

## Immunohistochemical Assessment of Ki-67 Expression in Adenoid Cystic Carcinoma of the Salivary Glands

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### Abstract

#### Objective

Adenoid cystic carcinoma (ACC) is a rare malignant tumor originating from the salivary glands, the rather bland histological appearance of which masks its ultimate biological aggressiveness. Evaluation of cell cycle and mitoses has been useful in predicting malignancy in many tumors. Ki-67 antigen is a human nuclear antigen that appears in all active phases of cell cycle. The study has been planned to find out (any kind of) relationships between Ki-67 expression ratio and the morphological growth pattern and the histological grade of the tumor.

#### Materials and Methods

Tissue samples of 19 ACC, were selected from the files in the archive of the Oral Pathology Department, of Mashhad University. All samples were picked up minor salivary glands including 11 men and 8 women with an average age of 46. One section was stained with H&E to confirm the diagnosis and the other with Ki - 67 monoclonal antibody. All samples were graded and scored for Ki-67 immunoreactivity, then the ratio of Ki-67 positive cells was calculated.

#### Results

The incidence of tumor was higher in 4<sup>th</sup> and 5<sup>th</sup> decades of age, particularly in women. The most common site of tumors was palate. Ki-67 expressed in 68% of all samples. The Ki-67 immunoreactivity ranged from 15% to 85%. Although the average percentage of Ki-67 expression seemed to increase with histological grade, the difference between grade III and grade I, and between grade III and mixed I / II was not statistically significant (p value = 0.3).

#### Conclusion

For ACC, Ki-67 immunostaining, regarding to histological grading, was not a reliable tool in predicting the intensity of tumor aggressiveness and seemed to have less value. Further studies with greater series of samples are needed to confirm this issue.

**Keywords:** Adenoid cystic carcinoma, Immunohistochemistry, Ki-67, MIB-1, Salivary gland.

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**Introduction**

Adenoid cystic carcinoma (ACC) is an almost rare and slow-growing epithelial tumor of the salivary glands. It comprises 5% to 10% of all salivary gland tumors. It is mostly located in minor salivary glands (31%). Nearly half of all intra oral ACC occurs in the palate (1). ACC may occur at any age, although most patients are middle aged or older (2). The peak incidence is in the fifth and sixth decades (1).

It is seen more frequently in women, when it occurs in the submandibular glands; but occurs equally in men and women, when found in minor salivary glands (2). Bill Roth was the first to describe ACC in 1856(3). Seifert describes ACC as an infiltrative malignant tumor having various histological features with three growth patterns: glandular (cribriform), tubular, and solid.

MIB-1, a monoclonal antibody that points at Ki-67 antigen in formalin-fixed, paraffin-embedded tissues was used to determine proliferative activity of tumors.

The purpose of this study was to evaluate the relationship between Ki - 67 expression morphological growth pattern and histological grade in ACC.

**Materials and Methods**

All patients with adenoid cystic carcinoma that were diagnosed within 1345-1384 (30 years) were selected from the archive of Oral Pathology Department of Mashhad Dentistry School in Iran. Histological sections of cancerous tissues and hematoxylin-eosine slides were reviewed by two pathologists and were graded

according to three growth patterns: cribriform, tubular, and solid.

The paraffin blocks of cases were cut into pieces with 4 microns thickness and dyed with immunohistochemical stains with using anti Ki - 67 (MIB-1 monoclonal antibody) labeled strepto-avidin-biotin method. Tonsil lymphoid tissues were used for positive controls and some sections with non-immune serum for negative controls. Slides were separately observed by two pathologists. All samples were scored for Ki-67 immunoreactivity.

The number of positive-stained cells were counted in 500 cells at × 400 magnification and the percentage of positive cells was calculated.

Statistical analysis was performed by SPSS program (ANOVA and T test). The results were considered significant when the p-value was <0.05.

**Results**

Patients included 11 men (57.9%) and 8 women (42.1%). Female to male ratio was 1:1.3. The average age of patients in this study was 49 years (ranged 19-80 years) in males and 42.5 years (ranged 30-65 years) in females. 52.62% of patients were in 4<sup>th</sup> and 5<sup>th</sup> decades of age.

All tumors originated from minor salivary glands, 11 (58%) were tumors located in palate, 2 in buccal mucosa, 2 in retromolar region and 4 tumors in nose, tuber region, sinus and mandibular angle (Table 1).

Table 1. Distribution of tumor location in patients with ACC (n=19).

