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The immunohistochemical expression Ki-67 In Polymorphous Low Grade Adenocarcinoma of the Salivary Glands

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Abstract

Background: Polymorphous Low Grade Adenocarcinoma is a rare malignant tumor of minor salivary glands. It has an unique histopathological, clinical and behavioural features. Most of its incidence has been presented in the palate. It shows a variety of growth patterns within the same one lesion, including cribriform, tubular, and solid areas that may mimic adenoid cystic carcinoma. The tumor cells histologically are characterized by lacking of nuclear atypical and hyperchromatism with rare mitoses. However, the prognosis of this tumor is good because of its low grade, although it has an unpredictable potential to metastasize.

Objectives: To evaluate the histopathological expression of Ki-67 proliferation antigen in Polymorphous low grade adenocarcinoma and compare the results with the clinicopathological parameters and grading of the tumors.

Materials and Methods: This study includes 20 cases with Polymorphous Low Grade Adenocarcinoma of minor salivary glands diagnosed histopathologically and treated surgically, were investigated for the immunohistochemical expression of Ki-67 antigen. Immunohistochemical staining was performed using a Labeled Strept- Avidin Biotin method (LSAB) and 0.05 (two-sided) was the level of significance.

Results: Twenty cases of Polymorphous Low Grade Adenocarcinoma of salivary glands were histologically diagnosed in this retrospective study to evaluate the expression of Ki-67. The mean age for patients with PLGA was nearly (50.6). (12) (60%) cases were males and (8) (40%) cases were females and also male to female ratio was (1.5:1).

In regarding to site distribution, the most common site was the palate followed by the submandibular gland and floor of the mouth.

Conclusions: it was observed that there is a higher rate of cellular proliferation in malignant tumors with high grade, indicating their role in the malignancy and aggressive behavior of these tumors.

Key words: Ki-67, Polymorphous Low Grade Adenocarcinoma, immunohistochemistry.

Introduction

Polymorphous low-grade adenocarcinoma (PLGA) is an uncommon epithelial malignant tumor which derived from reserve cells of salivary glands [1]. It is almost exclusively a malignant tumor of the

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minor salivary glands and the Palate is the most common site of this tumors followed by the buccal mucosa. The most common occurs predominantly during the sixth to eighth decades of life and has more ratio in female than male [2]. In 1990, the World Health Organization (WHO) stated that PLGA is a new entity of malignant salivary gland tumors [3]. PLGA frequently presents as an asymptomatic, slow-growing mass within the oral mucosa of the oral cavity and clinically its behavior similar to that of benign tumors. This will lead to delay in early diagnosis, so majority of PLGA cases are detected during routine oral diagnosis more than 30 years of age after onset of tumor as previously diagnosed [4]. There are various growth patterns of tumor cells such as cribriform or cordial, ductal, or solid patterns, thus the term "polymorphous" is applied to such malignancies [5]. Cell proliferation rate has been proposed as a useful adjunctive tool for detecting the progression of salivary gland tumors, but there is not a definitive agreement between authors on the most useful proliferative and prognostic marker for this type of tumor [6]. Because of the activity of proliferation is a reliable process to evaluate tumor biology, there has been many researches presented to find such biological markers [7]. Ki-67 is a widely accepted proliferating marker of tumor, with its immunostaining tightly associated with the cell cycle [8]. Ki67 is associated with cell proliferation and transcription of ribosomal RNA that is encoded by the gene MKI-67 [9]. The Ki-67 is used as a monitor marker for proliferation of cells and during inter-phase of cell cycle, the Ki-67 protein is appears in the cell nucleus. This proliferative protein is present in all active phases of the cell cycle but is not present resting cells [10].

Materials and methods

The retrospective study includes 20 cases with Polymorphous Low Grade Adenocarcinoma were randomly selected from the archives of oral pathology department, College of Dentistry, University of Baghdad for the period (1972 – 2006). The clinical and pathological information's such as age, gender, grade and lesion site were obtained from the patient files. The tissue blocks of tumor were fixed in formalin and embedded in paraffin wax were used in this study. In each block, one section was stained with hematoxylin and eosin for histopathological diagnosis and other section was prepared on adhesive slides for detection of Ki-67 antigen expression.

