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Research Article

Expression of Pcna in Salivary Gland Adenoid Cystic Carcinoma and Its Correlation with Clinicopathological Parameters

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Abstract:

Adenoid cystic carcinoma (ACC) is a one of the malignant salivary gland tumors in the head and neck region. Although complete surgical resection and addition radiotherapy have been shown to improve long term survival, the prognosis of ACC remains poor hence early diagnosis may diminish it. Aim. The aim of this study is to determine expression of PCNA in salivary gland adenoid cystic carcinoma and its correlation to clinicopathological parameters. Methods: the study was conducted by identifying the cases of salivary gland adenoid cystic carcinoma between 2013 – 2015. Clinical information was collected regarding age, gender, location, and histopathological grading of tumor. The expression of PCNA was assessed by using immunohistochemistry. Results. A total of 23 cases of salivary gland adenoid cystic carcinoma were identified. There was no significant difference between immune expression of PCNA and clinical parameters although there was a very strong correlation between expression of PCNA and histopathological grading ($p<0.01$). Conclusion. There was a correlation between expression of PCNA and histopathological grading in salivary gland adenoid cystic carcinoma. Thus, PCNA expression can be used to predict the prognosis of ACC.

Keywords: adenoid cystic carcinoma (ACC), clinical parameter, immunohistochemistry, PCNA.

INTRODUCTION

Adenoid cystic carcinoma (ACC) is one of the malignant salivary gland tumors in head and neck region.[1] The clinical and pathological characteristics include slow growth, perineural invasion, distant metastasis, and potential local recurrence.[1,2] It comprises 5% to 10% of all salivary gland tumors. It is mostly located in minor salivary glands (31%) which is nearly half of all intra oral ACC occurs in the palate.[3,4] ACC may occur at any age, although most patients are middle aged or older, the peak incidence is in the fifth and sixth decades. It is seen equally in men and women, when found in minor salivary glands.[3,4] Three histopathological type of ACC are known, namely, the cribriform (glandular), tubular, and solid type. All types may occur either separately or together in the same tumor, the solid type is the most aggressive among the three.[5]

Proliferating cell nuclear antigen (PCNA), is a 36 KD protein located on the chromosome 20p7. PCNA is a DNA clamp that acts as a polymerase factor in DNA-δ and is used for replication and expression during cell cycle. This PCNA is in the form of homo-trimer surrounding the DNA and has a functions as a framework for proteins to enter in the process of DNA replication, DNA repair and chromatin remodeling. PCNA is a nuclear protein expressed in the cell nucleus during the DNA synthesis phase of the cell cycle.[4,6] In malignancy, the detection of this protein immunohistochemically is a useful marker indicating the aggressiveness of a tumor and is associated with prognosis and survival rate.[4,7] PCNA expression is also present in salivary gland tumors, and the more aggressive of a tumor, its expression is increased and the prognosis is getting worse. In neoplasia, PCNA expression is regarded as a marker for the cellular proliferation rate, the detection of its expression is usually connected with a high frequency of metastasis and a unfavorable prognosis.[4,8] This research will use immunohistochemistry using a monoclonal antibody PCNA. By using the PCNA monoclonal antibody, cell proliferation can be detected because the antibody will only be expressed in cell proliferation.[4,8,9,10] Monoclonal antibody will react positively to the nucleus of tumor cells and this state is called immune reactive. Prognosis of a tumor is closely related to biological behavior of the tumor. The faster and higher the degree of cells tumor proliferation, the tumor will be more aggressive and its prognosis is getting worse.[4,8]

This study was conducted to evaluate PCNA as an immunohistochemistry (IHC) marker in ACC and assess the correlation between PCNA and clinical parameters data (age, gender, and tumor location) and its histopathological grading in determining the prognosis.

MATERIAL AND METHOD

This research is constituted retrospective conducted between 2013 to 2015. An absolute confidentiality of the patients' information was maintained for ethical purposes and an ethical approval was obtained from instructions in which the study was carried out.