Tumor site	Frequency	Percentage
Major salivary gland	0	0
Minor salivary gland, Palate	11	58
Buccal mucosa	2	11
Retro molar region	2	11
Sinus	1	5
Maxillary tuber region	1	5
Nose	1	5
Mandibular angle	1	5

Histologically, 13 (68%) tumors were classified in grade I, 4 (21%) of them in

grade II and 10.5% (2) in grade III. Of lesions of grade I and grade II, 62% and

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75% of tumors in grade I and II occurred in males. No tumor of grade III was found in men. Thus only in grade III, lesions were predominant in females. The relationship

between grade and gender is shown in Table 2. The mean age was 47.5 years in grade I, 39 years in grade II and 52.5 years in grade III.

Table 2. Frequency of grading according to gender.

Grade	Frequency	Percentage	Female		Male	
			frequency	percentage	frequency	percentage
I	13	68.6	5	26.3	8	42.1
II	4	20.9	1	5.3	3	15.8
III	2	10.5	1	10.5	0	0
	19	100	8	42.1	11	57.9

Average existence duration of lesion was 24 months (1 month to 8 years), 26 months in lesions of grade I, 30 months in grade II and 2 months in grade III. 13 (68.4%) tumors and 6 (31.6%) tumors revealed negative results. The range of positive-stained cells was from 15% to 85%. The mean percentage of Ki-67 immunoreactive cells was 38.84% in all of the positive samples.

Grading was carried out on the basis of histological growth pattern according to cribriform, tubular formation and solid pattern. The average percentage of Ki - 67 expression was 24. 23% in grade I, 26.25% in grade II and 42.5% in grade III (Table 3 and Figure 1,2). Histopathological data and Ki - 67 expression ratio of tumors are shown in Table 4.

Table 3. Correlation between grade and Ki-67 expression.

Grade	Frequency	Average of Ki-67 expression	Standard deviation	Standard error	Range of Expression
I	13	24.23%	26.13	7.25	0-85%
II	4	26.25%	22.87	11.43	0-55%
III	2	42.5%	24.75	17.5	25-60%

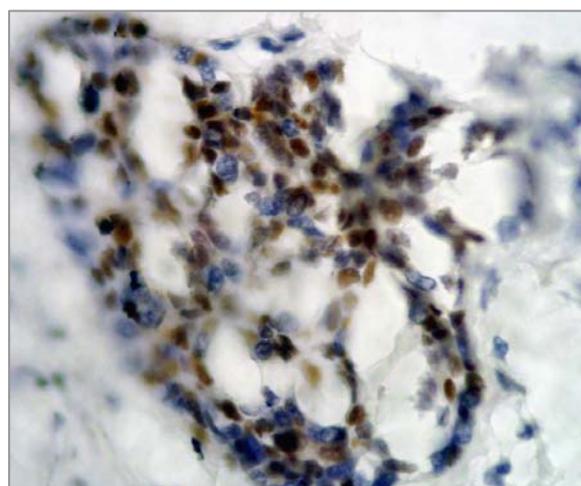


Figure 1. Ki - 67 immuno reactivity in nuclei of tumoral cells (grade I).

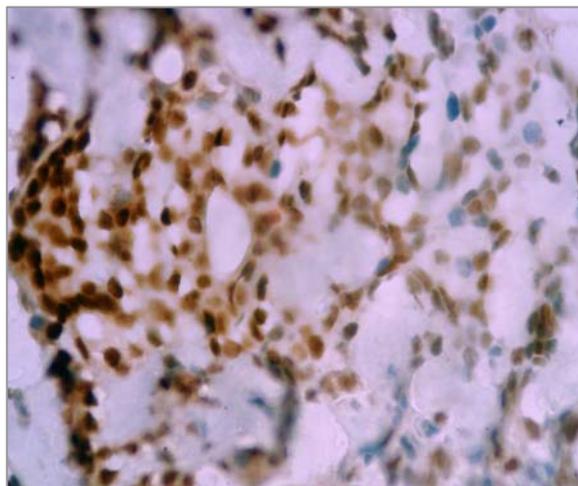


Figure 2. Ki - 67 immuno reactivity in nuclei of tumoral cells (grade II).

Table 4. Histopathological characteristics and Ki-67 expression ratio.

