



# The Immunohistochemical Expression Ki-67 In Adenoid Cystic Carcinoma of The Salivary Glands

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## Abstract

Adenoid cystic carcinomas are rare malignant tumors of salivary glands. They are occurring between the middle and elderly aged patients. They account for up to 30% of minor salivary gland tumors but only for about 6 % of parotid gland tumors. The aims were to evaluate the hisopathological immunopositivity of Ki-67 in Adenoid Cystic Carcinoma and to compare the results with Clinic pathological parameters and histological grades by using immunohistochemistry. Twenty cases have been studied histologically of Adenoid cystic carcinomas were retrieved for the period (2001 – 2011). The correlations between the clinical and pathological parameters were statistically analyzed by Chi-square test and the level of significance was 0.05 (two-sided). The high positivity expression of Ki-67 is independent prognostic factors of poor overall survival in ACC. In addition, more aggressive and different treatment should be provided to patients who show high expression of this marker.

**Keywords:** Ki-67; Immunohistochemistry; Salivary Glands Cancer

## Introduction

Adenoid Cystic Carcinoma (ACC) is a rare and slow-growing epithelial malignant tumor of the salivary glands. It commonly occurs in minor salivary glands (30%) and nearly half of all oral ACC cases occur in the palate, so it comprises 5% to 10% of all salivary gland tumors [1]. In minor salivary glands, the incidence is equal in both sexes, but when it occurs in the submandibular glands, high frequent in women [2]. The progression of disease becomes more rapid once metastatic disease is present, and nearly within 2 years after developing metastases one third of patients die [3].

It shows three hisopathological patterns, cribriform, tubular and solid; the most common pattern is the cribriform pattern, in which the polygonal cells are small and uniform which are arranged in epithelial components and less frequently the malignant tumor shows a tubular or solid pattern. The tubular pattern is formed from different ducts that can be presented by one or two layers of cells which similar to the myoepithelial cells. The solid pattern is composed of solid epithelial islands with necrosis of the central areas; the cells are small, basophilic and hyperchromatic with a densely granulated nucleus and mitoses are rarely seen [4].

The immunohistochemical expression of Ki-67 in malignant tumor of salivary glands is considered as a marker for the proliferation rate of cells; the high frequency of metastasis and an unfavorable prognosis is usually related to an increased immunohistochemical expression of Ki-67 [5]. In immunohistochemical study, expression of Ki-67 is showed poor prognostic factor of ACC; however, the relationship between the survival of patients and the high immunohistochemical expression of this marker is not well-known [6]. Our study showed that the assessment of the immunohistochemical expression of Ki-67 in 20 cases of Adenoid Cystic Carcinoma, aiming to correlate between the proliferative rate and the clinic pathological parameters.

## Materials and Methods

All cases with adenoid cystic carcinoma that were studied and diagnosed histologically within (2001- 2011) and then they were randomly selected from the file sheets of the department of Oral pathology, College of Dentistry, Baghdad University, Iraq. The clinical and pathological informations such as age, gender and lesion site were obtained from the

patient files (chart). 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 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 immunoreactivity 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  $\geq$ 90%). The assessments of statistical significance of the associations were done by Chi-square ( $\chi^2$ ) test of homogeneity.

