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Assessment of the Relationship between Ki-67 Expression and Different Clinicopathological Factors of Adenoid Cystic Carcinoma: A Retrospective Immunohistochemical Study

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Abstract

Background: Adenoid cystic carcinoma (ACC) is an aggressive cancer that can affect many glands in the body, including the salivary glands, lacrimal glands, and upper digestive tract. Although slow growing, ACC has a poor prognosis due to its local invasion as well as elevated recurrence rates. Ki-67 is a widely utilized nuclear indicator, a type of protein found in nature, including cell proliferative immunomarkers. The exact role of Ki-67 remains unclear despite its importance as a predictive and prognostic biomarker. The present study aimed to assess the significance of Ki-67 expression in ACC lesions and their correlation with clinicopathological characteristics.

Methods and Results: Thirty-three histopathology-confirmed ACC cases were included in this study. The expression of Ki67 was evaluated by immunohistochemistry. The immunohistochemical staining revealed brown nuclear positivity of Ki-67 in 33 study samples. Of these, 15(45.5%) had a weak score (+1), 13(39.4%) - a moderate score (+2), and 5(15.2%) - a high score (+3). The distribution of Ki-67 expression according to the grading of ACC lesions was weak and moderately positive for low-grading cases, low positive for moderate-grading cases, and moderately positive for severe-grading cases, with no significant correlation. Regarding the tumor site, the peak incidence for weak, moderate, and high positive staining of Ki-67 expression was at the palate site, followed by the parotid gland and orbit sites, and then the others.

Conclusion: There is a statistical association between Ki-67 level and tumor location parameters. The elevated Ki-67 labeling value relates to poor prognosis.(**International Journal of Biomedicine. 2025;15(1):141-145.**)

Keywords: adenoid cystic carcinoma • Ki-67 • immunohistochemistry

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Introduction

Malignant salivary gland tumors are very uncommon and can have a variety of morphologies, making diagnosis exceedingly challenging.[1] Adenoid cystic carcinoma (ACC) is an aggressive cancer that can affect many glands in the body, including the salivary glands, lacrimal glands, and upper digestive tract.[2] In the head and neck area, ACC accounts for 1% of cancers and 10% of salivary gland tumors.[3] Although slow growing, ACC has a poor prognosis due to its local invasion as well as elevated recurrence rates.[3,4] The low long-term survival rate of ACC is associated with the inability to manage distant metastases, which are more common in the lungs.[5] Several salivary gland cancers with tubular and cribriform structures, including polymorphic adenocarcinoma, as well as tumors with basaloid cell morphology, like basal cell adenocarcinoma or basal cell adenoma, are included in the differential diagnosis of ACC.[6] In comparison to the hyperchromasia of basaloid cells observed in ACC, polymorphous adenocarcinoma has cuboidal or columnar cells with round and pale nuclei and eosinophilic cytoplasm. While basal cell adenocarcinoma is part of the possible diagnoses of ACC with solid dominance, basal cell adenoma can be distinguished by the existence of a capsule and the lack of epithelial and perineural infiltration. However, columnar periphery cells with hyperchromatic nuclei and the lack of noticeable mitotic patterns are characteristics that distinguish ACC apart.[6]

Histopathological assessment can offer important predictive data, particularly if the solid growth structure predominates over the tubular and cribriform structures, which are linked to less aggressive biological action.[7] Four histopathological grading systems have been suggested

to determine estimations and therapy strategies for those suffering from ACC, depending on the dominant pattern of those characteristics.[8]

A dual cell group of luminal/epithelial cells and basaloid cells with myoepithelial/basal development distinguishes ACC. These cells may histologically develop certain architectural characteristics that identify three pathogenic categories.[9] Islands of little or cuboidal basaloid epithelial cells with a basophilic nucleus and scant cytoplasm, as well as many pseudocystic spaces packed with a basophilic substance associated with glycosaminoglycans, are precisely described in the cribriform pattern. Several multiple layers of basaloid cells or one layer composed of epithelial cells surround the ductal structures in the tubular structure.[10]

Some of the earliest identified prognostic indicators were the solid growth of tumor cells or the higher percentage of solid regions. This histological standard has been demonstrated to help determine the survival rates for individuals with ACC.[7] The solid variant of ACC is thought to be the most serious and has a terrible prognosis.[7,11] Additionally, ACC, which has a solid element that makes up over a third of the tumor, has an inadequate clinical course.

Ki-67 is a widely utilized nuclear indicator, a type of protein found in nature, including cell proliferative immunomarkers. Except for G0, it is found in all dividing, proliferating cells throughout the active stages of the cell cycle. Using standard immunohistochemistry staining, its antibody (MIB-1) is utilized to identify cancerous cells. This antibody's basic function is to identify the nuclei of proliferating cells, making it an effective treatment for diagnosing and predicting the growth of cancerous cells.[12]

It is frequently used as a mitotic marker due to its production varying across the cell cycle and increasing during mitosis. There are currently no established threshold criteria, and the exact role of Ki-67 remains unclear despite its importance as a predictive and prognostic biomarker.[13]

The present study aimed to assess the significance of Ki-67 expression in ACC lesions and their correlation with clinicopathological characteristics.