Patients diagnosed with salivary gland adenoid cystic

carcinoma at Department of Pathology Dr. Hasan Sadikin Hospital were identified. Clinical records for the patient were reviewed and information about their age, gender, location and histopathological grading of tumor were collected. The microscopic features were reviewed based on hematoxylin and eosin-stained section for routine histological examination. Histopathological grading consisted of the following categories: grade I, if there was a tubular and cribriform pattern without a solid component, grade II, when only cribriform pattern was present or mixed with a solid component comprising less than 30% and grade III, when there was a tumor with a predominantly solid component.[1] Paraffin-embedded tissues were sectioned (4 μm) and serially collected on glass slides coated with 2% 3-aminoprophyltriethoxysilane. The following monoclonal antibodies were used: anti PCNA

(Biocare, USA) is diluted 1:50. After being washed with Tris-buffered saline, the sections were labeled with streptavidin-biotin (LSAB kit K0492; Dako, Carpinteria, CA) and then incubated with 3'3-diaminobenzidine (K3468, Dako) for 2 to 5 minutes at room temperature. The results of the immunohistochemistry staining could be indicated as positive ones if there was a brownish color expression in the nucleus of tumor cells. The results of the staining were compared to both of the positive control using squamous cell carcinoma that was known to be positive with PCNA antibody and also the negative control that was obtained by omission of primary antibody, which were substituted with secondary antibody / "non-immune serum".

For the tissue evaluation of PCNA, each slide was scored based on the percentage of positively stained malignant nuclei. To determine the relationship of PCNA immune-expression with clinical characteristics (age, gender and location) and its histopathological grading, PCNA immune expression was categorized as: (+) low <25%, (++) moderate 26-50% and (+++) high >50% of positive tumor cells, counting at least 1000 cells under microscope CX-21 (Olympus America Inc. Melville, NY 11747) at high magnification (40x objective and 10x eyepiece).[11,12] Intensity of staining was not considered for evaluation. The quantitative analysis of PCNA positive cells were counted by two independent examiners. Both microscopically and immunohistochemically analysis performed blindly without any clinical information. After that, to determine the relationship of PCNA immune expression with clinical characteristics (age, gender and location), the data was classified into two categorized depend on cut-off point 50 % of positive tumor cells as >50%, and ≤50% of positive tumor cells.[13]

Statistical analysis was performed using the SPSS version 21.0 for windows. PCNA expression, as well as their correlation with clinical parameters (gender, age, and location) and histopathological grading were performed by Chi-square test. Significant differences were accepted at P<0.05.

RESULT.

Clinical Parameter and PCNA distribution in ACC

Among 23 cases of ACC, there were 13 males (56.5%) and 10 females (43.5%) with a mean age of 48,44 years old. (range 31-68 years). Female to male ratio was 1: 1,3. The location of tumors was various, namely, 4 (17%) cases in mayor salivary glands; 3 (13%) cases were located in submandibular glands, 1 (4%) case in parotid gland and 19 (83%) cases in minor salivary gland; 13 (56%) cases in palate, 2 (9%) cases in buccal mucosa, 2 (9%) cases in tongue, 2 (9%) cases in floor of mouth. (Table 1)

Table 1. Characteristics of Patients with ACC

No.	*Variable*	*n*	*%*
1	**Median of age (31-68 years, X = 48,44 years)**		
	<50	10	43,5%
	≥50	13	56,5%
2	**Gender**		
	Male	13	56,5%
	Female	10	43,5%
3	**Location**		
	Mayor salivary gland, 4 cases (17%)		
	Submandibular gland	3	13%
	Parotid gland	1	4%
	Minor salivary gland, 19 cases (83%)		
	Palate	13	56%
	Buccal mucous	2	9%
	Tongue	2	9%
	Mouth floor	2	9%

