Expression of Ki67 and p53 as proliferation and apoptosis markers in adenoid cystic carcinoma

Lehadh M. Al-Azzawi, B.D.S., M.Sc., Ph.D. [(1)]

ABSTRACT

Background: Adenoid cystic carcinoma (ACC) constitutes about 4% of salivary epithelial tumors and is the second common malignant epithelial salivary gland tumor involving both the major and minor salivary glands. Aims of the study is to evaluate immunohistochemical expression of Ki67 and p53 proteins in ACC.

Materials and Methods: immunohistochemical analyses of fifteen cases of formalin – fixed paraffin – embedded tissues blocks of ACC of salivary glands using ki67 and p53 antibodies.

Results: ki67 was expressed in 6 of 15 ACC (40%) while p53 aberration was demonstrated in 11 of tumor (73.3%). There was a statistically significant difference between the expression of ki67 and p53 proteins in ACC cases (p value = 0.041). Pearson's correlation test demonstrated a significant positive correlation between the numbers of percentage of ki67 and p53 positive cells in ACC cases (r = 0.042).

Conclusions: This study suggests that ki67 positive actively proliferating cells and p53 aberrations may play a role in ACC development and progression.

Keywords: Adenoid cystic carcinoma, Ki-67, p53. (J Bagh Coll Dentistry 2013; 25(2):76-79).

INTRODUCTION

Carcinomas of the salivary glands are rare, comprising less than 0.5% of all malignancies and about 5% of head and neck cancers [(1)]. They are characterized by morphological diversity between different tumor types or even within an individual tumor mass [(2, 3)].

Adenoid cystic carcinoma (ACC) is a common salivary gland cancer subtype accounting for 22% of salivary gland malignancies and 1% of all head and neck cancers [(4,5)]. Perineural invasion, delayed onset of hematogenous metastasis and poor response to traditional chemo- therapies are characteristics of this cancer [(6,7)]. ACC is histologically characterized by cribriform pattern. Tubular and solid patterns are also recognized. ACC is composed of epithelial and myoepithelial cells [(7,8)].

ACC was originally described by Lorain and Laboulbene in 1853. In 1859, Billroth suggested the name cylindroma. In 1930, Spies suggested the term adenoid cystic carcinoma to replace cylindroma and this name has been widely accepted. Until 1940s, the tumor was thought to be a benign variant of the mixed salivary gland tumor. In 1943, Dockerty and Mayo emphasized the malignant nature of this tumor [(9,10)].

Ki67 is a nuclear protein that is encoded by the gene MKI67 [(11)]. This protein is associated with cell proliferation and is associated with transcription of ribosomal RNA [(12,13)].

The Ki-67 is used as a marker for cell proliferation. During inter-phase, the Ki-67 protein is found specifically in the cell nucleus, whereas in mitosis most of the protein is transported to the surface of the chromosomes. This protein is present in all active phases of the cell cycle (G2, S, G1 and mitosis) but is absent in resting cells (G0) [(14,15)].

P53 is located at 17p 13.1, and it is altered in many types of cancer. The p53 protein stimulates the transcription of several genes that mediate cell cycle arrest, and this protein initiates apoptosis in response to DNA damage. While the wild-type p53 protein makes tumor differentiation possible, the mutant p53 protein blocks it [(16,17)]. Mutation in the p53 gene, which results in encoded non-functional protein, is considered as the most common genetic event in human cancer. It has been suggested that mutated p53 may lead to carcinogenesis, as the wild-type p53 contributes to tumour suppression through at least two mechanisms in response to DNA damage, arrest of cell proliferation and induction of apoptosis [(18)].

It is important to note that some P53 mutations do not result in positive immunostaining. However, p53 protein accumulation can occur in the absence of underlying gene mutations [(19)] in response to cellular stress that can result in stabilization, accumulation and activation of p53 in the nucleus [(20,21)]. Aim of this study is to evaluate the immunohistochemical expression of Ki67 and p53 proteins in ACC cases.

(1) Assistant professor, Department of Oral Diagnosis, Collage of Dentistry, University of Baghdad.

MATERIALS AND METHODS

Sample collection

A retrospective study was carried out on a fifteen of formalin fixed and paraffin embedded tissue blocks of adenoid cystic carcinoma of salivary gland origin, which were collected from different laboratories archives in Baghdad (Collage of Dentistry, Al – Shaheed hospital and private centre).

Tissue preparation and staining protocol

First, H and E stained sections were employed for confirmation of diagnosis. For immunohistochemical staining (IHC), Sections with 5µ thickness were prepared, deparaffinised and rehydrated. Antigen retrieval was performed for 30 minutes. Internal peroxidase activity was inhibited by 3% H2O2. Tissue sections were then incubated overnight with the anti-Ki67 monoclonal antibody (MIB-1, Dako, Denmark) and p53 (Do - 7, Dako, Denmark) at a 1/50 dilution separately.