Materials and Methods

Thirty-three cases of ACC were identified in the educational laboratory records at the medical city of the Ministry of Health. Formalin-fixed, paraffin-embedded, histopathology-confirmed biopsy specimens and appropriate individual clinical data (age, sex, and location) were obtained from the oral and maxillofacial reports. The lesions were graded based on the WHO guidelines, with mild, moderate, and severe grades. Two pathologists reviewed tissue sections stained with hematoxylin and eosin (H&E) to verify their diagnosis.

According to the product's datasheets, immuno-histochemical signal specificity was demonstrated by the absence of staining in negative control tissue samples and the appearance of a brown granular DAB dye structure in a certain cellular or tissue compartment for a particular antibody in positive control tissue slides. The nuclear positive staining for Ki-67 was assessed using a well-established quantitative scoring system ranging from negative to strong positive staining. The threshold for the absence of Ki-67 was established at a minimum of five tumors every 10 HPF. Positive expression was defined as low positive when 5%–19% of the cancerous cells were positive, moderately positive when 20%–49% were positive, and strongly positive when more than 50% of the cancerous cells were positive. However, we generated categorical variables like Ki-67 intensity using percentages and frequency values.

Statistical analysis was performed using the statistical software package SPSS version 19.0 (Chicago: SPSS Inc.). Baseline characteristics were summarized as frequencies and percentages. Inter-group comparisons were performed using chi-square tests or, alternatively, Fisher's exact test when expected cell counts were less than 5. A value of $P<0.05$ was considered significant.

Results

Age groups, gender, and other socioeconomic variables were dispersed, and correlations were shown to be essential to ensure that these two factors belonging to the subjects under study were spread randomly among their various classes. In agreement with the findings, the chance of ACC does not vary with age group distribution, and the influence of age diminished in the fifth decade with a mean age of 46.55±14.04. The age group distribution of the study samples revealed non-significant differences. Regarding gender, we also found non-significant differences (Table 1).

Table 1.

Demographic characteristics of study participants.

Variable	Class	No.	%	*P*-value
Age Groups	(20-29)	4	12.1	0.462
	(30-39)	7	21.2	
	(40-49)	7	21.2	
	(50-59)	8	24.2	
	(60-69)	5	15.2	
	(70-79)	2	6.1	
	Mean ± SD	46.55 ± 14.04		
Gender	Male	18	54.5	0.602
	Female	15	45.5	

For significant comparisons, we presented the probability distribution of location and grade data for individuals with ACC. These two factors for the people during the study were randomly distributed among different classes. The sample distribution of participants according to the tumor site demonstrated non-significant variations. The probability distribution of the disorders under research presented a considerable proportion of individuals with the maxilla site (27.3%), then parotid and orbit sites (18.2%), palate sites (15.2%), and floor of the mouth and tongue areas (12.1% and

9.1%, respectively). Individuals in the research showed non-significant differences in tumor grade: mild (51.5%), moderate (27.3%), and severe (21.2%) (Table 2).

Table 2.

Distribution of tumor site and grade among individuals with ACC.

Variable	Class	No.	%	*P*-value
Site	Palate	5	15.2	0.563
	Parotid	6	18.2	
	Maxilla	9	27.3	
	Orbit	6	18.2	
	Floor of mouth	4	12.1	
	Tongue	3	9.1	
Grade	Mild	17	51.5	0.078
	Moderate	9	27.3	
	Sever	7	21.2	
	Total	33	100	

The immunohistochemical staining revealed brown nuclear positivity of Ki-67 in 33 study samples. Of these, 15(45.5%) had a weak score (+1), 13(39.4%) - a moderate score (+2), and 5(15.2%) - a high score (+3) (Figure 1).

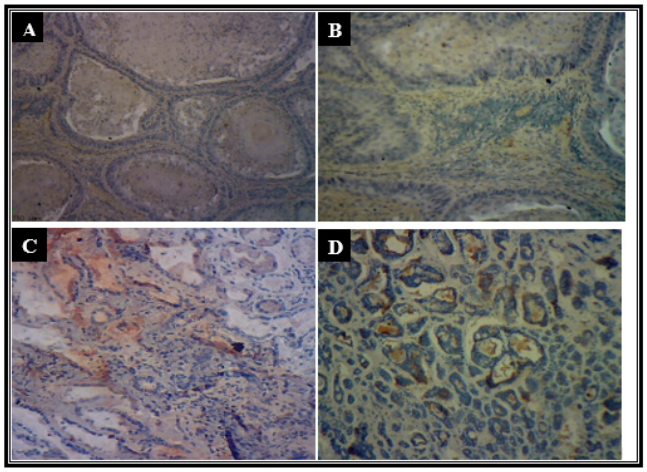

Fig. 1. *Positive nuclear immunohistochemical expression of Ki-67 in ACC*
A. Low staining, magnification 40X; B. Moderate staining, magnification 40X; C. High staining, magnification 20X; D. High staining, magnification 20X.

