Original Article

Immunohistochemical differentiation of proliferative activity in Warthin's tumor & different types of adenoid cystic carcinoma (p53, PCNA & CD34)

Kamal Abdelrahman[1], Mohamed Abd Elrahaman[1], Magdi Alazazi[2], Amr Saad Abdoalwahab[1]

[1] Department of Oral and Dental Pathology, Faculty of Dental Medicine, Al- Azhar University
[2] Oral biology Department, Faculty of Dental Medicine, Al-Azhar University
E-mail: drkamal96@yahoo.com

Abstract

Aim: To study the expression of p53, PCNA & CD34 and differentiate the proliferative activity and angiogenesis of Warthin's tumor & different types of adenoid cystic carcinoma. **Methods:** Hematoxylin and Eosin (H&E) and immunohistochemical staining techniques were used to detect p53, PCNA & CD34 proteins in 20 cases of Warthin's tumor & different types of adenoid cystic carcinoma (solid, tubular & cribriform pattern). Evaluation of immunostaining was done using computer image analyzer system & all data were statistically analyzed. **Results:** There was a statistically significant difference ($P<0.05$) in means of expression of p53, PCNA & CD34 between Warthin's tumor & different types of adenoid cystic carcinoma. **Conclusion:** The expression of p53, PCNA & CD34 have been associated with aggressive tumor progression and thus has been implicated as a predictive and prognostic marker and could provide useful prognostic markers for proliferative activity and good prognostic indicators for recurrence rate of adenoid cystic carcinoma.

Keywords: p53, PCNA, CD34, salivary gland

1. 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]. Papillary cyst adenolymphoma is the second most common benign cystic tumor of the salivary gland. This tumor was first described by Hilderbrand in 1895 as a form of congenital cyst of the neck. In 1929, Warthin's called this tumor as papillary cystadenoma lymphomatosum. Currently, this tumor is called as adenolymphoma, Warthin's tumor, and cystic papillary adenoma [2]. Warthin's tumors occur almost exclusively in the parotid glands, in its superficial lobe and rarely in the deeper lobe (10%). It presents as a slow growing node, firm or fluctuant at palpation, with indolent mass, multicentric (12%–20%), and bilateral (5%–14%) [3]. Ellis and Auclair [4], has shown adenolymphoma incidence between 5% and 11% of all parotid gland tumors, and Eveson and Cawson [5] has found 14%–30% of parotid tumors. Warthin's tumor has incomprehensible histogenesis of lymphoid stroma [6]. According to the 2005 World Health Organization classification of tumors, Warthin's tumor can be defined as a tumor composed of glandular and often cystic structures, sometimes with a papillary cystic arrangement, lined with characteristic bilayered epithelium, comprising inner columnar eosinophilic or

oncocytic cells surrounded by smaller basal cells. Lymphoid stroma often contains many germ centers, which may be the result of an immune response to neoplastic epithelium or may represent residual lymphoid tissue in the lymph nodes partially replaced by neoplastic epithelium [7]. Van der Wal et al.[8] have shown that the lymph nodes near the gland may be present within the healthy tissue of salivary gland, on the other hand salivary gland tissue may be present in the structure of lymph nodes. It is a common clinical observation that carcinomas initially spread via the lymphatic system while sarcomas spread via the hematogenous route. Adenoid cystic carcinoma (ACC) (cylindroma) of the salivary gland accounts for about 10% of all epithelial tumors and about 23% of all carcinomas occurring in this organ. ACC is a highly malignant tumor and preferentially metastasizes to distant organs, especially to the lung.[9,10]

Histologically, this tumor shows higher rates of local growth, perineural invasion and distant metastasis. Many cases of delayed recurrence after definite treatment have been reported, indicating the difficulty of treating and controlling this tumor. Factors related to the recurrence and prognosis of ACC includes tumor stage, histological classification, and status of the resected tumor margin, lymph node metastasis, and perineural invasion [11-13]. Although hematoxylin & 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, 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. 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]. Tumor growth and metastasis depends on its angiogenic activity. New blood vessels are essential for expansion and formation of macroscopic tumors [18]. The properties of tumor cells to release angiogenic and antiangiogenic factors are essential for tumor-induced neovascularization[19].

Counting tumor blood vessels by immunohistochemistry (IHC) is a common method for evaluating the angiogenic activity [20]. CD34 is a pan-endothelial marker that stains both "newly formed" blood vessels and normal ones trapped within tumor tissues. Tumors with high vascular density are associated with an increased metastatic potential and decreased survival. Thus, antiangiogenic drugs are considered as a potential target for cancer therapy[21]. The main purpose of the present study was to evaluate the expression of p53, PCNA & CD34 and differentiate the proliferative activity and angiogenesis of Warthin's tumor & different types of cylindroma.