Staining results and scoring system

Brown nuclear staining for Ki-67 and p53 was considered as positive. Immunoreactivity was expressed by determining the percentage of positive tumor cells, intensity of staining was not considered for evaluation.

At least 1000 cells per case in ten high power fields were considered. The Ki-67 and p53 nuclear expression in ≤5% of tumor cells was scored as negative, 6 – 25% (+), 26 – 50% (++), 51 -100% (+++).

Statistical analysis

Data were analyzed by SPSS (statistical package of social sciences) software for window 10. The percentage of variable was obtained by using Chi – square test. The $p \leq 0.05$ was considered statistically significant. Pearson's correlation test was used to verify any correlation between the percentage of positive cells of ki67 and p53 of ACC cases.

RESULTS

Sex of patients with ACC included nine females (60%) and six males (40%) with mean age of 55.5 ± 15.4 years. The majority of the cases were located in minor salivary glands mainly the palate (46.7%), followed by floor of the mouth (20%), upper lip (13.3%), and one case in the maxilla (6.7%) and only two cases were from major salivary gland (submandibular gland).

The histologic pattern of ACC case was mainly cribriform pattern (figure 1a and b) with some exception in few areas showed tubular pattern.

Forty percent (40%) of ACC cases expressed ki67 immunopositivity (figure 2). P53 immunopositivity was seen in 73.3% (figure 3), seven cases showed score (+) expression, P53 immunopositive cells tend to be evenly distributed within the tumor islands. In normal salivary gland tissue expressed positive ki67 while negative expression for p53 (figure 4 a and b respectively). There was a statistically significant difference between the expression of ki67 and p53 proteins in ACC cases (p value = 0.041) (figure 5).

Pearson's correlation test was also able to demonstrate significant positive correlation between the numbers of percentage of ki67 and p 53 positive cells in ACC cases (r = 0.042).

DISCUSSION

Adenoid cystic carcinoma is an uncommon form of malignant neoplasm that arises most commonly in the major and minor salivary glands of the head and neck. It is often slow to metastasize, but has a persistent and relentless growth with a poor long term prognosis. Major and minor salivary glands including seromucinous glands are the frequent sites of occurrence. The histological picture is variable. In its classic form, the tumor is composed of small uniform cells arranged in cribriform, tubular or solid growth patterns with interspersed globules and cylinders of hyaline basement membrane material [5, 6, 9, 10].

One of the most important biological mechanisms in oncogenesis is cell proliferation. Ki-67 is essential for cell proliferation. Ki-67 is required for the synthesis of ribosomes during the cell cycle. Therefore it is relevant to the rate of protein synthesis. Ki-67 was expressed at a rate of 40%. The difference in the expression of this marker in different studies may be related to the type of antibody used (monoclonal and polyclonal), and the differences in how the cells are counted. This relatively low expression Ki-67 rate may explain the present study and due to a relatively small number of patients and measuring the expression by using immunohistochemical staining, which effected by its a semi-quantitative nature, tissue aging effects, the staining technique, the enzyme antibody used, and single observer bias.

However in accordance with other reported, in all of revealed the frequency of Ki67 expression in ACC was lower and showed that this marker

has a role in determining the short-term prognosis [5,7,11,12,14-16,18,22].

In this study, 73.3% of cases were p53 positive and seven cases of them showed low expression and this finding is similar to previous reports [7,14,16-19,20-22]. This could be explained by the fact that in some tumors, for instance, p53 intronic mutations have been associated with stabilization of the p53 protein [21]. On the other hand, p53 protein can accumulate in the absence of underlying gene mutations [18] in response to cellular stress that can result in stabilization, accumulation and activation of p53 in the nucleus. The exact role of this p53 protein accumulation in tumors has not been completely clarified. In conclusion; this study suggests that ki67 positive actively proliferating cells and p53 aberrations may play a role in ACC development and progression.