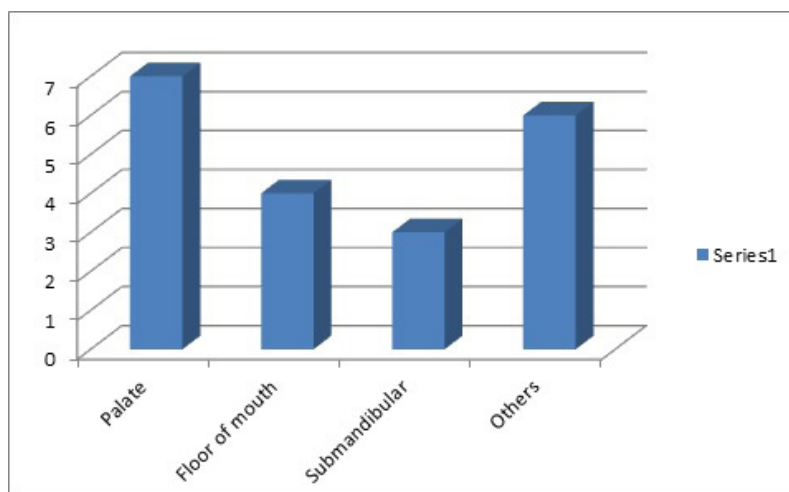
## Results

In this retrospective study, twenty cases of Adenoid Cystic Carcinoma of salivary glands were used to detect the Ki-67. The mean age for patients with ACC was (44.8 $\pm$ 12.5). According gender, (13) (65%) cases were males and (7) (35%) cases were females and also male to female ratio was (1.9: 1) (Table 1).

Age group	Number of Male	Percentage	Number of Female	Percentage	Total	Percentage	
(<40)	5	38.5	4	57.1	9	45	
(40-59)	5	38.5	2	28.6	7	35	
(60+)	3	23	1	14.3	4	20	
Total	13	65	7	35	20	100	
Mean $\pm$ -SD	44.8 $\pm$ 12.5						

**Table 1:** The incidence of Adenoid Cystic carcinoma according to age in both genders with percent

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

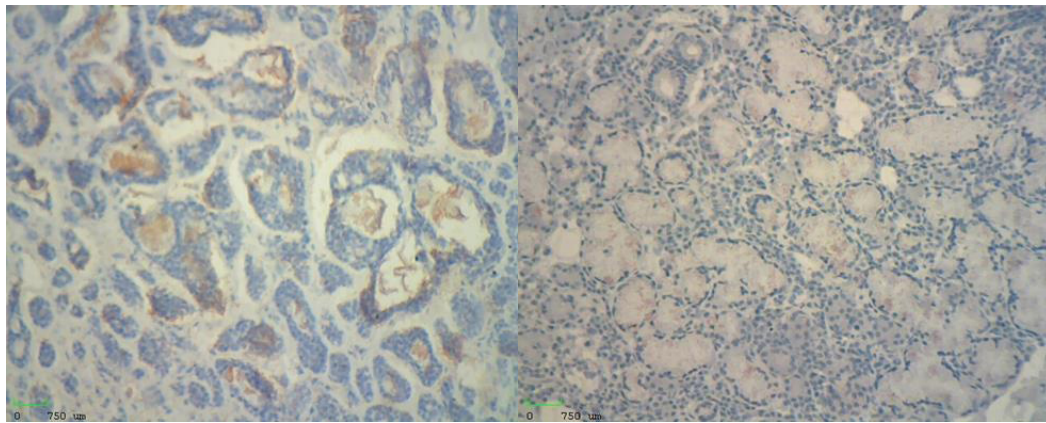


**Figure 1:** The difference in distribution of Adenoid Cystic carcinoma according to oral site

Twenty cases of ACC with histological grading categorized according to growth pattern into: (8) cases (40%) were grade (I), (9) cases (45%) were grade (II) and (3) cases (15%) were grade (III) (Figure 2).

From all cases of ACC, (17) cases expressed Ki-67 immunopositive cell. The tumor cells were evaluated as Ki-67 positive when their nuclei are stain in brown color, with a diffuse granular pattern, but (3) cases expressed Ki-67 immunonegative nuclei.

The higher frequency in both male and female was in grade I, 6 (46.2%) and 4 (57.1%) respectively, but the lower frequency was in grade III, 3 (23.1%) for male and no tumor of grade III was found in female (Table 2).