2. Materials and Methods

Twenty formalin-fixed paraffin embedded blocks were used, 5 of them were previously diagnosed as Warthin's tumor & 15 cylindroma (5 solid, 5 tubular & 5 cribriform pattern), were collected from the archives of the Pathology Department, National Cancer Institute (Cairo University), Oral Pathology Department, Al-Azhar University, Faculty of Dental Medicine (Cairo - Boys). Tissue sections using rotary microtome of 4μm thickness were cut, mounted on glass slides and stained with hematoxylin and eosin stain (H&E) for light microscopic examination. For immunohistochemical (IHC) markers, other tissue sections were cut at 4μm and put on positive charged slides for the application of standard labeled streptavidin- biotin method to apply each antibody used separately (p53, PCNA & CD34 antibodies) (Lab Vision Corporation, USA). The sections were deparaffinized in xylene and rehydrated through graded ethanol (100 %, 95 % and 70 %) each run for 5 minutes. Slides were washed in distilled water then in phosphate buffered saline (PBS), each for 5

minutes. Endogenous peroxidase activity was blocked using 3% solution of hydrogen peroxide (H_2O_2) in methanol for 30 minutes at room temperature. Slides were then washed in PBS. Slides were then immersed in plastic jars containing 200 ml of 10 M citrate buffer (pH 6) (ready to use from DAKO). The jars were put in microwave at maximum power at 100°C for 3 intervals, each one 5 minutes. Slides were left at room temperature to coal gradually. Slides were then washed in distilled water followed by PBS for 5 minutes. Tissue sections were received one or two drops of the primary antibodies in a dilution of 1:100 for (PCNA & CD34) and a dilution of 1:200 for p53 then incubated in a humid chamber at room temperature overnight. Slides were then washed in distilled water, followed by PBS for 5 minutes. Biotinylated secondary antibody was added and incubated at room temperature for 30 minutes. Tissue sections were then washed in PBS for 5 minutes. One or two drops of peroxidase-labeled streptavidin were applied for 30 minutes at room temperature then washed in PBS. The tissue sections were received DAB for 2-4 minutes to develop color, followed by putting in distilled water. Tissue sections were counterstained using Mayer's hematoxylin for one minute and then washed in tap water. The slides were placed in two changes of 95% alcohol followed by two changes of absolute alcohol, each for 3 minutes then mounted with DPX and covered with plastic covers in order to be examined. The immunostained sections were examined using light microscope to assess the prevalence of positive cases and the localization of immmunostaining within the tissues. A section was considered either positive or negative according to the presence or absence of brown staining in the nuclei of tumor cells; regarding p53 & PCNA, in the nuclei of endothelial cells of blood vessels regarding CD34. Area percentage of positive staining for p53, PCNA and CD34 stains of tumor was measured in an area with reference to a standard measuring frame of area per 5 fields using a magnification (x 200) by light microscopy. These areas were counted and the positive index (PI) was calculated by image analyzer computer system to assess area percentage of positive cells, the image analysis was performed using a computer system (Software Leica Quin 500, Wetzlar, Germany) consisting of color video camera, color monitor, CBU of IBM personal computer connected to the microscope. The image analyzer was first calibrated automatically to convert the measurement units (pixels)

produced by the image analyzer program into actual micrometer units. Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation, unpaired student t-test, analysis of variance (ANOVA) tests and Tukey's test by SPSS V20. Significant level: Non Significant >0.05 Significant <0.05*.