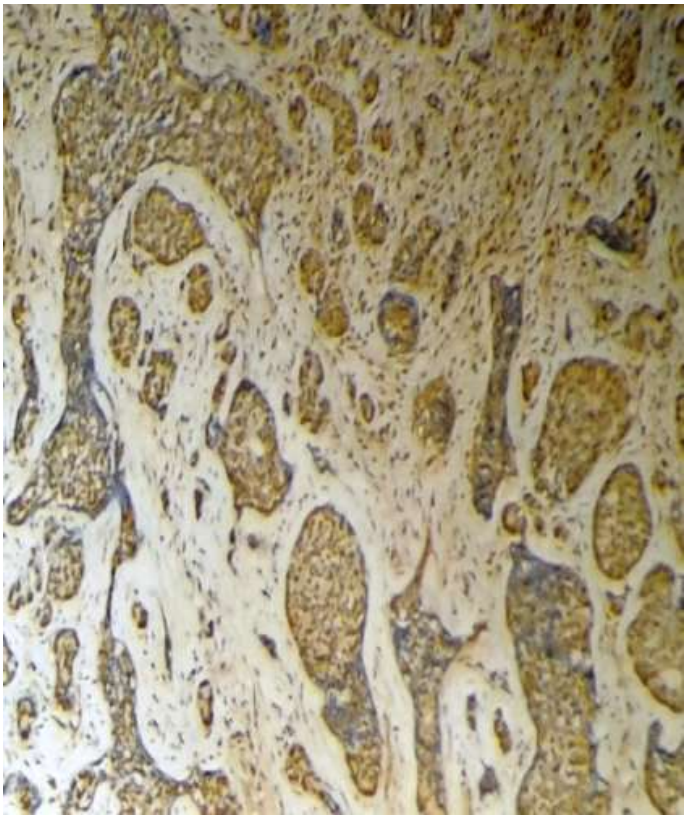
PCNA distribution in ACC

The percentage of PCNA positive cells in the tissues from 23 patients, there were 7 males with positive tumor cells ≤50% and 6 males with positive tumor cells > 50%. Nine females with positive tumor cells ≤50% and 1 female with positive tumor cells > 50%. There were 10 persons below 50 years with 8 persons ≤ 50% positive tumor cells and 2 persons with > 50% positive tumor cells, and 13 persons above 50 years with 8 persons ≤ 50% positive tumor cells and 5 persons with > 50% positive tumor cells. There were 2 cases in mayor salivary gland with ≤ 50% positive tumor cells and 2 cases with > 50% positive tumor cells, and 14 cases in minor salivary gland with ≤ 50% positive tumor cells and 5 cases with > 50% positive tumor cells.

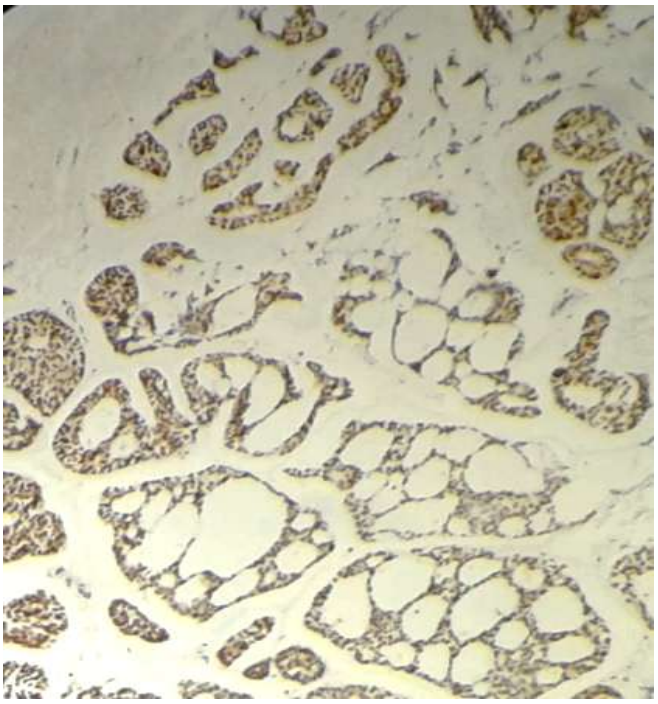
Significant statistical was observed between PCNA expression and clinical parameters. No significant statistical correlation was found between PCNA expression and clinical parameters like age (p= 0.340), gender (p= 0.062), location of tumor (p= 0.349). (Table 2 and figure 1)

Table 2. Distribution of PCNA and their correlation with clinical parameters in ACC

No	*Characteristic*	*Expression of PCNA (%)*		*n*	*p*
		< 50%	*> 50%*		
1	**Gender**				0,062
	Male	7	6	13	
	Female	9	1	10	
2	**Median of age (31-68 years, X = 48,44 years)**				0,340
	<50	8	2	10	
	≥50	8	5	13	
3	**Location.**				
	Major SG (submandibular, parotid)	2	2	4	0,349
	Minor SG (palate, buccal, tongue, mouth floor)	14	5	19	



