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Evaluation of Immunohistochemical Expression of P53 and Pcna in Pleomorphic Adenoma, Mucoepidermoid and Adenoid Cystic Carcinomas of Salivary Glands

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Key words
mucoepidermoid carcinoma, adenoid cystic carcinoma, pleomorphic adenoma, p53, PCNA.

Abstract

Salivary gland tumours are uncommon with a broad heterogeneity. The most common benign tumour is the pleomorphic adenoma, whereas mucoepidermoid carcinoma and adenoid cystic carcinoma predominate among the malignancies.

The aim of this study was to describe the tissue expression of p53 and PCNA protein in pleomorphic adenoma, mucoepidermoid carcinoma and adenoid cystic carcinoma and to compare their expression among the studied tumors. The study enrolled (45)formalin –fixed, paraffin- embedded tissue blocks of salivary gland tumors, diagnosed as pleomorphic adenoma, mucoepidermoid carcinoma and adenoid cystic carcinoma (15 cases for each) and evaluated the immunohistochemical expression of P53 and PCNA proteins.

The study revealed positive p53 protein expression in (60%) of muocoepidermoid carcinoma cases and (20%) of adenoid cystic carcinoma cases, while only one case (6.7%) of pleomorphic adenoma was P53 positive. The immunopositivity of PCNA protein expression was found in (100%) of both mucoepidermoid carcinoma and adenoid cystic carcinoma cases and (66.7%) of pleomorphic adenoma cases.

The results of this study demonstrated that inactivation of p53 protein may play an important role in the activation of PCNA with increasing the proliferation activity of MEC and ACC.

Introduction

The salivary glands are exocrine organs comprising ducto-acinar units that produce and secrete saliva. Tumors commonly arise in the salivary glands, and these comprise approximately 1% of all neoplasms in the whole body [(1)]. Pleomorphic salivary adenoma (PA) is the most common neoplasm of salivary gland [(2,3)] , accounts for 54 to 65 % of all salivary gland tumors [(4)] and was shown sometimes to undergo malignant transformation in its natural course [(5)]. PA, also known as a benign mixed tumor, is a benign neoplasm which shows a remarkable degree of morphological diversity. Microscopically PA is showing varying combination of epithelial and myoepithelial cells in a mesenchymal or stromal background. The duct-like formation exhibit ductal luminal cells in

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the inner layer and a luminal cells in the outer layer. The capsule varies in thickness and presence [6,7]. Mucoepidermoid carcinoma (MEC) is one of the most common malignant salivary gland tumors. MEC is composed of various proportions of mucous, epidermoid (e.g. squamous), intermediate, columnar, and clear cells often demonstrates cystic growth. It is the most common malignant neoplasm observed in the major and minor salivary glands. Microscopic grading of MEC is important to determine the prognosis. MECs are graded as low grade intermediate grade and high grade [8, 9, 10]. Adenoid cystic carcinomas (ACCs) are rare tumors of the head and neck accounting for approximately (10-12%) of malignant tumors of the salivary glands [11]. They are characterized by rather slow growth pattern and have a perineural spread. ACC is a second most common malignant tumor of salivary gland. It is considered as an intermediate grade of salivary gland neoplasms [12]. Morphologically, three growth patterns have been described cribriform, or classic pattern; tubular; and solid, or basaloid pattern. The tumors are categorized according to the predominated pattern [13].Although hemotoxylin-eosin (HE) staining is still the gold standard method used for diagnosing the salivary gland tumor, immunohistochemistry (IHC) can enhance the accuracy of such analysis, while its role may be limited. IHC can be a helpful tool to investigate the subjects that cannot be assessed by histological examination, such as the cell nature and differentiation status, cell proliferation, and tumor protein expression [1].Apoptosis is a highly regulated active process, characterized by cell shrinkage, chromatin condensation and DNA fragmentation promoted by endonucleases. Apoptosis is frequently deregulated in human cancers, being a suitable target for anticancer therapy [14]. P53 is a tumor suppressor gene which acts as a tumor suppressor in human being in normal form.Mutation of P53, located on the short arm of chromosome 17, is among the most commonly detected genetic abnormalities in human neoplasia [15]. The current studies of the molecular biology of cancer have demonstrated that the loss of function of tumor suppressor gene such as P53 may lead to the development of many different cancer types [16]. The expression of genes related to cell proliferation and oncogenesis seems to be associated with the prognosis of some oral tumors. Numerous studies involving proliferating cell nuclear antigen (PCNA) has been conducting to determine the process related to cell proliferation and, consequently, the susceptibility of some tumors to malignant transformation [17]. The evaluation of cell proliferation using PCNA is comparable to and, under certain condition, superior to the traditional methods of mitotic figure count using optical microscopy , tritiated timidin uptake and flow cytometry [18]. This study aimed to describe the tissue expression of p53 and PCNA protein in pleomorphic adenoma, mucoepidermoid carcinoma and adenoid cystic carcinoma and to compare their expression among the studied tumors.