3.Results

p53 and PCNA were expressed in all specimens of Warthin's tumor, weak immunoreactivity was observed mainly in neoplastic epithelial cells and the lymphoid cells component of the tumors, but not in the cells lining cystic spaces, weak positive CD34staining was also noted in vascular endothelial cells (Fig.1). In ACC cases, with cribriform, tubular and solid pattern showed nuclear p53 and PCNA reaction, high positive expression was observed in of basaloid epithelial cells of solid pattern, while weak expression was observed in cribriform and tubular pattern, also all pattern of ACC showed nuclear weak CD34 expression located in the nuclei of endothelial cells of blood vessels around tumor nests (Fig.2). Statistical analysis results were revealed that, there were significant differences in the mean of p53, PCNA & CD34 expression in Warthin's tumor and ACC (solid, tubular & cribriform pattern), where P value was < 0.05 (Table 1 , Fig. 3). In ACC cases, in regard to expression of p53, PCNA & CD34, solid pattern had recorded the highest mean area percentage (28.14%, 65.38%, 50.16%) respectively, while cribriform pattern had recorded the lowest mean area percentage (14.63%, 21.65%, 20.22%) respectively and ANOVA test revealed significant difference between solid, tubular & cribriform pattern where P value was <0.05(Table 2 , Fig. 4). Statistically, Tukey's test revealed significant difference between (solid pattern & tubular pattern), (solid pattern & cribriform pattern) in regard to of PCNA expression where P value was <0.05, also there was significant difference between (solid pattern & cribriform pattern) in regard to of CD34 expression where P value was <0.05, while there was no significant difference between (solid pattern & tubular pattern) (solid pattern &cribriform pattern), (tubular pattern &cribriform
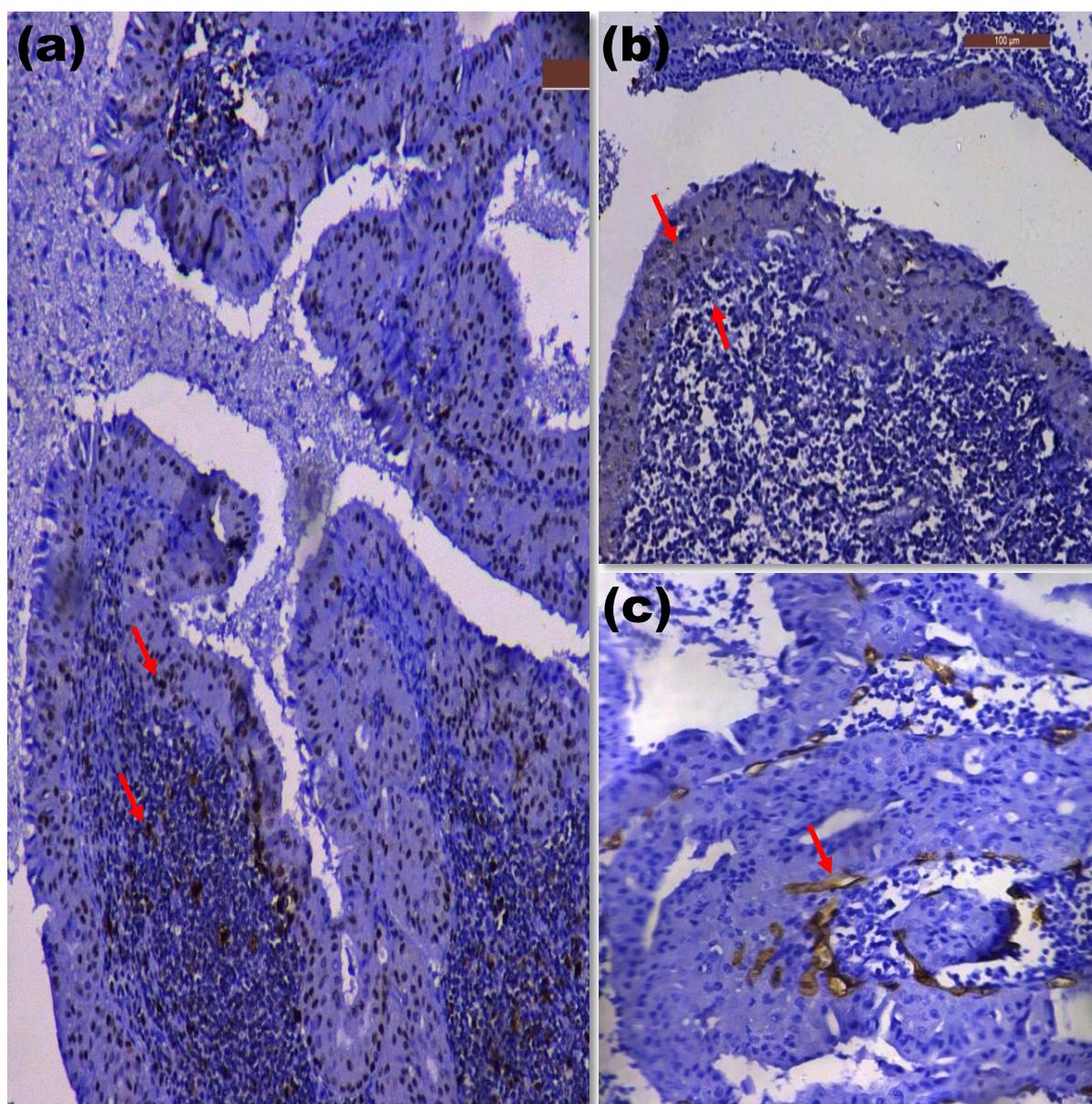
Figure 1: Immunohistochemical expression of (a) PCNA (x200) and (b) p53 (x200) in Warthin's tumor showed positive nuclear staining throughout neoplastic epithelial cells and in the lymphoid cells (c) Positive CD34 expression located in the nuclei of endothelial cells of blood vessels around tumor nests (x200).