The immunohistochemical sections with malignant tumor tissues and hematoxylin-eosin slides were diagnosed by two pathologists and the grading of tumors were according to three growth patterns: cribriform, tubular, and solid. The tissue with paraffin blocks of cases were cut into pieces with 4 microns in thickness and dyed with immunohistochemical stains by using the method of anti-Ki - 67 (MIB-1 monoclonal antibody) labeled strepto-avidin-biotin. The positive controls had been done by using breast tissues and the negative controls by using some sections with non-immune serum. The scoring of all samples for Ki-67 immuno reactivity and Statistical analysis were performed by SPSS program (ANOVA and T test). When the p-value was <0.05, the results were considered significant. The score of intensity to estimate the immunostaining of Ki-67 was represented by four score such as (1, no staining; 2, weak; 3, moderate; 4, strong). The proportion score was done by estimated fraction of positively stained tumor cells with this marker (1

<10%; 2 = 10 to 50%; 3 = 50 to 90%; 4 ≥90%). The assessments of statistical significance of the associations were done by Chi-square ($\chi 2$) test of homogeneity.

Results

Twenty cases of Polymorphous Low Grade Adenocarcinoma of salivary glands were histologically diagnosed in this retrospective study to evaluate the expression of Ki-67. The ages for patients were divided into three groups; the first group < 40, the second group between (40−59) and the third group ≥ 60 years. The mean age for patients with PLGA was 50.6± 15.8. According to gender, (12) (60%) cases were males and (8) (40%) cases were females and also male to female ratio was (1.5:1) (Table 1).

With regards to growth pattern of tumor cells, twenty cases of PLGA with histological grading categorized into: (5) cases (25%) were grade (I), (7) cases (35%) were grade (II) and (8) cases (40%)

The immunohistochemical positive expressions with Ki-67 were 16 cases. The tumor cells were evaluated as Ki-67 positive when their nuclei are stain in brown color, with a diffuse granular pattern, but only (4) cases expressed Ki-67 immunonegative nuclei.

In both male and female, the grade III was the higher frequency 8 (40%), the grade I was the lowest frequency 5 (25%) and the grade II was 7 (35%) (Table 2). The grading was evaluated according to the histological growth pattern such as well, moderately and poorly differentiated tumor.

The immunohisochemical expression results of Ki-67 in Polymorphous Low Grade Adenocarcinoma were (10) cases (50%) with weak positive expression, (2) cases (10%) with moderate positive expression, (4) cases (20%) with strong positive expression and (4) cases (20%) with the negative expression. The relationship between Ki-67 immunostaining and the grade level of malignant tumor (P= 0.59) was non-significant that seemed to be not going together with the increase in proliferation of tumor cells (Figure 3).

Discussions

Salivary gland neoplasms comprise the most heterogeneous group of tumors of any body site, with frequent overlap of histological parameters and biological outcome [11].

The Polymorphous Low grade adenocarcinoma(PLGA) has clinical behavior differs from other malignant tumors of salivary glands because of its features were characterized by the rate of growth is slow, absence of symptoms, less aggressiveness, low metastatic potential and favorable prognosis[12]. Previously, the terms such as lobular carcinoma and terminal duct carcinoma were used for PLGA and it is associated with a slow growth and indolent biology [13]. It is generally an asymptomatic, slowly growing mass or swelling of the palate, cheek, or upper lip. Multiple synchronous occurrences have been reported [14].

Most of the tumors with PLGA will not lead to difficulty in diagnosis if careful attention is paid to some characteristic features of the tumor cells such as lack of pleomorphism and arrangement of the tumor cells. However, sometimes tumors may be diagnosed as an adenoid cystic carcinoma or a cellular mixed tumor. The growth patterns of tumor are different and lack of deep-staining tumor cells assist in diagnosis of PLGA and recognized it from adenoid cystic carcinoma, which has tubular, cribriform, and solid growth patterns. [15]. The rapid proliferation rate is one

of the features common to most neoplasms. Identification of a proliferating fraction within the tumor cell population has been useful in diagnosis and/or prognosis in a many of human cancers. Tumor markers indicative of cell cycle state has proven useful for determining growth patterns [16].

In the present study, Ki-67 protein was examined in breast cancer as a positive control tissue as well as in PLGA as malignant salivary gland tumors. All normal control sections of salivary gland tissues revealed negative immunoreactivity to Ki-67.

Polymorphous Low Grade Adenocarcinoma exhibited intense diffuse total cell immunepositivity in the almost all malignant cells, these findings are in agreement with Tadbir et al [17], and Trandafirescu et al, who stated that, Ki-67 is useful in assessing the intensity of Ki-67 in Salivary gland tumors and it gives indications on the risk of malignancy [18].