Materials and Methods

Archival formalin – fixed, paraffin – embedded tissue blocks of 45 cases of salivary gland tumors diagnosed as pleomorphic adenoma, mucoepidermoid carcinoma and adenoid cystic carcinoma (15 cases for each) were obtained from the department of oral and maxillofacial Pathology, college of Dentistry – university of Baghdad and other centers in Baghdad dated from(1980 – 2010). Sections of 5 µm thick of the paraffin embedded tissue were cut and stained with hematoxylin and eosin for routine histopathological examination. The MECs were graded according to Ellis & Auclair (1996) criteria [19]. Another 5 µm paraffin sections were cut and mounted on coated glass slides for immunohistochemial analysis. Antigen retrieval was done using citrate buffer (pH 6.0) by microwave digestion .Endogenous peroxidase was blocked with 0.05% hydrogen peroxide for 30 min. After incubation with a 1:20 dilution of normal horse serum to reduce non-specific binding, the slides were incubated overnight at 48C with primary antibodies against p53 (Dako, CloneDO-7;

1:80 and PCNA (Dako-patts, PC-10, 1: 50). Secondary antibodies associated with a streptavidin–biotin–peroxidase method was used (Dako A/S، Strept ABComplex Duet, mouse/rabbit), complemented with diaminobenzidine as the cromogen. All slides were counterstained with hematoxylin. After each step the sections were washed with phosphate buffered saline. Negative controls sections were obtained using non-immune serum instead of the primary antibody. Samples of squamous cell carcinoma were used as positive control. Immunoreactivity was classified as: (-) negative ≤5%, (+) low 6–25%, (++) moderate 26–50% and (+++) high >50% of positive tumour cells, counting at least 1000 cells at high magnification (40x objective and 10x eyepiece) [17]. Intensity of staining was not considered for evaluation. The quantitative analysis of P53 and PCNA positive cells were counted by two independent examiners. Both microscopical and immunohistochemical analysis performed blindly without any clinical information.

Statistical Analysis

Data were analyzed by SPSS software for window 10. The percentage of variable was obtained by using Chi – square test. The one way ANOVA test was employed for analyzing the data. The student t test was used for the comparison between two variables. The $p \leq 0.05$ was considered statistically significant.

Result

Regarding p53 protein expression almost most of PA cases were negative (93.3%) except one case (6.7%) which showed weak positive expression. In MEC (60%) of the cases showed positive immunoreactivity while (40%) were negative. Whereas for ACC cases P53 immunopositivity was found in (20%) (table 1). The rate of p53 positivity was significantly higher in MEC and ACC groups than PA group (p=0.048).PCNA immunopositivity was seen in (66.7%) of PA cases, (100%) of MEC cases with high positivity in (60%) of cases, and (100%) of ACC cases of which (46.7%) showed high positivity (table 1). The rate of PCNA positivity was significantly higher in MEC and ACC groups than PA group (p=0.046). The immunopositivity of PCNA protein was greater in MEC, ACC and PA cases than that of p53 protein and the differences were statistically significant (p=0.047, 0.047 and 0.048 respectivily). Figure 1 showed positive nuclear immunohistochemical staining of p53 and PCNA in MEC and ACC cases. The association of p53 and PCNA expression in each pair of the studied groups is summarized in table (2). Accordingly, there was a statistically significant difference between the expression of p53 and PCNA proteins in PA and MEC cases (p=0.042, 0.041 respectively), between PA and ACC cases (p=0.041, 0.042 respectively) and between MEC and ACC cases (p=0.042, 0.043 respectively). Microscopical examination of the H&E sections of MECs showed that 11 cases (73.3%) were high grade, 3 cases (20%) intermediate grade and only one case (6.7%) was low grade. There was no statistically significant differences regarding p53 and PCNA expression in relation to low and intermediate grades of MEC cases, while there was a significant difference in relation to the high grade (p=0.042).The expression of p53 protein in relation to the grade of MEC showed highly significant difference (p<0.01) whereas there was a significant difference for the expression of PCNA protein in relation to grade of MEC cases as demonstrated in figure 2 and table 3.