	Warthin tumor (n=5)		Cylindroma (n=15)		T-test	
	Mean	**SD**	**Mean**	**SD**	**t**	**P-value**
p53	5.75	1.80	23.00	12.59	2.999	0.008*
PCNA	12.50	4.14	42.35	22.48	2.901	0.010*
CD34	7.27	4.71	34.52	18.68	3.174	0.005*

Table(1) Mean, standard deviation (SD), P-values and results of comparison between expression of p53, PCNA 4 in Warthin's tumor & cylindroma.
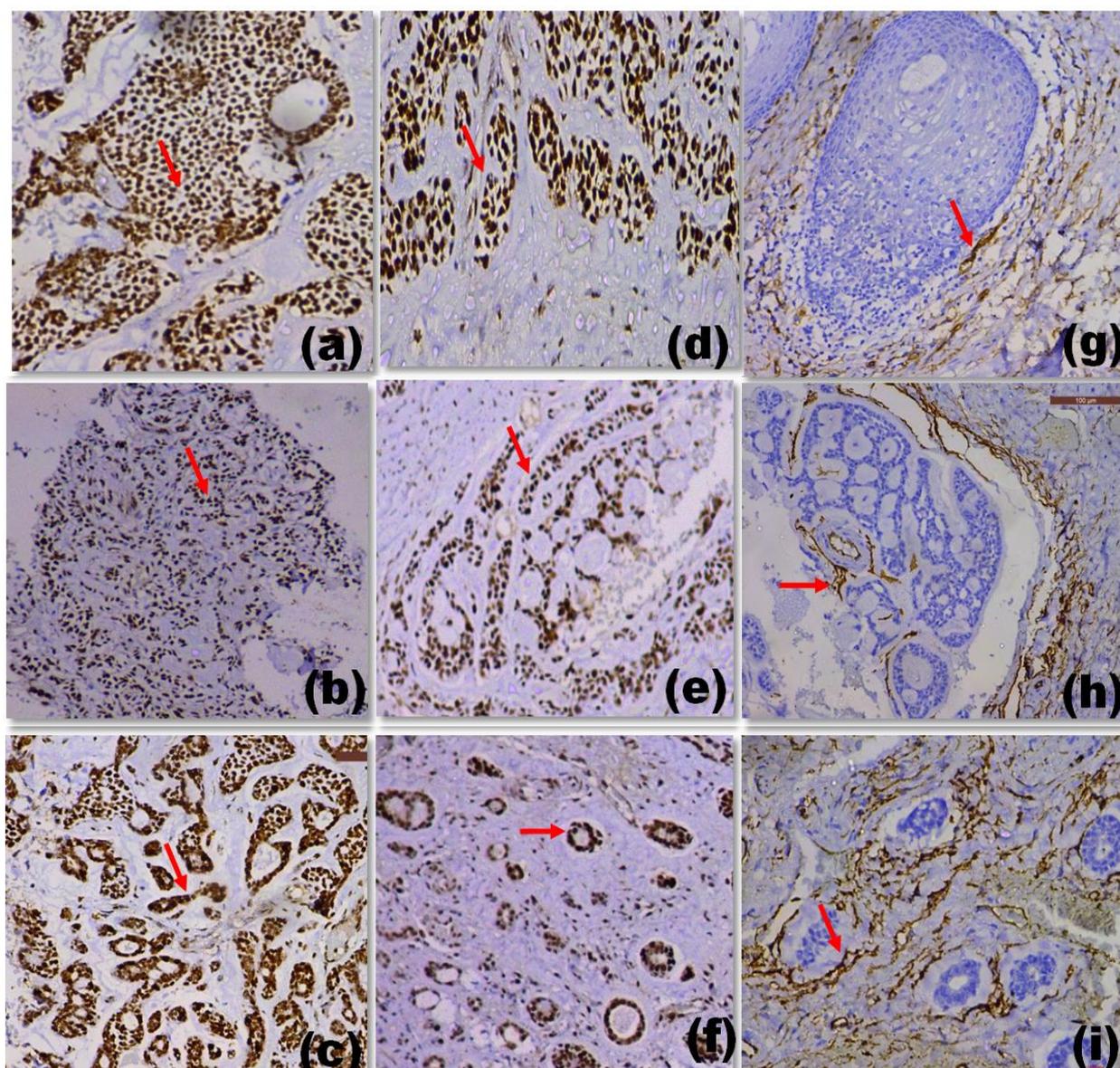
Figure 2: Immunohistochemical expression of (a, b, c) PCNA and (d, e, f) p53 in different types of ACC showed positive staining throughout neoplastic epithelial cells of solid, cribriform and tubular pattern respectively and (g, h, i) CD34 expression located in the nuclei of endothelial cells of blood vessels around tumor nests (x200).

