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Silver Staining Efficiency in Differential Diagnosis of Adenoid Cystic Carcinoma (ACC) from Polymorpheus Low-Grade Adenocarcinoma (PLGA)

Mahbube Valipour Tahamtan[a], Shahrzad Shahbeik[b], Alireza Abdollahi[c], Gita Rezvani[d]

[a] Undergraduate Student, Dental School, Shahed University, Tehran, Iran.
[b] Postgraduate Student, Dental School, Shahed University, Tehran, Iran.
[c] Dept. of Pathology, Imam Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran.
[d] Dept. of Oral & Maxillofacial Pathology, Dental School, Shahed University, Tehran, Iran.
Correspondence to Gita Rezvani (e-mail: gitarez@yahoo.com).

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Objectives The aim of this study is finding a practical and easy way to differentiate, between two malignant tumors Adenoid Cystic Carcinoma (ACC) and Polymorphous Low-grade Adeno Carcinoma (PLGA) histologically. In this regard, We proposed silver nitrate staining (AgNOR).

Methods In this cross-sectional study, 30 parrafin-embedded blocks of ACC and 9 paraffin-embedded blocks of PLGA with the most acceptable standards elected and stained with silver nitrate. Then the number and quality (size and pattern) of the stained spots in 5 random microscopic fields at 100× magnification (at least 100 cells) were evaluated. To compare the number of dots between the two tumors T-test was used and the quality of dots, compared with Mann-Whitney U test.

Results The mean AgNOR counts for ACC (3/45), and for PLGA (2/45). Significant differences were observed in the number of dots between the ACC and PLGA (p=0.004), but in terms of quality, there was no statistically significant difference between the two tumors.

Conclusion AgNOR count can be useful as an available method in confirm the diagnosis of ACC from PLGA.

Keywords Adenoid Cystic Carcinoma (ACC), Adeno Carcinoma, Silver nitrate, Cell proliferation

Introduction

Salivary gland tumors are less pervasive compared to other body tumors and make up less than 3% of head and neck neoplasm, but they form a significant percentage of tumors of the mouth, jaw, and face. [1] These tumors have diverse morphologies sometimes making diagnosis difficult or even impossible in small biopsies. In such cases, precise clinical and pathological features are important and helpful in many cases, but there are cases requiring specific techniques for diagnosis.[2,3] Adenoid cystic carcinoma (ACC) and Polymorphous low-grade adenocarcinoma (PLGA) can be given as examples of these cases that are two adenocarcinoma with different prognosis that overlap in histological features.[4, 5]

ACC is one of the most pervasive and most recognized salivary gland malignancies described by Robin for the first time in 1853.[6] It is a brutal tumor with infiltrative nature and slow growth[1], prone to local recurrence, and eventually distant metastases and poor prognosis. The preferred treatment is usually surgical removal of the tumor, but in some cases, radiation can help improve patient survival.[7,8]

PLGA is a malignant, invasive and persistent tumor, but is slow in metastasis and is almost exclusive to salivary glands [(1)]. This tumor has been known since the early 1980s prior to its recognition as a distinct tumor. Its samples used to be classified as ACC and sometimes pleomorphic adenoma. However, it was found that the tumor has distinct clinical and pathological features and has recently emerged as a distinct malignant tumor of the salivary glands.[9, 10]

The best treatment for PLGA is wide excisional surgery sometimes involving resection of the bone. Unfortunately, local recurrence is not a rare phenomenon and 9 to 17% of the patients reported to have it up to 5 years after surgery, but usually with wide excisional surgery, it is successfully controlled in more than 50% of the cases.[1]

Similarity of histological features may cause confusion in the diagnosis of this tumor, particularly if the sample is a small biopsy of the minor salivary glands.[1, 11] The use of advanced diagnostic methods can help histopathology diagnosis of these two tumors, but most of these techniques are practically not used in the pathology laboratory due to the need for training of experts, expensive kits, and lack of access to kits. Silver staining is a rapid, simple, cost-effective, available, and a one-stage method used as a helpful way in Oral Pathology with H & E staining.[12, 13] Several studies have introduced this staining as a useful prognostic indicator as well as a method for diagnosis of premalignant and malignant lesions, and as an indicator to diagnose various histological degrees in malignant tumors indices.[14-17]

According to the examinations by researchers of this study, so far there have been no studies examining silver staining to differentiate between malignant tumor ACC and PLGA.[18] Therefore, the present study was conducted to introduce a simple and accessible method for differentiation between ACC and PLGA by using silver staining.