Discussion

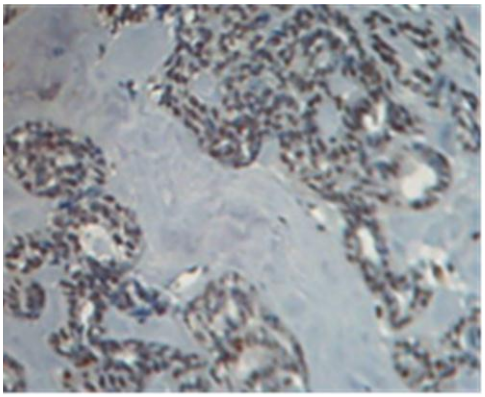
Many authors have correlated the expression of normal and mutant p53 protein with the aggressiveness, differentiation and prognosis of salivary gland tumors, but the results are still controversial [20]. PA is the most common salivary gland tumor, with well-known clinical and microscopical characteristic. Nevertheless, its pathogenesis is still unclear, as its expression of oncogenes and the factor that influence its transformation to malignancy. It is

interesting that although PA is considered negative for p53, mutation of this suppressor gene seems to be involved in the transformation of PA to carcinoma ex-PA [21] Tsuji et al [22] showed PCNA expression in about (70%)of PAs and only one out of 11 cases was positive for p53. Lazzara &Cleveland [23] analyzed the expression of p53 in benign and malignant intra-oral salivary gland tumors. Most benign tumors were negative for p53 and some cases showed a low expression of p53. Most studies indicated that p53 had low expression in PA of the parotid and minor salivary glands. However, Jorge et al., [24] showed that p53 expression was negative in PAs of minor salivary glands of children and PCNA is weakly positive .According to Tom et al., [25] study p53 positive immunoexpression was observed in 5 of 41 cases of benign pleomorphic adenoma. In the present study , most cases showed negative labeling for p53, in agreement with the findings of some studies (Lazzaro and Cleveland 2000,Weber et al 2002)[23&26],emphasizing that this protein is not implicated in the pathogenesis of PA. In contrast, most cases were positive for the p53 protein in the studies of Ohki et al [27] and Ohtake et al [28], indicating, according to these authors a tendency toward malignant transformation of pleomorphic adenoma. In this study the results showed that about (66.7%) of PA cases being positive for PCNA and this is in agreement with Alves et al (2004)[29] , and Tsuji et al [22], while it is in disagreement with the results of another study(Gorden et al 2008)[30] which observed weak PCNA labeling (42.1%) which concluded that PCNA can favor the proliferative activity of pleomorphic adenoma. The results of this study concerning the PCNA expression may indicate a greater proliferative activity of these tumors and suggests a tendency toward recurrence and possible susceptibility of these lesions to malignant transformation. MECs showed immunopositivity for p53 in 60% of cases, with the remaining six cases (40%) being negative. In the literatures different expressions have been demonstrated for p53 in MEC. Ehab [31] and saad[32] found higher expression of p53 in MEC (80%, 94% respectively).However, Dor et al,[33] found low expression. The variation in the expression of p53 in this study and the aforementioned studies may be due to different scoring systems, fixation times and concentration of antibodies, and the sensitivity of the technique used. PCNA was positive in all MEC cases of which (60%) were classified as high positive. Cardoso et al., [34] evaluated the relationship between the grade of MEC with PCNA expression. There was significant difference in PCNA expression in high grade MEC and intermediate and low grade MECs. However, there were no differences between the intermediate and low grades. In the current study 8 out of 11 cases of high grade MECs and one out of three intermediate grade MECs presented high PCNA expression. In the only single low grade MEC the PCNA expression was low. Although most of the study cases were high grade MECs, these data could confirm that these tumours present slow proliferative activity as do most of the salivary gland tumours, also they suggest that the evaluation of PCNA expression in MEC of salivary glands could be used as complementary procedure for appropriate classification of these tumours. P53 expression was negative in 80% of ACCs, in 13% was low positive and in 6.7% was high positive. Lazzals and Cleveland [23] compared p53 and ki67 expression in benign and malignant intra oral and perioral salivary gland tumours including ACCs which showed low or no expression for p53 in 76.5% of the cases. In contrast, Gallo et al,[35] described higher positivity for p53, 8 out of 10 cases for ACC of parotid gland, these authors reported that the differences was probably due to the antibody used. In this study, the expression of p53 and PCNA were higher in ACCs than in PA but lower than MEC. Daniele et al.,[36] also found the PCNA index was lower in PA than in ACC. The current results regarding PCNA expression confirms that PA has a slower proliferation rate compared to that of MEC and ACC. Cell proliferation is related to tumour aggressiveness and prognosis, although this has not yet been confirmed for all types of cancer using PCNA markers. MEC showed higher