	Warthin tumor (n=5)		Cylindroma solid pattern (n=5)		Cylindroma tubular pattern (n=5)		Cylindroma cribriform pattern (n=5)		ANOVA	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**f**	**P-value**
p53	5.75	1.80	28.14	15.78	26.23	12.46	14.63	4.15	5.182	0.011*
PCNA	12.50	4.14	65.38	19.69	40.01	11.43	21.65	6.72	18.748	<0.001*
CD34	7.27	4.71	50.16	17.86	33.18	17.59	20.22	5.43	9.880	<0.001*

Table (2) Mean, standard deviation (SD), P-values and results of comparison between expression of p53, PCNA & CD34 in Warthin's tumor & different types of cylindroma (solid, tubular & cribriform pattern).

Tukey's test	Warthin tumor			solid pattern		tubular pattern
	Solid Pattern	**Tubular Pattern**	**Cribriform Pattern**	**Tubular Pattern**	**Cribriform Pattern**	**Cribriform Pattern**
p53	0.016*	0.029*	0.539	0.991	0.204	0.318
PCNA	<0.001*	0.011*	0.635	0.020*	<0.001*	0.115
CD34	<0.001*	0.029*	0.422	0.208	0.011*	0.421

Table (3) Mean, standard deviation (SD), P-values and results of comparison between expression of p53, PCNA & CD34 in Warthin's tumor & different types of cylindroma (solid, tubular & cribriform pattern).
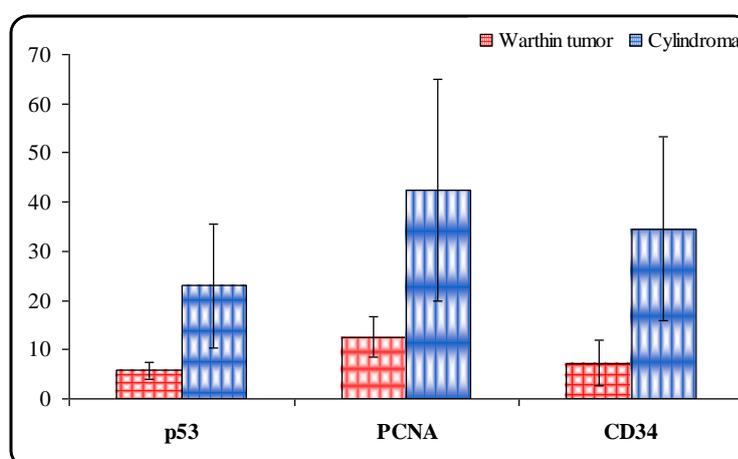
Figure 3: Bar chart representing mean of p53, PCNA & CD34 expression in Warthin's tumor & cylindroma
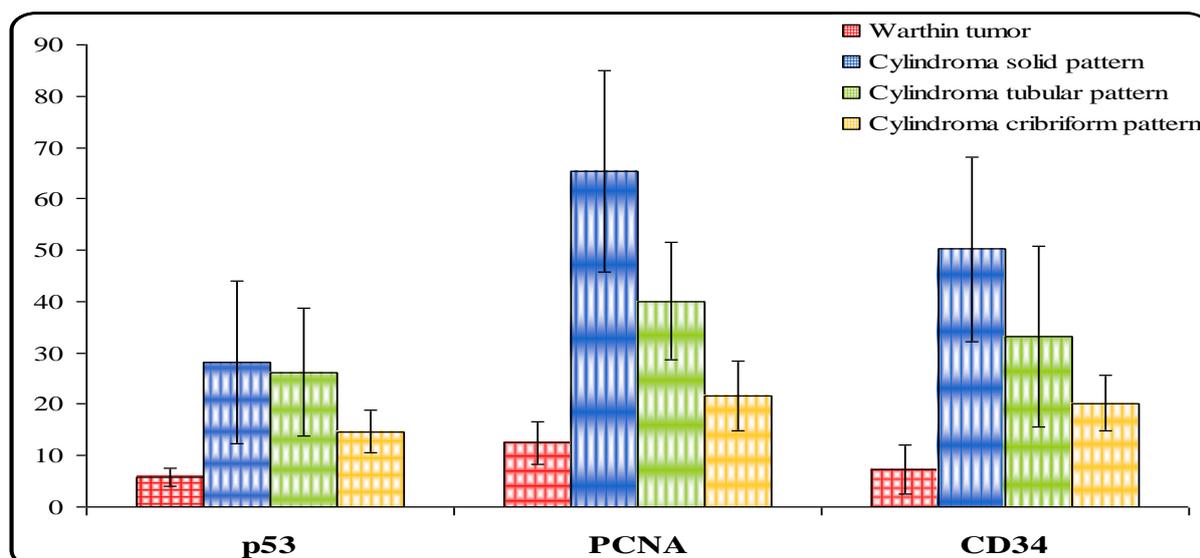
Figure 4: Bar chart representing mean of p53, PCNA & CD34 expression in Warthin's tumor & different types of cylindroma (solid, tubular & cribriform pattern).