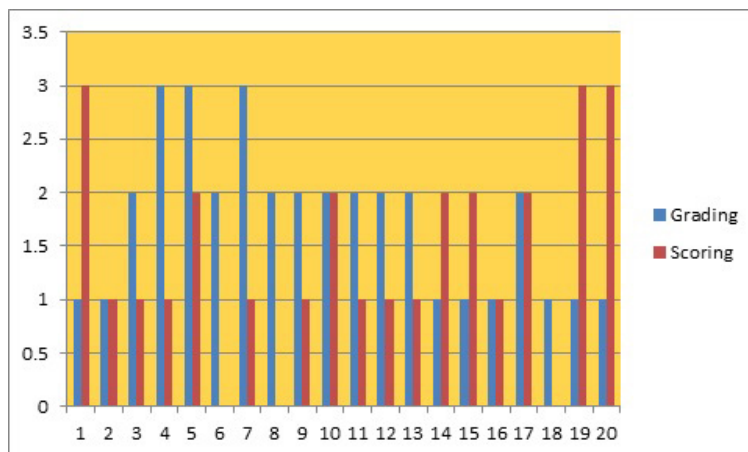
**Figure 2:** (A) The expression of Ki-67 in Adenoid cystic carcinoma (Cribriform type) (B) The expression of Ki-67 in Adenoid cystic carcinoma (Tubular type) (20X)

The grading was evaluated according to the histological growth pattern such as cribriform, tubular and solid pattern. The average percentage of Ki – 67 immunostain was 35% in both grade I and grade II, while in grade III was 15% (Table 2). The range of cells with positive stain was from 10% to 80%. In all of the positive stained samples, the mean percentage of Ki-67 immunoreactive cells was 33%.

The relationship between Ki-67 immunostaining and the grade level of malignant tumor (P= 0.83) was non-significant that seemed to be not going together with the increase in proliferation of tumor cells (Figure 3).

Grade	Male		Female		Total	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
Grade I	6	46.2	4	57.1	10	50
Grade II	4	30.7	3	42.9	7	35
Grade III	3	23.1	0	0	3	15
Total	13	65	7	35	20	100

**Table 2:** The frequencies of Adenoid cystic carcinoma grading in both Male and Female



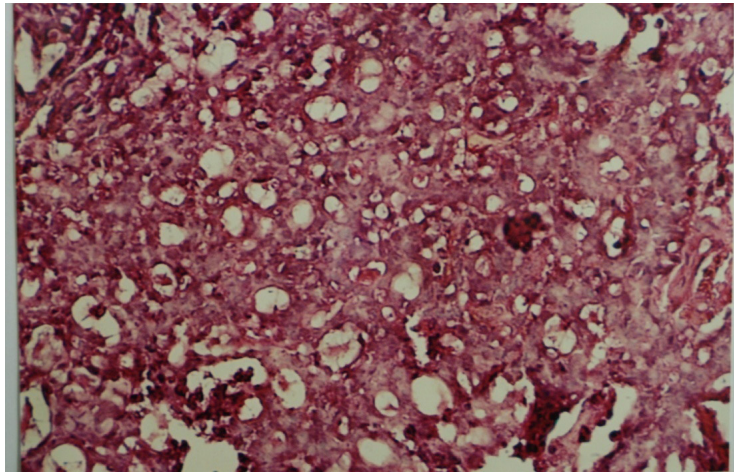
**Figure 3:** The relation between the Ki - 67 expression ratio of ACC and the grading of Adenoid cystic carcinoma

## Discussion

The aim of this study was to identify prognostic marker of Adenoid cystic carcinoma by evaluating the relationship between immunohistochemical expressions and clinic pathological data. ACC is extremely malignant rare tumor [7]. Its incidence may reach to up to 30% of minor salivary gland tumors and the palate is the most common site of this type of tumor [8]. According to age, ACC has a peak incidence in the fourth to sixth decades of life which comes in agreement with the results of other studies [9]. This may be defined as a result of prolonged exposure to chemicals and other predisposing factors of cancer that lead to development of cancer. The mean age of patients with Adenoid cystic carcinoma was 44 years that accordance with many other studies such as Triantafyllidou K et al., 2006 [1].

In regarding to gender, the frequency of male more than female; however, there are studies presenting a male predominance in ACC, but other studies presenting an equal gender distributions [10]. The neurological manifestations are a reflection

of the predilection of the tumor to infiltrate and spread along and around the nerves which are often prominent features and may be extensive. The tumor cells of Adenoid cystic carcinoma are two types: cells of the lining of the duct and cells of myoepithelial type [1] (Figure 4).