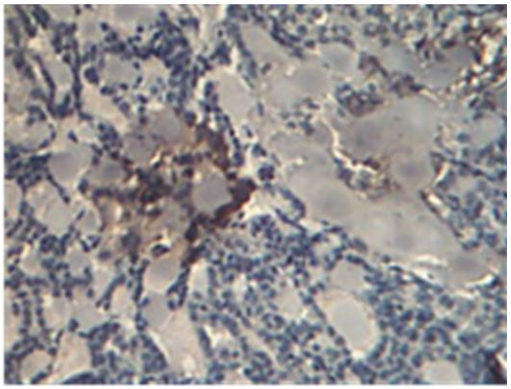
expression of these markers than those of the other salivary gland. This could be in part explained by the fact that most of our MEC cases were high grade tumours. In conclusion; the expression of p53 and PCNA were higher in ACCs than in PA but lower than MEC. The results of this study demonstrated that the inactivation of p53 protein may play an important role in the activation of other markers like PCNA with increasing the proliferative activity of MEC and ACC.

List of Abbreviations

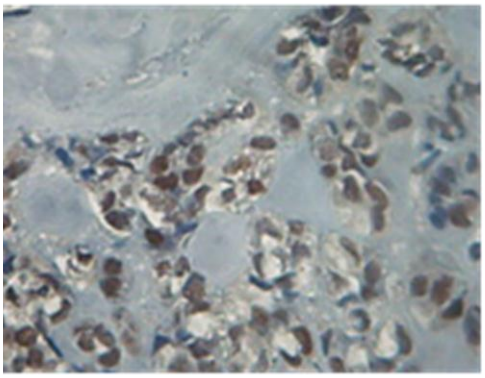
PA: pleomorphic adenoma
MEC: mucoepidermoid carcinoma
ACC: adenoid cystic carcinoma



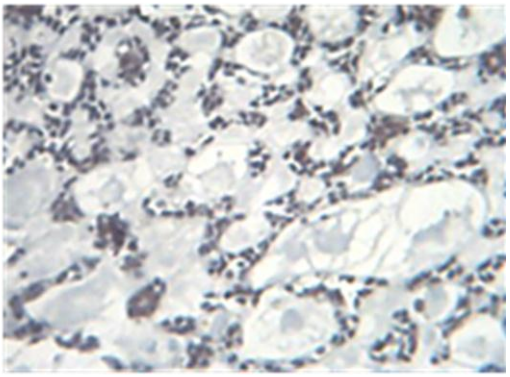
A. MEC x20 p53



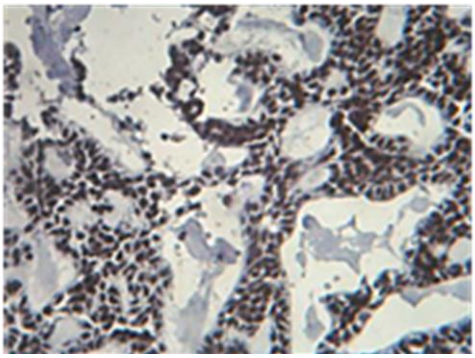
C. MEC x20 PCNA



B. MEC x20 p53



D. ACC x20 p53



E. ACC x20 PCNA

Fig.(1):- Immunohistochemical staining with p53 and PCNA protein expression in MEC (A: P53 X200, B: x400 and C: PCNA X200) and ACC (D: P53 X200 and D: PCNA X200).