Microscopically, PLGA shows histopathologic features characteristic of many benign and malignant salivary glands neoplasms, particularly several overlapping histological patterns with pleomorphic adenoma (PA) and adenoid cystic carcinoma (ACC). It is difficult to confirm PLGA only by histopathology, so it is important to perform immunohistochemical analysis [19]. In previous studies, Simpson et al. (2002) reported two cases of PLGA with transformation to high-grade carcinoma, and found that the low-grade areas with the Ki-67 labeling index of 2% and 3% were typical, in stark contrast to 38% and 30% in the high-grade elements While, in present study 7 cases with high grade and 5 cases with low grade [20].

Several studies have shown the relationship between cell proliferation, and aggressive behavior or prognosis of tumors. Nordgard et al has studied the relationship between Ki-67 and the prognosis in 44 adenoid cystic carcinoma and showed that this marker has a role in determining the short-term prognosis [21]. In this study, due to unavailability of information associated with prognosis, survival and follow up of the patients, it was impossible to assess the relationship between Ki-67 marker and factors associated with prognosis.

Finally, the ki67/MIB1 index was thought either of overall utility in predicting the clinical behavior of SGT, or it was considered significant only when coupled to TUNELevaluation and p53 staining [22]. This was in-line with the reported association between the hyper-expression of the protein and the deregulation of the cell proliferation in malignant tumors of breast, tongue and prostate and in malignant melanoma, and was extremely significant in terms of statistical evaluation, besides tumor histotype, grade and stage at diagnosis [23].

In conclusion, in this study, it was observed that there is a higher rate of cellular proliferation in malignant tumors with high grade, indicating their role in the malignancy and aggressive behavior of these tumors.

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Recomendation

It is important to use large number of patients with this type of cancer and the results of other different studies should be correlated with our study to predict many other feathers of malignant cells.

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prostatic cancer. Histopathology 54: 580- 589, 2009.

Table (1): The frequency of Polymorphous Low Grade Adenocarcinoma according to age in both genders with percent.

Age group	Number of Male	Percentage	Number of Female	Percentage	Total	Percentage
(<40 **)**	3	25%	2	25%	5	45
(40-59)	4	33.3%	4	50%	8	35
(60+ **)**	5	41.7%	2	25%	7	20
Total	12	60%	8	40%	20	100
Mean+/-SD	52.6 +/-15.8					

F- test = [0.9] Non-significant

According to Site, Palate was the most affected site followed by the floor of the mouth and the submandibular glands (Figure 1).

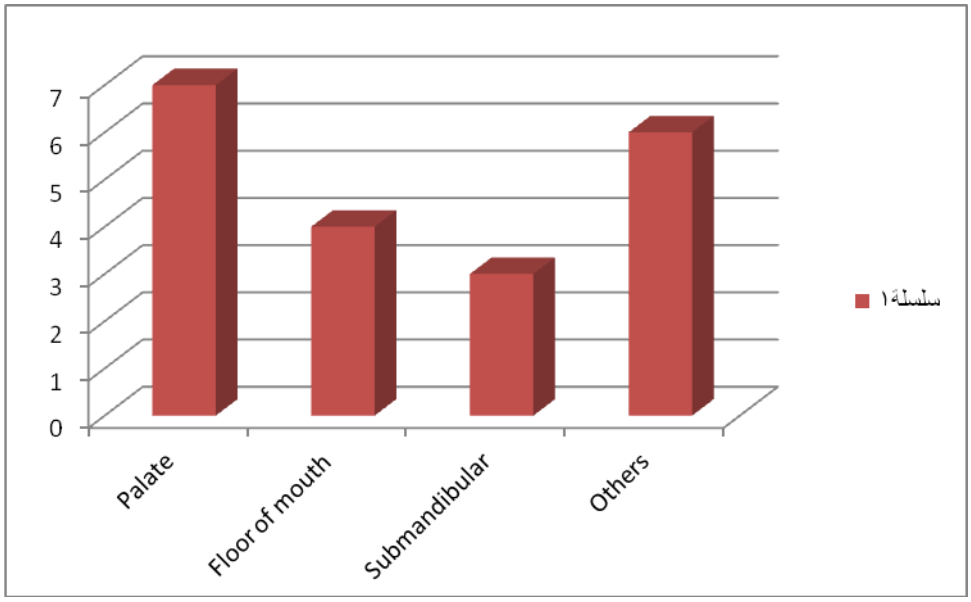

Figure 1: The incidence of Polymorphous Low Grade Adenocarcinoma according to oral site.