The figure 1. A



The figure 1. B

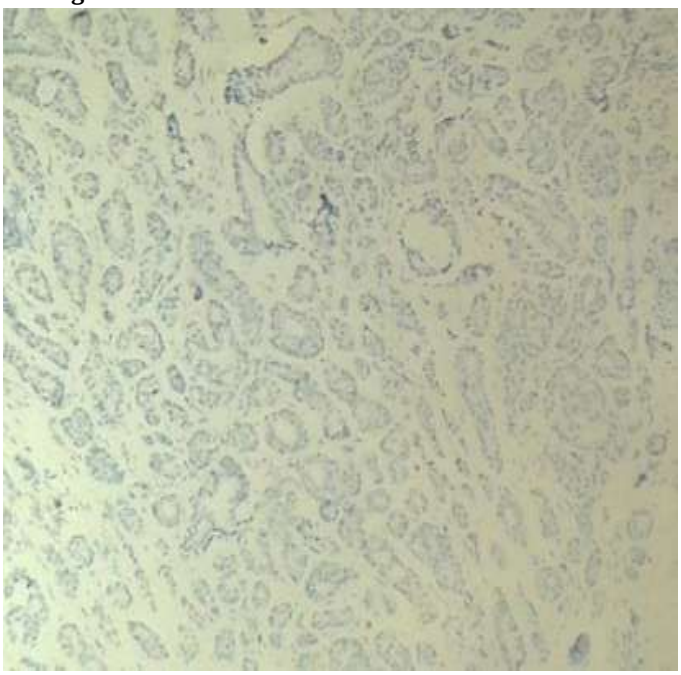


Figure 1. C

When studying the correlation between PCNA and histopathological grading, it was observed a positive and significant coefficient of correlation of Pearson r = 0.706 (p=0.000)

The figures 1 demonstrates PCNA expression in ACC consist of figure A that shows a solid type, figure B is cribriform type and figure C is a tubular type. In this research, gradation I adenoid cystic carcinoma was present in 10 cases, gradation II in 8 cases and gradation III in 5 cases. The immune expression of PCNA in tumor cells varied from ≤25% to > 50%, i.e. 6 cases +1 (≤25%),10 cases +2 (26% -50%),7 cases +3 (> 50%) as shown in table 3, figure 1 and figure 2.

Table 3. Distribution of PCNA and their correlation with histopathological grading in ACC

No	*Histopathological Grading*	*+1 <25%*	*+2 26-50 %*	*+3 >50 %*	*n*	*p-value*
1	Grading I	6	4	-	10	
2	Grading II	-	6	2	8	0.000*
3	Grading III	-	-	5	5	r = 0.706

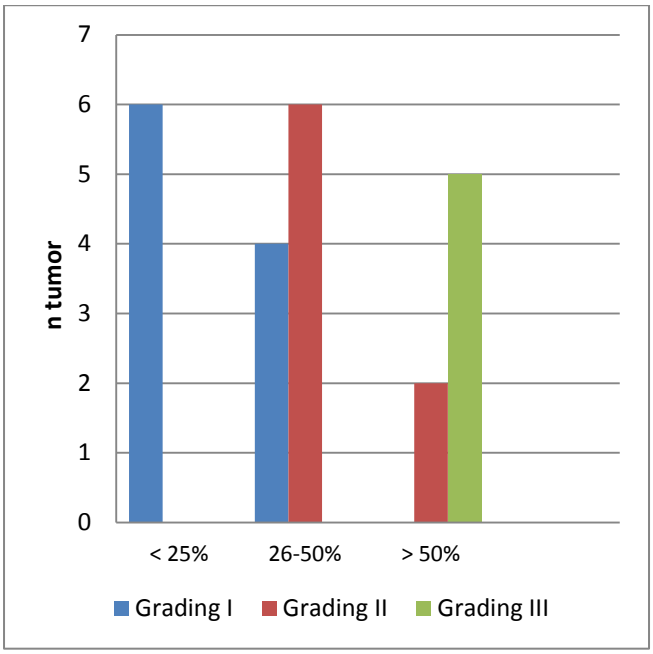


Figure2. Expression of PCNA

DISCUSSION

ACC is a rare tumor, constituting approximately 10% of all head and neck malignancies.[3,5,14,15] This kind of tumor is most common in the minor salivary glands, and the palate is the most common site.[2,15] In this study, 56% of tumor occurred in palate. In most studies a 1:1.3 male to female is observed for ACC.[15,16].