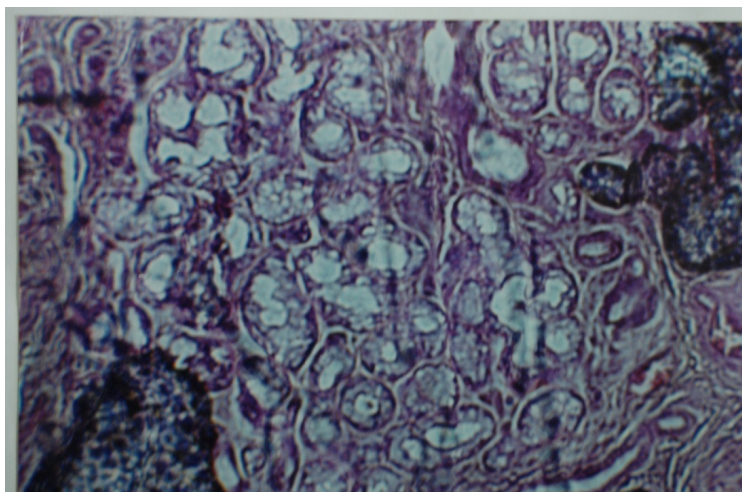
**Figure 4:** Adenoid cystic carcinoma, cribriform growth pattern. H & E stain, grade (I). (X20)

Histologically, ACC can be classified into three growth patterns: cribriform, tubular and solid or busload pattern. Most of these tumors have architecture of all these three patterns more than one pattern and it is then categorized according to the more predominant histological pattern [11]. In addition to grading, there are many other important histological features are presence of tumor at surgical margins of lesion, primary location [12]. According to grading, 40% of ACC cases were detected in grade I, 45% were detected in grade II and 15% in grade III. There is not any correlation between the gender and grade of tumors and also no relationship between the age and grade of tumors. In the most other malignancies, the degree of malignancy of tumors can be related to the activity of carcinoma cells [13].

MIB-1 index (the numerical percentage of Ki-67 antigen positive cells used for evaluation of proliferative activity of Ki-67 antigen. In this retrospective study, 17 cases (85%) were immunohistochemically positive for Ki-67 and 3 cases (15%) were negative. The immunoreactive cells of Ki-67 ratio were 10% to 80%. This range is different in many other studies such as Kiyoshima *et al.* stated that this range from 0.4% to 27.2% of their cases [14]. While, Carlinfante, *et al.* showed low labeling index Ki-67 in 42% of their cases ranged from 3% to 15% [15].

In other than Adenoid cystic carcinoma such as (mucoepidermoid carcinoma and acinic cell carcinoma) with the histological pattern of tumor are related to Ki-67 expression in malignant cells have been demonstrated, but this disagreement with this study between Ki-67 immunoreaction and grading of ACC [16]. In addition, other study stated results similar to our results such as: in 2001, Kiyoshima *et al.* did not find significant relationship between immunohistochemical expression of Ki-67 and morphological growth pattern or clinical course of the tumors [15].

While, Norberg *et al.* showed that the immunopositivity of Ki-67 antigen correlated strongly with tumor grade ( $p=0.053$ ), and stating ratio greater than 10% [17] (Figure 5).



**Figure 5:** Adenoid cystic carcinoma, tubular growth pattern. H & E stain, grade (II). (X10)

Many of articles revealed that Ki-67 expression in ACC most correlated with clinic pathological factors such as age, sex, site of primary tumor, and grading of tumor. It was seen that Ki-67 expression to have non prognostic significance with grading of tumor [15]. Some studies stated that this index is a reliable marker for distinguishing between benign and malignant tumors of salivary glands [18].

In conclusion, the high immunohistochemical expression scoring of Ki-67 is not related to prognostic factors of poor overall survival of patients with ACC. Therefore, more aggressive and differentiated treatment should be provided to patients who show high expression of these markers.

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## Recommendation

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 features of malignant cells.

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