Tumor no	Prominent microscopic pattern	Grade	Ki-67
1	Tubular	I	15%
2	Tubular	I	--
3	Mixed	II	-
4	Solid	III	60%
5	Mixed	II	20%
6	Tubular	I	25%
7	Tubular	I	40%
8	Tubular	I	30%
9	Mixed	II	55%
10	Tubular	I	40%
11	Tubular	I	25%
12	Mixed	II	30%
13	Solid	III	25%
14	Cribriform	I	-
15	Tubular	I	-
16	Cribriform	I	55%
17	Tubular	I	-
18	Tubular	I	85%
19	Tubular	I	-

## Discussion

Adenoid cystic carcinoma is a rare tumor accounting for 2% to 4% of all head and neck malignancies (1). It most commonly occurs in minor salivary glands, and palate is the most common site ( $\geq 50\%$  of cases) (1-3). In this study 58% of tumors occurred in palate. In most studies a 1:1.3 male to female ratio was seen, which showed female predominance for ACC and other salivary gland tumors (3). However, there are studies reporting a male predominance in ACC (4), and others reporting an equal gender distribution (5,6). Male to female ratio in this study was 1:0.73 with male predominance. The mean age of patients was 46 years and 53% of them were in 4<sup>th</sup> and 5<sup>th</sup> decades. This finding correlated with other references (1,2,7). Tumor in most patients (18 of 19 cases) presented as swelling with or without pain. That was the same as the previous report (2).

Microscopically, the tumor cells are two types: duct-lining cells and cells of myoepithelial type. Perineural or perivascular spread without stromal reaction is a characteristic feature (1). Szanto, *et al.*

described three histologic grades for ACC: Grade I, tumors with tubular and cribriform areas but without solid components; Grade II, cribriform tumors that were either pure or mixed with less than 30% of solid areas; Grade III, tumors with a predominantly solid pattern (8). Histologically, tubular pattern (58% of cases) was the most common pattern, despite the references indicating predominance of cribriform pattern with incidence of 50% (7,9).

In addition to grading, other important prognostic features are primary location, presence or absence of tumor at surgical margins, and the anatomic structures it involves (8). Another study revealed that stage (especially size) of the lesion is one of the most important prognostic factors (10).

68% of tumors in this study were detected in grade I, 21% in grade II and 11% in grade III. We did not find any correlation between gender and grade or age and grade. In many of tumors the proportions of cycling tumor cells are important in predicting outcome and have prognostic significance.

In most malignancies, the activity of carcinoma cells is generally considered to be

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related to the degree of malignancy (11). MIB-1 index (the numerical percentage of Ki-67 antigen positive cells) is one of the tools that can be used for evaluation of this proliferative activity. In this study 13 tumors (68.4%) were immunohistochemically positive for Ki-67 and 6 tumors (31.6%) were negative. The Ki-67 immunoreactive cells ratio was 15% to 85%. This range is variable in different studies. In Norberg *et al.* study the proportions of cycling tumor cells differed from 0.3 to 55% (12). Carlinfante, *et al.* found low labeling index Ki-67 in 42% of their cases ranged from 3% to 15% (13). Kiyoshima *et al.* found this range to be from 0.4% to 27.2% (14).

We observed Ki-67 immunoreactivity in 61% of grade I, 75% of grade II and 100% of grade III, lesions. The mean percentage of Ki-67 expression in all samples was 26.58%. The mean percentage of this expression was 24.23%, 26.25% and 42.5% in grade I, grade II, and grade III, respectively. Although Ki-67 expression ratio seems to increase with the increase of grade, but statistical analysis revealed no significant relationship between Ki-67 expression ratio and grade (p value= 0.6). Furthermore, t- test showed no significant difference between Ki-67 expression ratio between grade I and grade III (p value= 0.37) and also grade I, II and grade III (p value= 0.34). ANOVA test showed immunoreactive cell ratio is not affected by histological grade of ACC.

Relationship between Ki-67 expression, histological pattern and clinical course in malignant salivary gland tumors (including mucoepidermoid carcinoma and acinic cell carcinoma) has been demonstrated (14-16), but

this agreement is not seen between Ki-67 immunoreaction and grading of ACC. For example Kiyoshima *et al.* in 2001, did not find significant relationship between immunostaining ratio of Ki-67 and morphological growth pattern or clinical course (14).

Carlinfante *et al.* also found no relation of this marker to histologic type, clinical staging and survival (13). In contrast, in study of Norberg *et al.* cycling tumor cell population (Ki-67 positive) correlated strongly with tumor grade (p= 0.053), and labeling ratio greater than 10% indicated more aggressive tumors (12). Similar finding was observed by Triantafillidou *et al.* (1) and Giannoni *et al.* (17).

Review of articles indicated that Ki-67 expression in ACC most correlated with clinical factors such as failure of treatment, site of primary tumor, and involved surgical margins. It was seen that Ki-67 expression to have prognostic significance but its relationship with grading has not been agreed on (14,18). Some investigators claimed that this index is a reliable marker for discriminating between benign and malignant tumors of salivary glands (19).