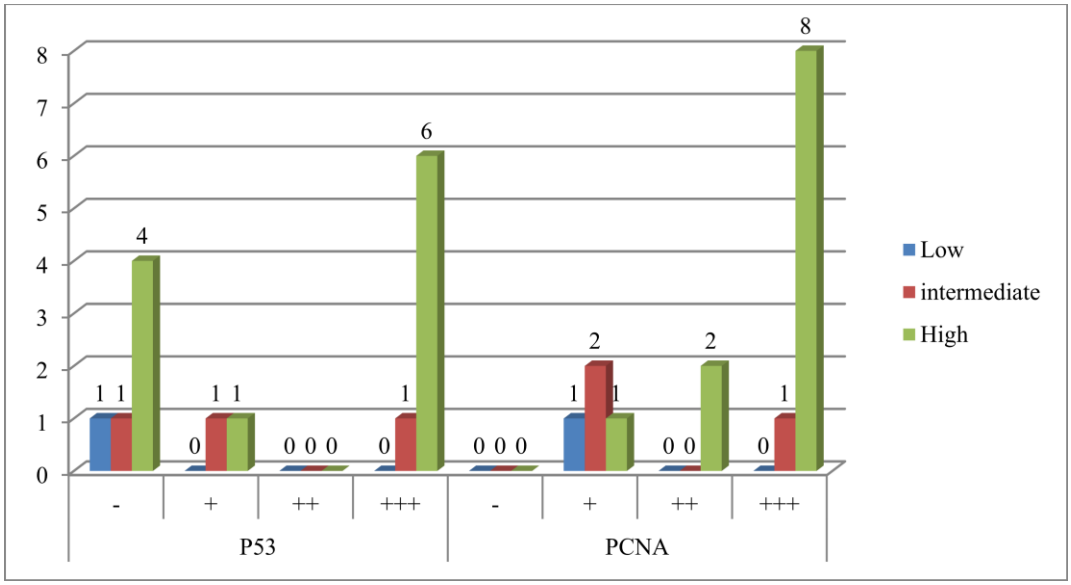


Fig.(2):-Frequency distribution of malignancy grade of MEC cases with IHC analysis of p53 and PCNA.

Table(1):-Immunohistochemical expression and chi – square test statistically analyzed in PA, MEC and ACC.

Variables		P53				PCNA				P-value
		-	+	++	+++	-	+	++	+++	
PA	No.	14	1	0	0	5	4	6	0	0.048
	%	93.3	6.7	0	0	33.3	26.7	40.0	0	S*
MEC	No.	6	1	0	8	0	4	2	9	0.047
	%	40.0	6.7	0	53.3	0	26.7	13.3	60.0	S*
ACC	No	12	2	0	1	0	3	5	7	0.047
	%	80.0	13.3	0	6.7	0	20.0	33.3	46.7	S*
P-value		0.048 S*				0.046 S*				

*P<0.05 Significant

Table(2):- Comparison between immunohistochemical expression of P53 and PCNA proteins in studies groups.

Variables	P53		PCNA	
	P-value	Sig	P-value	Sig*
PA&MEC	0.042	S	0.041	S
PA&ACC	0.041	S	0.042	S
MEC&ACC	0.042	S	0.043	S

*P<0.05 Significant

Table(3):-Correlation of malignancy grade of MEC with IHC analysis of p53 and PCNA.

variable		P53				PCNA				P-value
		-	+	++	+++	-	+	++	+++	
Low	No	1	0	0	0	0	1	0	0	0.989
	%	100	0	0	0	0	100	0	0	NS**
Intermediate no		1	1	0	1	0	2	0	1	0.892
%		33.3	33.3	0	33.3	0	66.7	0	33.3	NS**
High	No	4	1	0	6	0	1	2	8	0.042
	%	36.4	9.1	0	54.5	0	9.1	18.2	72.7	S*

P<0.05 Significant **P<0.05 Non Significant**.

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