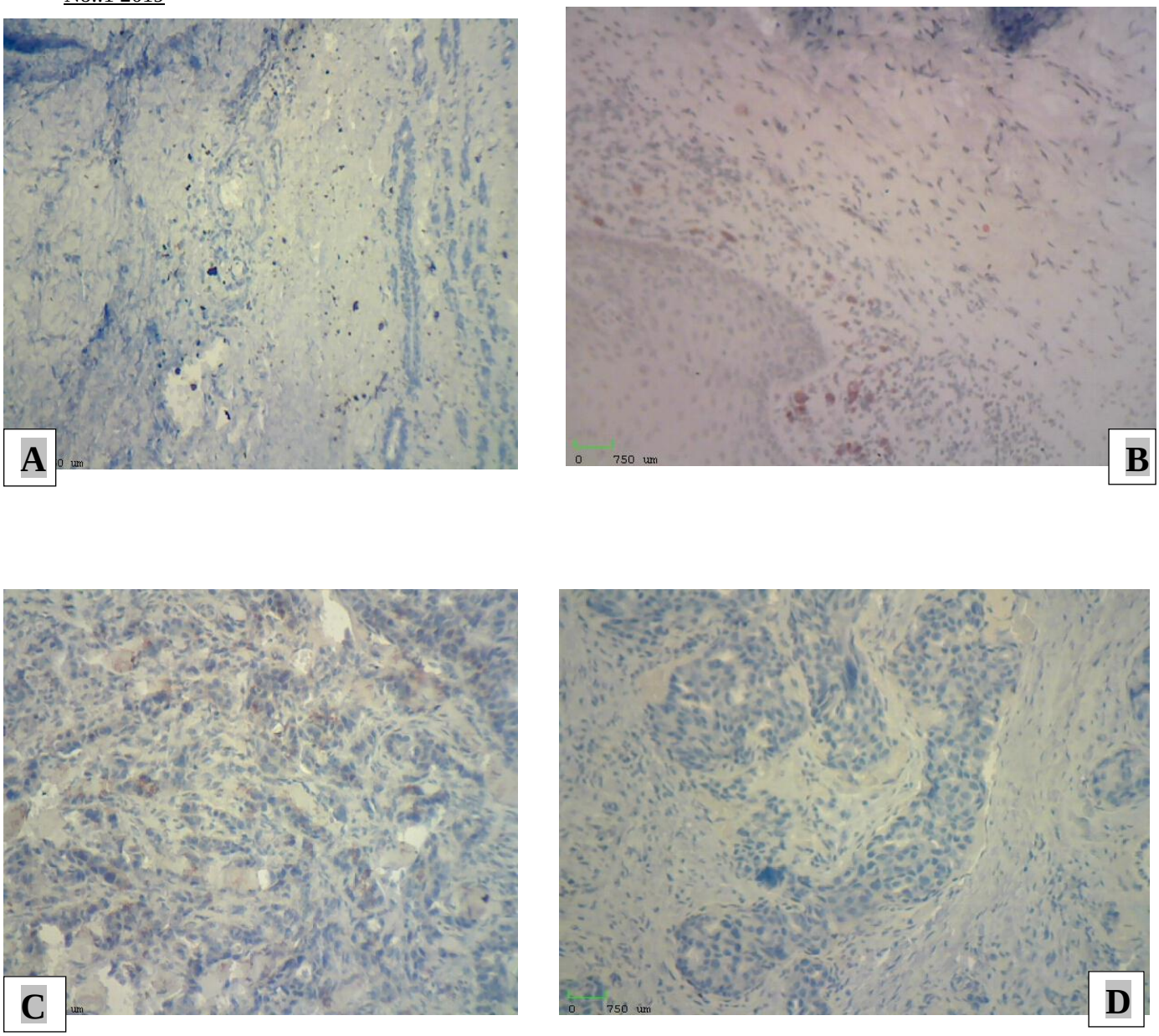

Figure (2): (A) The expression of Ki-67 in Polymorphous Low Grade Adenocarcinoma (poorly differentiated) (X20) , (B) The expression of Ki-67 in breast cancer (20x), (C) The expression of Ki-67 in Polymorphous Low Grade Adenocarcinoma (moderately differentiated) (X20), (D) The expression of Ki-67 in Polymorphous Low Grade Adenocarcinoma(well differentiated).

Table (2): The frequencies of grading in Polymorphous Low grade Adenocarcinoma according to gender.

Grade	Male		Female		Total	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
Grade I	3	25	2	25	5	25
Grade II	3	25	4	50	7	35
Grade III	6	50	2	25	8	40
Total	12	60	8	40	20	100 %