

Immuno Histochemical Expression of Ki-67 in Adenoid Cystic Carcinoma of Salivary Gland Tumors

Expression of Ki-67 in Adenoid Cystic Carcinoma of Salivary Gland

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ABSTRACT

Objective: To determine expression of Ki-67 in Adenoid cystic carcinoma of salivary glands.

Study Design: Descriptive study.

Place and Duration of Study: This study was conducted at the Department of Oral Pathology, de'Montmorency College of Dentistry, Lahore from May 2014 to December 2016.

Materials and Methods: Thirty two cases of Adenoid cystic carcinoma (ADCC) of salivary glands were selected from Departments of Surgery, Lahore General Hospital, Mayo Hospital, and de'Montmorency College of Dentistry, Lahore. Slides were prepared by routine hematoxylin and eosin (H & E) staining, as well as by Immunohistochemistry (IHC) for Ki-67. Grading of ADCC was done as low, intermediate and high grades on H&E sections. Scoring of Ki-67 expression was determined on Ki-67 immunohistochemical stained slides. Data was entered into SPSS version 22 and descriptive statistics were determined.

Results: Out of 32 cases of Adenoid cystic carcinomas 68.1% (22) reported in female and remaining in male (10) 31.2%. Total cases of intermediate grade ADCCs were 43.8%, high grade ADCCs were 31.3%, and low grade ADCCs were 25%. Strong positive expression was observed in 93.8% cases and only 6.3% showed moderate positive expression. A significant association of Ki 67 with grades of Adenoid cystic Carcinoma was observed (p 0.041).

Conclusion: Ki-67 is expressed in Adenoid cystic carcinoma. In most of the cases ADCC expressed strong positive expression of Ki-67. Its expression is helpful in grading small biopsies, predicting behavior, and planning target therapy of Adenoid cystic carcinoma.

Key Words: Ki-67, salivary gland tumors, immunohistochemistry, Adenoid cystic carcinoma

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INTRODUCTION

Benign tumors are common in females whereas malignant tumors are more common in males.¹ Malignant salivary gland tumors are 0.3 % of all malignancies.² There are 24 types of malignant salivary gland tumors having diverse morphology.^{2,3,4} Adenoid Cystic Carcinoma (ADCC) is a rare malignancy of secretory glands which accounts for only 1% of all malignant tumors of the head and neck region and 10% of all salivary gland neoplasms.

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It occurs more commonly in minor as compared to major salivary glands.⁵ This tumor can be diagnosed in salivary glands, breast, tracheobronchial tree, lacrimal gland, skin, female genital tract and prostate. Out of all these Salivary Gland (SG) of the oral cavity is the most common site.^{6,7,8} In the oral cavity palate is the most common site ranking 39.9% and tongue is the second most common site ranking 19.8%.⁹ Its frequency is much lower in major SGs as compared to minor SGs, 23.5 % and 76.5 % respectively.^{10,11} The majority of patients with ADCC present clinically with a slowly enlarging palpable mass, or a 'mass' that produces local 'obstructive symptoms' when located in a minor salivary gland. An important reported characteristic of ADCC is neural invasion which is considered as an unfavorable prognostic factor indicating association with local recurrences and the development of distant metastases. Perineural invasion is diagnosed in up to 46% of ADCC cases. The rate of distant metastasis reported ranges between 20 and 52% and most frequently detected in the lungs, bones, liver and brain.¹² The carcinoma is usually diagnosed on histopathology which shows tissue composed of inner ductal epithelial cells and outer myoepithelial cells. Three growth patterns have been described: the

cribriform or glandular type, the tubular type and the solid type with the solid type showing more aggressive behaviour and poor prognosis. The other diagnostic tools are initial needle biopsy, core needle biopsy and Immunohistochemistry Ki-67 is the most commonly used cell proliferation markers.¹² Its Immunohistochemical expression increases with cell cycle progression and reaches its peak during the G2 and M phases suggesting high biological aggressiveness of tumour with poor prognosis.¹³ A considerable number of researches have linked Ki-67 expression with the aggressiveness and prognosis of adenoid cystic carcinoma, although the results are still inconclusive.¹⁴ The aim of this study was to determine expression of Ki-67 in ADCC of the salivary glands tumors.

