis process. This characteristic is related to their involvement in the angiogenesis process as well as in cell proliferation stimulation (7, 9). It appears, therefore, that increasing presence of such cells in a tumor is accompanied by a more invasive behavior of the tumor. Since ACC exhibits a more invasive biologic behavior than PLGA, the more noticeable presence of mast cells in this tumor compared to PLGA is justifiable.

Among effective factors in the tumorigenic behavior of mast cells are the presence of factors such as histamine in mast cell granules, which is a factor for vasodilation, angiogenesis, and mitogenesis, and of

growth factors including CSF, FGF, NGF, TGF[1], and the production of enzymes involved in tissue destruction and metastasis such as kinase and tryptase. On the other hand, such factors as interleukins, tumor necrosis factor (TNF) alpha, transforming growth factor (TGF) beta, and other inflammatory factors secreted from mast cells prevent tumorigenesis. However, it is not clear which factor dominates the other and how mast cells play their ultimate role in each tumor, and requires further studies (3-6). The present study demonstrated that mast cells in these tumors play a more tumorigenic role.

Histopathological similarities between ACC and PLGA, as well as their common physical features, i.e. the simultaneous appearance of epithelial and myoepithelial markers, make the diagnosis of these tumors difficult. Researchers have used a variety of biomarkers to detect these tumors and have found such markers as CD34 and c-kit useful for the differential diagnosis of these two tumors (1). However, these studies have all been carried out by using relatively expensive and complex techniques, the simplest of which is immunohistochemistry method.

Although Vidal (2013) could not find a general significant relationship between capillary density and mast cells in salivary gland tumors, yet he maintained, based on the data collected from his study that, compared to other ACC tumors, pleomorphic adenoma, and PLGA, mucoepidermoid carcinoma (MEC) had a higher density of mast cells and capillaries. He added that, next to MEC, solid ACC tumors displayed higher density levels compared to other subgroups (10)

Katpadi et al. (2004) examined the number and dispersion of mast cells in pleomorphic adenoma of salivary glands. Results revealed that polymorphic stromata could influence mast cell density. Mast cell density, in turn, results in variations in tumors arising from major and minor glands. According to the researcher of the present study, the greater presence and higher density of mast cells in salivary gland tumors is instrumental in the transformation of benign tumors to malignant ones or of less invasive (e.g. PLGA) to more invasive types (e.g. ACC) (11).

In their study on gastric cancer, Yano et al. observed a significant difference in the number of mast cells in tumors favoring distant metastasis compared with those without metastasis. They also found a positive relationship between the number of mast cells and poor prognosis, which is in line with the findings of the present study (12).

In their study on colon cancer, Xia et al (2011) found no relationship between mast cells and tumor invasion and did not regard them involved in the progression of stage BIII colon cancer. Such disagreement with the findings of this study could possibly be attributed to the dual function of mast cells, i.e. defending the body against tumors and promoting their growth at the same time (13).

Results of this study and those of previous ones show that conducting more research on the location of mast cell infiltration inside tumors, the types of mast cells and their granulation or degranulation, and stages of tumors, can provide more assistance in understanding the process of tumorigenesis, and the mechanism of action of these cells in various types of tumor.

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