pattern), in regard to of p53 expression where P value was (0.991, 0.204, 0.318) respectively Table 3 , Fig. 4).

4.Discussion

In the present study, p53 and PCNA expression for Warthin's tumor tissue sections showed weak positive staining in nucleus of neoplastic epithelial cells and in the lymphoid cells, similar results were obtained by Yang et al,[22] who reported that Warthin's tumor had actively dividing cells in the lymphoid follicles in addition to a proliferating fraction of cuboidal cells in the basal epithelial component. It is suggested that growth occurs in the basal layer of the epithelium and in the lymphoid cells, and that an interaction between the two cell groups is responsible for the tumor growth. In contrast to Faur et al,[23] who detected the presence of p53 and PCNA positive nuclei in the tumoral cells of the epithelial component. In the lymphoid stroma of Warthin's we did not detect immunopositivity for this marker. In our study, there were significant differences among the mean numbers of p53 and PCNA in all types of ACC compared to Warthin's tumor (p ≤ 0.05), these results regarding PCNA and p53 expression confirms that Warthin's tumor has a slower proliferation rate compared to that of ACC and cell proliferation is related to tumor aggressiveness and prognosis. This result similar to the observations of Gallo et al,[24] described higher positivity for p53, 8 out of 10 cases for ACC of parotid gland. In contrast, Lazzals and Cleveland [25] compared p53 and ki67 expression in benign and malignant intra oral and perioral salivary gland tumors including ACC which showed low or no expression for p53 in 76.5% of the cases, also Al-Ani and Al-Azzawi (2011) [26] noted p53 expression was negative in 80% of ACCs, in 13% was low positive and in 6.7% was high positive, 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. Tumor growth and metastasis depends on its angiogenic activity. New blood vessels are essential for clonal expansion and formation of macroscopic tumors.[18] The properties of tumor cells to release angiogenic and antiangiogenic factors are essential for tumor-induced neovascularization.[19] Counting tumor blood vessels by immunohistochemistry is a common method for evaluating the angiogenic activity.[27] CD34 is a pan-

endothelial marker that stains both "newly formed" blood vessels and normal ones trapped within tumor tissues.[21] Tumors with high vascular density are associated with an increased metastatic potential and decreased survival. In this study, there were significant differences in the mean of CD34 expression in Warthin's and ACC (solid, tubular & cribriform pattern), where P value was < 0.05 which was in agreement with other investigations.[28-30] This indicates that development of blood vessels is associated with invasiveness and malignant behavior. It has also been stated that the stroma of Warthin's tumors always presents poor vascularization. The present study in agreement with Moghadam, et al. (2015) [31], who reported that vessels of ACC frequently formed a rim of capillaries around tumor nests. It seems that large blood vessels in ACC are needed to compensate for decreased angiogenesis. Zhang and Peng[28] stated that the microvessels around the solid tumor nests of ACC were much denser than that around cribriform and tubular nests. Microvessel density (MVD) in ACC had also significant correlation with tumor size, clinical stage, vascular invasion, recurrence, perineural invasion and distant metastasis. However, a study by Costa et al. [19] did not reveal any significant increase in MVD in metastatic group. In the present there were significant difference between (solid pattern & tubular pattern), (solid pattern & cribriform pattern) in regard to of PCNA expression where P value was <0.05, also there was significant difference between (solid pattern & cribriform pattern) in regard to of CD34 expression where P value was <0.05, these results may be an explained for the more malignant behavior of solid type than the other two types. These results in agreement with previous studies [25, 32- 34] that supports the concept of solid variant ACC as a high-grade tumor, this suggests that the over expression of these markers may be an excellent indicator of biologically more advanced tumors.