MATERIALS AND METHODS

It is a descriptive study conducted in Postgraduate Medical Institute, Lahore after the approval of ethical board during period of May 2014 to December 2016. Total thirty two cases of ADCC of salivary glands were included in this study after taking written and verbal consent from the patients. These cases were collected from Departments of Surgery, Lahore General Hospital, Mayo Hospital, and de'Montmorency college of Dentistry, Lahore. Routine processing and slides preparation was done in the Histopathology Laboratory of PGMI, Lahore. Slides were stained with routine Hematoxylin and Eosin (H&E) stain. Grading of ADCC was done as low, intermediate and high grade. Immunohistochemical staining for Ki-67 antigen was also done and scoring of Ki-67 expression was determined on Ki-67 immunohistochemical stained slides. Ki-67 immunoreactivity was divided into four groups as; score zero (0); negative [when neoplastic cells stained less than 5%], score one (1): + weak positive (WP) [when neoplastic cells stained 5-19%], score two (2): ++ moderate positive [when neoplastic cells stained 20-50%] score three (3): +++ strong positive (SP) [when neoplastic cells stained more than 50%]. Observations were recorded based on intensity of nuclear staining. The intensity was graded in all the cases with 0, 1, 2 and 3 to represent negative, weak positive, moderate positive and strong positive staining respectively. Care was taken to decrease the subjectivity by ensuring (a) two observations per field area of slide and (b) by intra-lesional comparison with a positive control.¹⁵ Data was entered into SPSS version 22 and descriptive statistics were determined. P value <0.05 was taken as significant.

RESULTS

Out of 32 cases of Adenoid cystic carcinomas 68.1% (22) reported in female and remaining in male (10) 31.2%. Total cases of intermediate grade ADCCs were 43.8%, high grade ADCCs were 31.3%, and low grade ADCCs were 25%. Strong positive expression was observed in 93.8% cases and only 6.3% showed moderate positive expression. A significant association

of Ki 67 with grades of Adenoid cystic Carcinoma was observed (p 0.041).

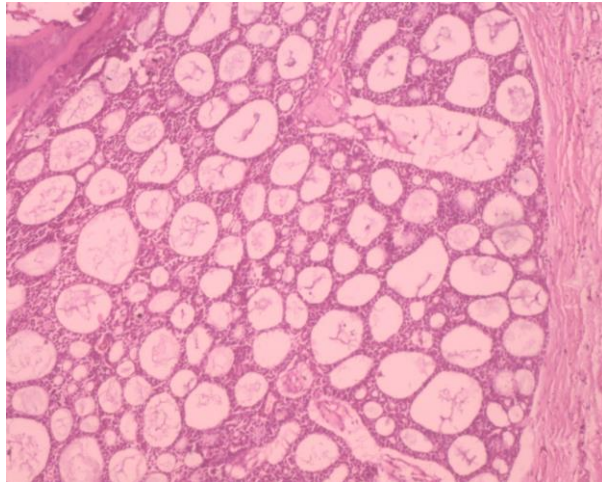
Table No.1: Clinicopathological characteristics of ADCC

Clinicopathological characteristics of ADCC	No. (f)	%age
Age: 20- 40	10	31.2
41-60	10	31.2
61-80	12	37.5
Total	32	100.0
Gender: Male	10	31.2
Female	22	68.8
Total	32	100.0
Hospital		
Mayo hospital	14	43.7
Lahore General	12	37.5
de'Montmorency College of Dentistry/ PDH,	6	18.8
Total	32	100.0
Site		
Parotid Gland	14	43.7
Submandibular salivary Gland	2	6.3
Minor salivary gland on palate	6	18.8
Minor salivary gland on labial mucosa	2	6.2
Minor salivary gland on Buccal mucosa	8	25.0
Total	32	100.0
Laterality		
Right	12	37.5
Left	20	62.5
Total	32	100.0
Specimens		
Incisional	14	43.8
Excisional	14	43.8
Resection ☐	4	12.4
Total	32	100.0
Size		
less than 1cm maximum diameter	2	6.3
1cm to 2cm maximum diameter	4	12.4
2-5cm	18	56.3
more than 5 cm in maximum diameter	8	25.0
Total	32	100.0
Mass: Solid	32	100
Grade : Low Grade	8	25.0
Intermediate	14	43.8
High Grade	10	31.2
Total	32	100.0
Ki-67 Expression		
++ moderate positive [staining in 20-50% of neoplastic cells]	2	6.3
+++ strong positive [staining in more than 50% of neoplastic cells]	30	93.7
Total	32	100.0