The distribution of Ki-67 expression according to the grading of ACC lesions was weak and moderately positive for low-grading cases, low positive for moderate-grading cases, and moderately positive for severe-grading cases, with no significant correlation (Figure 2).

Regarding the tumor site, the peak incidence for weak, moderate, and high positive staining of Ki-67 expression was at the palate site, followed by the parotid gland and orbit sites, and then the others (Figure 3).

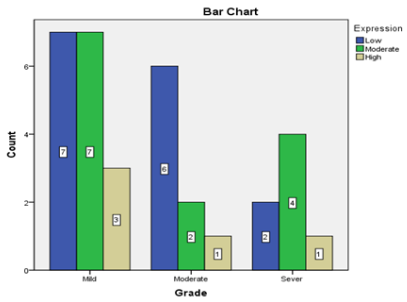

Fig. 2. *The bar chart shows the distribution of Ki-67 expression according to the grading of ACC.*

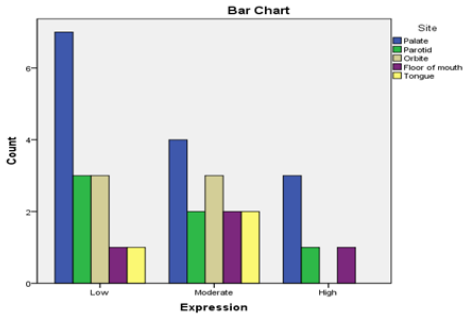

Fig. 3. *The bar chart shows the distribution of Ki-67 expression according to the ACC site.*

Discussion

ACC occurs mainly in secretory glands, particularly the salivary glands, and accounts for around 1% of all types of head and neck cancers. Despite their generally slow development, ACCs frequently have metastatic spread and local recurrence, which worsens the prognosis during a prolonged period.[15]

The variable shape of salivary gland cancer makes diagnosis challenging, yet specificity is essential for effective therapy. The shape of cells and cell division are necessary for the final diagnosis of cancers of ACC. Even while stromal alterations, neural spread, and the structure and development pattern of the tumor restrictions are important aspects of histological diagnosis, these tumors can nevertheless provide diagnostic challenges in some situations. Immunohistochemistry can be employed when it is required to evaluate the type of cells, differentiation, and growth. One of the fundamental biological processes in carcinogenesis is the proliferation of cells.[16]

The mean age of patients in the present study was 46.55 with a range of 20-77. This finding differed from the findings of two other studies,[17,18] which stated that the mean age of their patients was 58.8 and 61, respectively. Regional and ethnic disparities and the small size of the study sample might explain these differences in mean age.

According to gender, our study revealed that the men-to-women ratio was 1.2:1, while in most other studies, the ratio was 1:1 or showed women more predominant,[19] which may be due to hormonal features.

ACC has been reported to exhibit three distinct histopathological structures: tubular, cribriform, and solid. Simple tubules made up of border myoepithelial and interior ductal cells define the tubular structure. Myoepithelial cells with myxoid or hyalinized particles and isolated solitary ductal components comprise most of the cribriform pattern. The solid pattern is characterized by solid nests made of layers of basaloid cells. In typical ACC, a mixture of tubular and cribriform patterns is frequently seen. A poorer prognosis and a more extensive tumor stage have been linked to solid structures.[20]

The majority of the ACC specimens (51.5%) of this immunohistochemical study were in low grade, while the smallest instances (21.2%) were in high grade, based on tumor grading. This outcome was consistent with prior research.(20) In contrast, several researchers had different results from the current study's conclusion that most ACC cases in our group are high grade.[21,22] According to the present research, there is a non-significant association between the tumor grade and Ki-67 expression.

According to the tumor site, the findings of our study showed that the most frequent location was in the minor salivary gland of the palate for mild, moderate, and high immunostaining Ki-67 expression, followed by the parotid gland and the other sites. This result follows many other research studies.[23,24] However, it disagreed with several studies stating that the parotid and sublingual glands were the most common sites.[25,26]

In conclusion, Ki-67 displayed almost mild positive expression in most ACC patients, indicating that it may play a role in the occurrence of ACC cases. There is a statistical association between Ki-67 level and tumor location parameters. The elevated Ki-67 labeling value relates to poor prognosis.

Competing Interests

The authors declare that they have no competing interests.

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