Conclusion

The expression of p53, PCNA & CD34 have been associated with aggressive tumor progression, and thus has been implicated as a predictive and prognostic marker and could provide useful prognostic markers for proliferative activity and good prognostic indicators for recurrence rate of cylindroma.

References

1. **Nagao T, Sato E , Inoue R, Oshiro H, Takahashi RH, Nagai T, et al**. Immunohistochemical analysis of salivary gland tumors. Application for Surgical Pathology Practice. Acta Histo chem Cytochem. 2012; 45: 269-82.

2. **Mylarappa DA, Haragannvar VC, Gopalkrishna AH, Reshma Joy A, Latha D, Archana VK.** Papillary cyst adenoma lymphomatosum: A case report and review. J Med Radiol Pathol Surg.2015; 1: 17–19.

3. **Faur A, Lazar E, Cornianu M, Dema A, Lazureanu C, Costi S, et al**. Malignant transformation of the epithelial component in Warthin's tumor. Rev RomânăMed Lab. 2009; 17: 51–56 .

4. **Ellis GL, Auclair PL.** Tumours of the salivary glands. 3rd ed (1996). Armed Forces Institute of Pathology, Washington.

5. **Eveson JW, Cawson RA.** A review of 2410 cases with particular reference to histological types, site, age and sex distribution. J Pathol. 1985; 146: 51–58 .

6. **Howard DR, Bagley C, Batsakis JG.** Warthin's tumor: A functional immunologic study of the lymphoid cell component. Am J Otolaryngol. 1982; 3: 15–19 .

7. **Barnes L, Eveson JW, Reichart P, Sidransky D.** World Health Organization Classification of Tumours Pathology and Genetics of Head and Neck Tumours (2005). International Agency for Research on Cancer (IARC), Lyon, France.

8. **Van der Wal JE, Davids JJ, van der Waal I.** Extraparotid Warthin's tumors – Report of 10 cases. Br J Oral Maxillofac Surg . 1993; 31: 43–44.

9. **El-Naggar AK, Huvos AG.** Adenoid cystic carcinoma.In: Barnes L, Eveson JW, Reichart P, Sidransky D (eds). WHO Classification of Tumours: Pathology and Genetics of Head and Neck Tumours. IRAC Press: Lyon, 2005, pp 221–222.

10. **Regezi JA, Sciuba JJ.** Oral Pathology: Clinical Pathologic Correlations, 5th edn (2008). Saunders: St Louis, pp 207–9.

11. **Chen AM, Bucci MK, Weinberg V, Garcia J, Quivey JM, Schechter NR, et al.** Adenoid cystic carcinoma of the head and neck treated by surgery with or without postoperative radiation therapy: prognostic features of recurrence. Int J Radiat Oncol Biol Phys. 2006; 66: 152–59.

12. **Garden AS, Weber RS, Morrison WH, Ang KK, Peters LJ.** The influence of positive margins and nerve invasion in adenoid cystic carcinoma of the head and neck treated with surgery and radiation. Int J Radiat Oncol Biol Phys. 1995; 32: 619–26.

13. **Mendenhall WM, Morris CG, Amdur RJ, Werning JW, Hinerman RW, Villaret DB.** Radiotherapy alone or combined with surgery for adenoid cystic carcinoma of the head and neck. Head Neck. 2004; 26: 154–62.

14. **Cotter TG.** Apoptosis and cancer : the genesis of a research field. Nat Rev Cancer.2009; 9:501- 7.

15. **Tarakji B, Baroudi K, Hanounch S, Nassani MZ, Alotaibi AM, Kharma MY, et al.** Expression of P21 independent on or independent of P53 in carcinoma ex pleomorphic adenoma (undifferentiated and adenocarcinoma types) . Pol J. Pathol. 2012; 63 :286-91.

16. **Bau DT, Tsai MH, Lo YL, Hsu CM, Tsai Y, Lee CC, et al.** Association of P53 and P21 (CDKN1A/WAF1/CIP1) Polymorphisms with oral cancer in Taiwan patients. Anti Cancer Res. 2007; 27: 1559-64.