However, there are studies reporting a male predominance in ACC and others reporting an equal gender distribution.[3,13] Male to female ratio in this study was 1 : 0.77 showing male predominance. The mean age of patients was 48,44 years. This finding correlate with other references.[2,13] Cell proliferation is biological process that is essential to all living organisms due to its role in the growth and maintenance of tissue homeostasis. The control of this important process is dysregulated in some type of neoplasm, and the assessment of cell proliferation activity in tumours has become a common tool used by histopathology's expert to provide useful information for assessing diagnosis and clinical behaviour.[4] Today, the use of immunohistochemistry techniques, which are increasingly being applied in routine pathology.[4] PCNA is a marker protein that is necessary for DNA synthesis which is elevated during the G1/S phase of cell cycle.[4] PCNA expression may be used as a marker of cell proliferation. Various cell proliferation markers have been used in several studies as diagnostic and prognostic tools.[17] Currently, new markers are being added to evaluate cell proliferation, however, PCNA is still used as a marker of cell proliferation.[4] Furthermore, several studies have been performed to evaluate cell proliferation using PCNA. Many investigations of tumour-cell proliferative activity have used PCNA to evaluate cell proliferation in oral tumours.[4]PCNA is also a target for cancer therapy, and PCNA inhibitors are currently being developed as potential anti-cancer agents. Many authors have correlated the expression of PCNA with the aggressiveness and prognosis of ACC in salivary gland, but the results are still controversial.[7,12,15,17] Owing to the rarity of these tumours, there is little data on whether specific ethnic, geographic, or other factor predispose to the development of the disease.[2]

In many of tumors the proportions of tumor cells cycle are important in predicting outcome and have prognostic significance.[3] In most malignancies, the activity of carcinoma cells is generally considered to be related to degree of malignancy. More and more studies focused their case studies on PCNA expression and prognosis of the malignant tumors.[8,15] In salivary gland tumors, 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.[12] In all type of ameloblastoma and ameloblastic carcinoma, the percentages for PCNA is high.[4] Although cell proliferation is related to tumors aggressiveness and prognosis, there are few studies describing the expression of proliferative markers in salivary gland cancer.[7]The degree of proliferation of a tumor is closely related to the biological behavior of the tumor, so the faster and higher tumor proliferation, cause the more aggressive tumor and the prognosis is worse.[18]

In recent years, the development molecular biology has been done and show results very valuable. Various molecular markers have been identified and their relation to the development of tumors of the oral cavity has been discussed a lot.[19] PCNA is one of the tools that can be used for evaluation of this proliferative activity, the correlation between PCNA expression and survival probabilities of salivary gland tumor may make proliferation markers helpful tools in patient follow-up.[4,7] In this research, we attributed PCNA immuno-expression to clinical parameters (sex, age and location), but the results did not show any correlation between PCNA and clinical parameters. This result is also in accordance with the results of other references which have found that clinical parameters with an unfavorable effect on survival in ACC include age and location.[15] Likewise there was no significant difference between PCNA in major and minor salivary gland. Nevertheless, it is still unclear whether the major or minor salivary glands have a poorer clinical outcome.[15] Some study reported that histological grading is associated with disease-free survival, recurrence, and have reported that ACC patients with solid type are more likely to

have worse prognosis.[2,3,15] There was a significant relationship between PCNA and histopathological grading in this research. This was in accordance with Stenner's research (2012) which states that, the PCNA expression is the prognostic factor in ACC.[7]

CONCLUSION

In conclusion, there was no significant correlation between PCNA immune expression with clinical parameters in adenoid cystic carcinoma, making clinical parameters unusable to determine the prognosis of adenoid cystic carcinoma. Although, there was a very strong correlation between expression of PCNA and histopathological grading in salivary gland adenoid cystic carcinoma. Thus, PCNA expression could be used to predict the prognosis. The correlation between PCNA expression and prognosis of ACC might make proliferation markers helpful tools in patient follow-up and targeted therapy in salivary gland tumor in the future.

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