## Conclusion

This study examined the immunohistochemical expression of Ki-67 antigen in the ACC arising from salivary glands, and showed there was no significant relationship between Ki-67 expression ratio and grade of tumor. However, further examinations with larger sample series are needed to confirm this issue.

## References

1. Triantafillidou K, Dimitrakopoulos J, Iordanidis F, Koufogiannis D. Management of adenoid cystic carcinoma of minor salivary glands. *J Oral Maxillofac Surg* 2006; 64: 1114-1120.
2. Gnepp DR. Diagnostic surgical pathology of the head and neck. Philadelphia: WB Saunders. 2001; 379.
3. Rapidis AD, Givalos N, Gakiopoulou H, Faratzis G, Stavrianos SD, Vilos GA, *et al.* Adenoid cystic carcinoma of the head and neck, Clinicopathological analysis of 23 patients and review of the literature. *Oral Oncolog* 2005; 41: 328-335.
4. Stallmach I, Zenklusen P, Komminoth P. Loss of heterozygosity at chromosome 6q23-35 correlates with clinical and histological parameters in salivary glands adenoid cystic carcinoma. *Virchows Arch* 2002; 440: 77-84.

5. Van Der, Waal JE, Becking AG, Snow GB, Van Der Waal I. Distant metastases of adenoid cystic carcinoma of the salivary glands and the value of diagnostic examinations during follow-up. *Head Neck* 2002; 779-83.
6. Konkemueller H, Eckardt A, Brachvoget P, Hausamen JE. Adenoid cystic carcinoma of the head and neck a 20 years experience. *Int J Oral Maxillofac Surg* 2004; 33: 25-31.
7. Neville BW, Damm DD, Allen CM, Bouquot JE. *Oral and maxillofacial pathology*. Philadelphia: WB Saunders; 2002. 406-30.
8. Szanto PA, Luna MA, Tortoledo ME, White RA. Histologic grading of adenoid cystic carcinoma of the salivary glands. *Cancer* 1984; 54: 1062-9.
9. Mils SE, Stenberg S. *Diagnostic surgical pathology*. Philadelphia: Lippincott Williams and Wilkins. 2004; 946-947.
10. Santucci M, Bondi R. Histologic-prognostic correlations in adenoid cystic carcinoma of major and minor salivary glands of oral cavity. *Tumori J* 1986; 72: 293-300.
11. Hideo K, Min Z, Shinobu M, Yoshihiro Y, Toshiko T, Taiki T, *et al*. The relationship of the histologic grade at the deep invasive front and the expression of Ki-67 antigen and p53 protein in oral squamous cell carcinoma. *J Oral Pathol Med* 2005; 34: 602-7.
12. Norberg-Spaak L, Dardick I, Ledin T. Adenoid cystic carcinoma: use of cell proliferation, BCL-2 expression, histologic grade and clinical stage as predictors of clinical outcome. *Head Neck* 2000; 22: 489-97.
13. Carlinfante G, Lazzaretti M, Ferrari S, Bianchi B, Crafa P. P53, bcl-2 and Ki-67 expression in adenoid cystic carcinoma of the palate. A clinico-pathologic study of 21 cases with long-term follow-up. *Pathol Res Pract* 2005; 200: 791-9.
14. Kiyoshima T, Shima K, Kobayashi I, Matsuo K, Okamora K, Komatsu S, *et al*. Expression of P53 tumor suppressor gene in adenoid cystic and salivary glands. *Oral Oncology* 2001; 37: 315-322.
15. Vacchi Suzi M, Alessi A, *et al*. Prognostic relevance of cell proliferation in major salivary gland carcinoma. *Acta Otorhinolaryngol Ital* 2005; 25:161-68.
16. Skalova A, Lehtonen H, Boguslawsky K, Leivo I. Prognostic significance of cell proliferation in mucoepidermoid carcinoma of the salivary gland. *Hum Pathol* 1994; 25: 929-35.
17. Giannoni C, El-Naggar AK, Ordonez NG, Tu ZN, Austin J, Luna MA, Batsakis JG. C-erbB-2/neu oncogene and Ki-67 analysis in the assessment of palatal salivary gland neoplasms. *Otolaryngol Head Neck Surg* 1995; 112: 391-8.
18. Nordgard S, Franzen G, Boysen M, Halvorsen TB. Ki-67 as a prognostic marker in adenoid cystic carcinoma assessed with the monoclonal antibody MIB1 in paraffin section. *Laryngoscope* 1997; 107: 531-6.
19. Zhu Q, Tipoe GL, White FH. Proliferative activity as detected by immunostaining with Ki-67 and proliferating cell nuclear antigen in benign and malignant epithelial lesions of the human parotid gland. *Anal Quant Cytol Histol* 1999; 21: 336-42.