17. **Alves FA, Perez DE, Almeido OP, Lopes MA , Kowalski LP.** Pleomorphic adenoma of the submandibular gland: clinicopathological and immunohistochemical features of 60 cases of Brazil. Arch Otolaryngol. Head – Neck Surg. 2002; 128: 1400-3.

18. **Tadbir AA, Pardis S, Ashkavandi ZJ, Najvani AD, Ashraf MJ, Taheri A, et al**. Expression of Ki67 and CD105 as proliferation and angiogenesis markers in salivary gland tumors. Asian Pac J Cancer Prev. 2012; 13: 5155-59.

19. **Costa AF, Demasi AP, BonfittoVL, BonfittoJF, Furuse C, Araújo VC, et al.** Angiogenesis in salivary carcinomas with and without myoepithelial differentiation. Virchows Arch. 2008; 453: 359-67.

20. **Shieh YS, Hung YJ, Hsieh CB, Chen JS, Chou KC, Liu SY.** Tumor-associated macrophage correlated with angiogenesis and progression of mucoepidermoid carcinoma of salivary glands. Ann Surg Oncol. 2009; 16: 751-60.

21. **Soares AB, Juliano PB, Araujo VC, Metze K, Altemani A.** Angiogenic switch during tumor progression of carcinoma ex-pleomorphic adenoma. Virchows Arch. 2007; 451: 65-71.

22. **Yang L, Hashimura K, Qin C, Shrestha P, Sumitomo S, Mori M.** Immunoreactivity of proliferating cell nuclear antigen in salivary gland tumours: an assessment of growth potential. Virchows Arch A Pathol Anat Histopathol. 1993; 422: 481-86.

23. **Faur AC, Sas I, Motoc AG, Cornianu M, Zamfir CL, Lazăr DC, et al.** Ki-67 and p53 immunostaining assessment of proliferative activity in salivary tumors. Rom J Morphol Embryol. 2015; 56:1429-39.

24. **Gallo O , Franchi A, Bianchi S, Boddi V, Giannelli E, Alajmo E.** p53 oncoprotein expression in parotid gland carcinoma is associated with clinical outcome. Cancer.1995; 75: 2037-44.

25. **Lazzaro B, Cleveland D.** p53 and ki 67 antigen expression in small oral biopsy specimens of salivary gland tumors. Oral Surg Oral Med Oral Pathol Oral Endod. 2000; 89: 613-17.

26. **Al-Ani L, Al-Azzawi L.** Evaluation of Immunohistochemical Expression of p53 and PCNA in Pleomorphic Adenoma, Mucoepidermoid and Adenoid Cystic Carcinomas of Salivary Glands. Tikrit Journal for Dental Sciences. 2013; 1: 1-8.

27. **Shieh YS, Hung YJ, Hsieh CB, Chen JS, Chou KC, Liu SY.** Tumor-associated macrophage correlated with angiogenesis and progression of mucoepidermoid carcinoma of salivary glands. Ann Surg Oncol. 2009; 16: 751–60.

28. **Zhang J, Peng B.** NF-kappa B promotes iNOS and VEGF expression in salivary gland adenoid cystic carcinoma cells and enhances endothelial cell motility in vitro. Cell Prolif. 2009; 42: 150–61.

29. **Luukkaa H, Laitakari J, Vahlberg T, Klemi P, Stenbäck F, Grénman R.** Morphometric analysis of CD34-positive vessels in salivary gland adenoid cystic and mucoepidermoid carcinomas. J Oral Pathol Med. 2009; 38: 695–700.

30. **Vidal MT, de Oliveira Araújo IB, Gurgel CA, Pereira Fde A, Vilas-Bôas DS, Ramos EA, et al.** Density of mast cells and microvessels in minor salivary gland tumors. Tumour Biol. 2013; 34: 309–16.

31. **Moghadam SA, Abadi AM, Mokhtari S.** Immunohistochemical analysis of CD34 expression in salivary gland tumors. J Oral Maxillofac Pathol. 2015; 19: 30-3.

32. **El-Nagdy S, Salama NM, Mourad MI.** Immunohistochemical clue for the histological overlap of salivary adenoid cystic carcinoma and polymorphous low-grade adenocarcinoma. Interv Med Appl Sci. 2013; 5: 131-39.

33. **Chundru NS, Amudala R, Thankappan P, Nagaraju CD**. Adenoid cystic carcinoma of palate: A case report and review of literature. Dent Res J 2013; 10: 274-78.

34. **Zarbo RJ.** Salivary gland neoplasia: a review for the practicing pathologist. Mod Pathol. 2002; 15: 298-323.