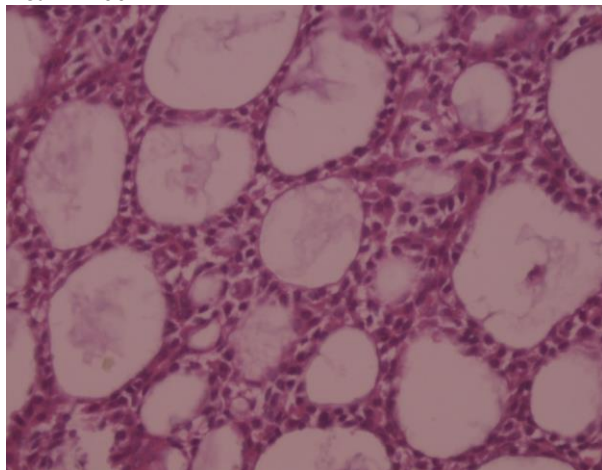
Table 2: Association of Ki-67 with grades of Adenoid cystic Carcinoma

Grade	Ki-67		Total
	++ moderate positive	+++ strong positive	
Low Grade	2	6	8
Intermediate	0	14	14
High Grade	0	10	10
Total	2	30	32

P value 0.041



Photomicrograph No: 1, Adenoid Cystic Carcinoma, H&E X100

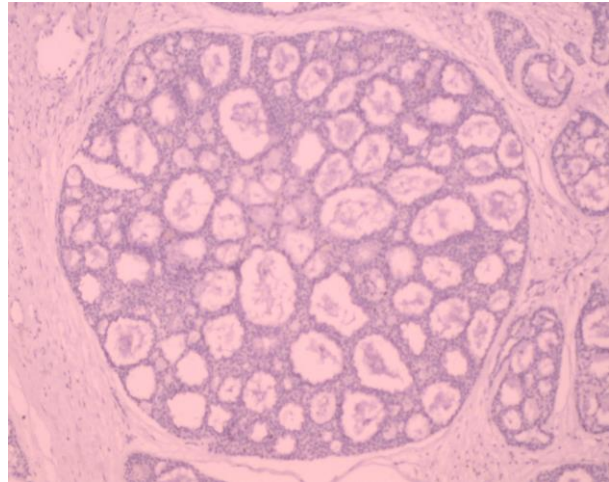


Photomicrograph No: 2, Adenoid Cystic Carcinoma, H&E X400

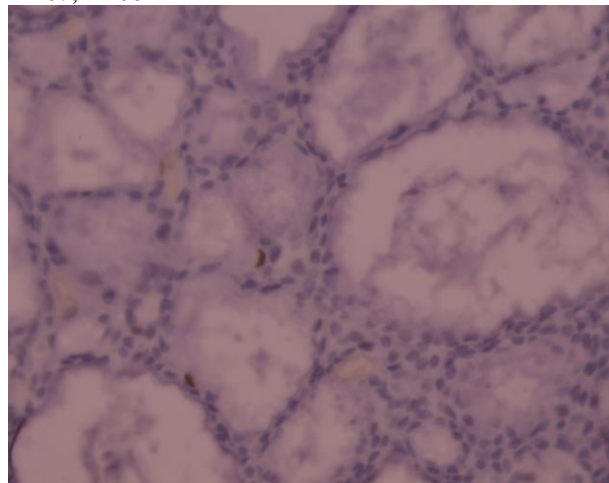
DISCUSSION

Salivary gland neoplasms are a heterogeneous and important group of tumors. Among these ADCC is an important and challenging due to its various histopathological subtypes. Different markers have always been extensively studied in ADCC hoping to find any diagnostic or screening help in identification of its histopathological subtype or to label the type or grade of carcinoma, but, mostly these studies have