Materials and Methods

Forty paraffin-embedded blocks, which had proper fixation and texture and no edema, hemorrhage, and high necrosis were selected and 4µm thick sections were prepared and stained by silver nitrate (Merck, Germany) according to ploton method.[19] Breast cancer was considered as a positive control. Qualitative and quantitative evaluations were done by light microscopy (Olympus CHS model, Japan) in a double blind manner by two observers in areas with the lowest cell overlapping and stain deposition. Slides stained poorly were excluded and the remaining slides that contained 39 samples (30 ACC and 9 PLGA) were evaluated.

Quantitative assessment took place using standard methods Crocker [(9)], so that on each slide, 100 cells per smear in 5 random microscopic fields at 100 × magnification optical microscope were selected and the number of NOR (Nuclear Organizer Regions) was counted. Cells at the core which had one or more dots were selected and cells lacking these points were not counted. Accumulate points which was not distinguishable and the cases where the structure of the nucleus was stained rings, were considered as one point. Then the average Nucleolar organizer regions (NORs) in 100 cells were calculated per slide.

Qualitative assessment included the size and distribution points.[20]

The ranking of the dots size: 0 same size dots, +1 two different sizes, +2 three different sizes, +3 includes all sizes.

Scale distribution of dots: 0 Limited to the nucleolus, +1 rarely out of the nucleolus, +2 medium dispersion out of the nucleolus, +3 wide dispersion outside the nucleolus.[20]

To compare the number of stained points between the two tumors T-test was used and to check the size and pattern, non-parametric test Mann-Whitney U Test was used.

Results

Analysis of data was performed by two observers on 30 ACC samples and 9 samples of PLGA. Repeatability coefficient with respect to the number of Silver stainable nucleolar organizer regions (AgNORs) was of ICC type equal to 0.998 and in relation to the size and distribution was of Kappa type equal to 1.

In reviewing the quantitative results, according to table 1, AgNORs dots, mean, and SD of the number of nucleolar organized regions in ACC was 3.45±1.26 (Fig 1) and in PLGA 2.45±1.25 (Fig 2) that showed a statistically significant difference (P=0.004).

Table 1- The number of nuclear organization region in the ACC and PLGA

Lesion	Minimum Num spot	Maximum Num spot	Mean Num spot	P-value
ACC	1.00	7.00	3.45±1.26	0.004
PLGA	1.00	5.00	2.45±1.25	

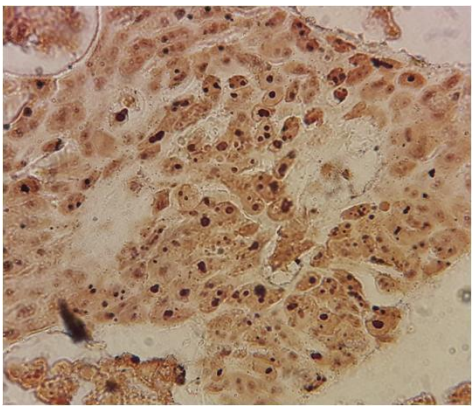

Figure 1- AgNORs dots in ACC, X100 magnification

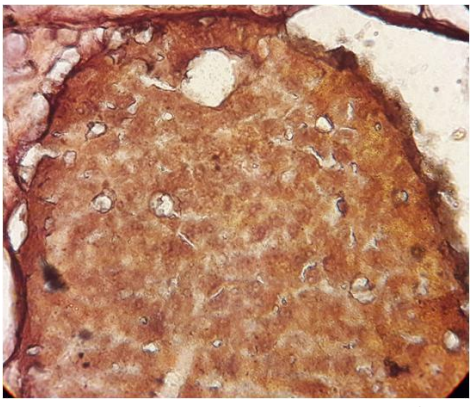

Figure 2- AgNORs dots in PLGA, X100 magnification

As is seen in tables 2 and 3, the size and distribution pattern of the points did not show statistically significant differences between ACC and PLGA (P=0.30 and P=0.42). The sensitivity and specificity of this test are 0.967 and 0.667 respectively.