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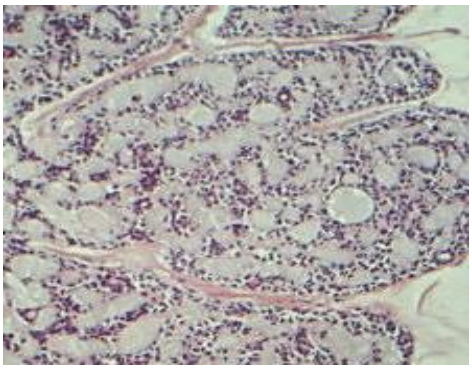
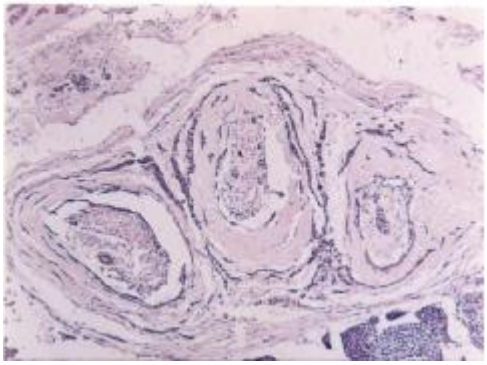


A



B

Figure 1.a Adenoid cystic carcinoma, cribriform histological subtype and b. Neural infiltration (invasion) in adenoid cystic carcinoma. Original magnification 100x – H&E.

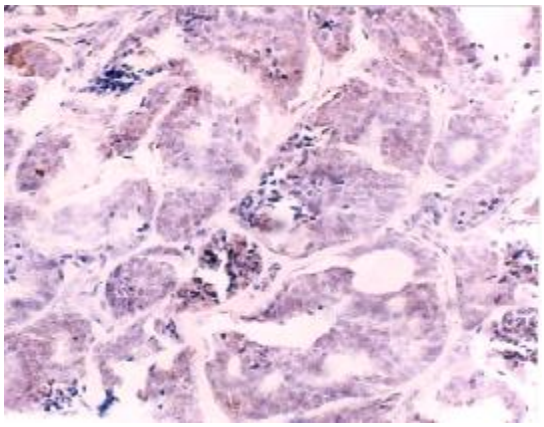


Figure 2. Adenoid cystic carcinoma, cribriform histological subtype demonstrating nuclear immunostaining for ki67. Original magnification 200x.

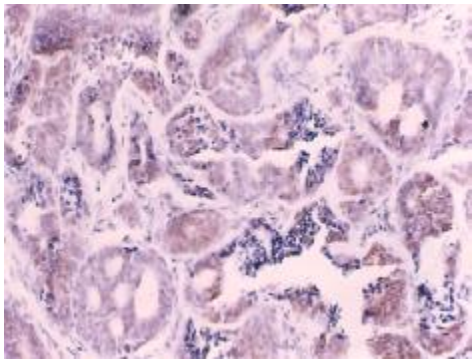


Figure 3. Adenoid cystic carcinoma, cribriform histological subtype demonstrating nuclear immunostaining for p53. Original magnification 200x

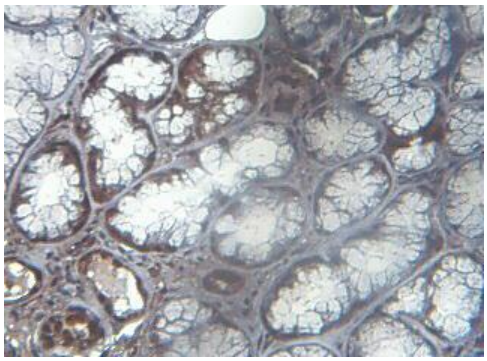


Figure 4a. Normal salivary gland tissue demonstrating nuclear immunostaining for ki67. Original magnification 200x.

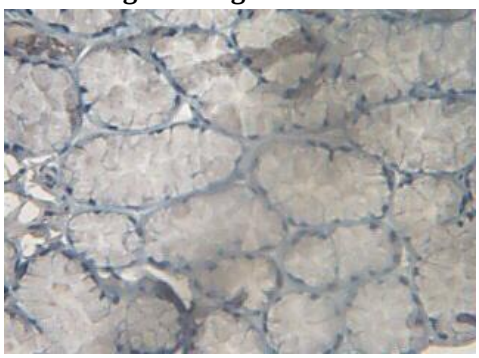


Figure 4b. Normal salivary gland tissue demonstrating negative staining for p53. Original magnification 200x.

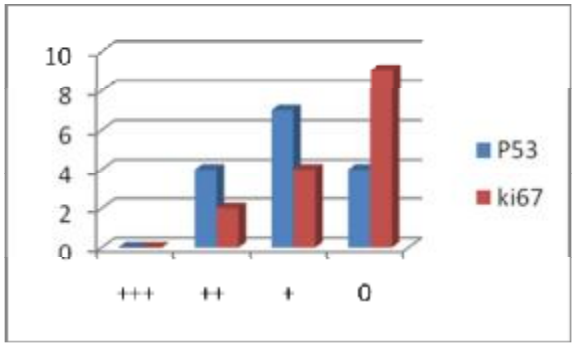


Figure 5. Expression of Ki67 and p53 in ACC.