inconclusive results, possibly due to the small number of cases in studies.¹⁶



Photomicrograph No: 3, Adenoid Cystic Carcinoma, Ki-67, X100



Photomicrograph No: 4, Adenoid Cystic Carcinoma, Ki-67 X400 (Slide No. 4369)

Al-Azzawi¹⁷ assessed immune-histochemical expression of proliferative and apoptotic proteins (Ki-67 and p53) in ADCC. Total 15 cases were included in the study. Ki-67 was expressed 40% in adenoid cystic carcinoma. The expression of p53 was demonstrated in 73.3% of the total cases. It was concluded that both proteins (Ki-67 and p53) played role in tumorigenesis of ADCC. In our study ADCC has also the strongest positive correlation with Ki-67 expression scores.

Bu et al. (2015)¹⁸ showed that there was a strong positive expression of Ki-67 in all growth patterns of ADCC. Our study revealed that only 2 of the 8 low grade cases showed a moderate positive score and the other 30 low, intermediate and high grade ADCC tumors show a strong positive expression for Ki-67. In the study by Fujii et al.(2017)¹⁹, a high Ki-67 index is seen in 24.2% of all ADCC tumors, however in present study a high Ki-67 index is seen in 93.7% of the ADCC tumors. Iyogun et al. (2017)²⁰, also studied the

expression of Ki-67 in ADCC and found that there was a strong positive expression of marker in 75% cases. This was more than that found by Fujii et al, but less than the current study.

Kintawati et al¹⁴ in 2017 concluded that the expression of Ki-67 varies as the grade of ADCC changes. They found that as the grade of Ki-67 becomes higher, the stronger is the expression of Ki-67. This is supported by our current study, where intermediate and high grade ADCC tumors all show a strong positive expression of Ki-67. Moderate expression of Ki-67 is only seen in low grade ADCC.

Kungoane²¹ in 2015 did a research to evaluate expression of proliferation marker Ki-67 among others in SG tumors to correlate it with tumor type. His results showed Ki-67 expression was significantly higher amongst the five SG tumors. The expression of Ki-67 was significantly higher in ADCC than in MEC, ACC, PA and PLGA. This result is also similar to the result of our study where ADCC has also been found the strongest positive correlation with Ki-67 expression scores. In another study MCM2 expression was higher as compared to Ki-67 in ADCC, however in current study MCM2 was not determined.²²In another study expression of Ki-67 was strong positive in large size ADCC however in this study majority ADCC expressed strong positive.²³ Expression of Ki-67 was determined on 44 ADCC and it was observed that its expression was more in solid variant as compared to other variants.²⁴ In another study total 67 ADCC evaluated for Ki-67 expression, out of them 60 % expressed weak positive expression and 40% cases expressed strong positive expression which is in contrary to this study.²⁵In another study mean Ki-67 index was 30-35% in ADCC of salivary gland, however current study showed higher values.²⁶

CONCLUSION

ADCC expressed strong positive expression of Ki-67 in this study. Ki-67 expression in ADCC showed that it has definitive role in its development. There is need to determine its expression in high grade and low grades ADCC on large sample size. In small biopsies where grading is difficult on routine H&E staining Ki-67 expression might be helpful in determining its aggressive variants.

Author's Contribution:

Concept & Design of Study:	Sultan Muhammad Wahid
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Data Analysis:	Muhammad Talha Haseeb, Rozina Jaffar, Memona Ansari
Revisiting Critically:	Sultan Muhammad Wahid, Zainab Rizvi
Final Approval of version:	Sultan Muhammad Wahid

Conflict of Interest: The study has no conflict of interest to declare by any author.

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