Table 2-The size of nuclear organization region in ACC and PLGA

Lesion	Same Size	Two Different Size	Three Different Size	All Size	Total
ACC	1.7%	61.7%	36.7%	0.0%	100.0%
PLGA	0.0%	77.8%	22.2%	0.0%	100.0%
P-value			0.30		

Table 3- Distribution pattern of nuclear organization region in ACC and PLGA

Lesion	Limited to the nucleolus	Rarely out of the nucleolus	Medium out of the nucleolus	Wide dispersion out of the nucleolus	Total
ACC	0.0%	56.7%	43.3%	0.0%	100.0%
PLGA	0.0%	66.7%	33.3%	0.0%	100.0%
P-value			0.42		

Discussion

Histological similarity of ACC and PLGA tumors despite their different prognoses and biological processes calls for a method to help differentiate between tumor diagnosis[(18)]. Although the current known methods sometimes have high sensitivity, they require high cost and time. The results showed that the rate of cell proliferation in the ACC is significantly higher than in PLGA. Therefore, AgNOR staining technique can be suggested as a simple method available to differentiate between cases of suspected tumor before any sophisticated techniques. According to the study, what is reliable is the number of points stained while the quality of the stained points (size and distribution points) has little diagnostic value.

Numerous studies in the same field on a variety of tumors suggest that NORs in the nucleus of hyperplasic and malignant cell expresses their proliferative activity, so it is thought that NORs are a reflection of synthetic activity of hyper plastic cells and increase the speed of cell cycle and malignancies.[21-24] Thus, it seems that the higher presence of these cells in a tumor is along with its more aggressive behavior of the tumor. In other words, higher cell proliferation indicates higher growth rate, recurrence, and metastasis of the tumor. Since ACC compared with PLGA has more aggressive biological behavior, more presence of NOR's in the tumor relative to the PLGA is justified.

Silver staining and its diagnostic value have been used as indicators to determine the cellular proliferation and its relationship with the diagnosis of benign, premalignant, malignant, grade and prognosis of tumors in other parts of the body.[25-30]

One of the problems that existed in the present study was unstable staining of samples by AgNOR, which resulted from factors such as fixer solution, temperature, reaction time, concentration levels of silver and formic acid.[19] To fix this problem, in the study, Pluto modified method was used.[6, 31] *Yamamoto et al.* stated the number of AgNOR points in ACC solid pattern more than trabecular and screening, and proved that solid pattern that has the poorest prognosis is along with cellular proliferation. They concluded that AgNOR staining is a proper method to assess cell proliferation in different patterns ACC.[32]

Matsumura et al. (1989) proposed AgNOR staining as appropriate in differential diagnosis of benign and malignant tumors.[12] On the other, researchers found no relationship between AgNOR counts with prognosis and differentiation of malignant ACC tumors that could be due to the difference in the nature of the tumors examined, the lack of specimens and mismatch between them, or differences in the methodology of counting.[33] No statistically significant differences between the average number of AgNOR points and degrees of malignant neoplasm as well as the clinical course of malignant or benign masses in salivary glands.[15] They stated that AgNOR staining is useful in distinguishing benign from malignant, but not in distinguishing between histology types and degrees of malignant neoplasm of slivery glands and predicting prognosis.[14, 15]

Conclusion

The results showed that silver staining techniques could be used as an aid in the differential diagnosis of ACC and PLGA tumors. Since the technique used in this study is low cost, fast, easy and accessible, it could be an alternative to complex and expensive methods. It suggested that in future studies, AgNORs were assessed in ACC according to the histological pattern.

Conflict of Interests

None Declared ■

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