

# GATA 3 Reactivity in Adenoid Cystic Carcinoma and Pleomorphic Adenoma of Salivary Glands

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## Abstract

**Objectives:** The main targets is evaluate clinicopathological distribution and comparison of immunohistochemical (IHC) expression of GATA 3 between in adenoid cystic carcinoma (ACC) and pleomorphic adenoma (PA) of salivary . **Design and Methods:** This retrospective analysis, open surgical specimens of salivary gland that were done , and consisting of 150 cases that divided into three groups: normal salivary gland tissues (n=50), adenoid cystic carcinoma(n=50), and pleomorphic adenoma(n=50). Clinical data (age, sex, previous diseases ), physical examination, and investigations are done for all patients. In addition. These 150 cases of paraffin embedding blocks of salivary tissue were stained by ordinary stained (hematoxylin and eosin staining ). Manual IHC staining of GATA 3 was done. **Results:** Age groups is younger for normal salivary gland tissues than PA, and ACC . Sex distribution was slightly increase in male in comparison to female in normal salivary gland tissues and PA. GATA 3 staining was showing more positivity in normal salivary gland tissues (43/50 patients) than PA (27/50 patients) and ACC (14/50 patients). This positivity in GATA 3 IHC staining were more in male sex, regarding 55.8% in normal salivary gland tissues than ACC 50% and PA 51.9% . Age group 31-40, 40-50 and 61-70 years were showing more positive GATA 3 IHC staining in normal salivary gland tissues, PA, and ACC respectively. there was insignificant association between GATA 3 IHC staining with either age groups or sex groups distribution . **Conclusion:** GATA3 IHC is good marker and can used for searching salivary gland tumor origin, assisting in diagnosis Salivary gland tumors subtypes but GATA3 IHC staining has no association to specific sex or age groups in Salivary gland tumors. Usage of surgical operations for histopathological specimens preparation are better and give good efficiency for IHC markers staining.

**Keywords:** pleomorphic adenoma, adenoid cystic carcinoma, GATA-binding protein 3 , immunohistochemistry.

## Introduction

Salivary gland tumors (SGT) are infrequent diseases, 5% from all head and neck neoplasm<sup>(1)</sup>, the its incidence about 1.7/100,000 people in united states of America (USA) <sup>(2)</sup>, and these tumors grow from one of the major or minor salivary glands<sup>(3)</sup> .

SGT are characterize by various histopathological pictures between people and in the same patients, and treatment lines are also differs between the patients<sup>(4)</sup>. These tumors show many problems regarding their studying because difficult in getting their patients specimens, little amount of both cell line culture and animal patterns<sup>(5)</sup>.

Pleomorphic adenoma (PA) and Adenoid cystic carcinoma (ACC) are commonest benign and malignant neoplasm of Salivary gland respectively<sup>(3,6-7)</sup> .

ACC is characterized by basaloid cancer and consisting of mixed epithelial and myoepithelial tissues with different histopathological pictures<sup>(3)</sup>. ACC is 2<sup>nd</sup> main cancer (about 10%) in SGT , originate approximately 60% of minor salivary tumor<sup>(8-9)</sup>, and its metastasis mainly (about 50%) to skeleton bone, hepatic and brain tissues<sup>(10)</sup>.

PA is described a different degree of capsulation and representing around 60% from the total SGT<sup>(3)</sup>, with several histopathological configurations that resulting in

diagnostic troubles<sup>(3,9)</sup>.

GATA-binding protein 3 (GATA3) is responsible for differentiating of many tissue through their transcriptional function<sup>(11)</sup> and existing in wide range of tissues such transitional cell carcinoma, SGT, epidermis of skin, T-lymphocyte, and basal layer of prostate<sup>(12)</sup>.

SGT should regarded as potential primary location when facing cancer positive for GATA3, but GATA3 cannot discriminate between breast and salivary tumor primary site<sup>(13)</sup>.

The main aims of this research is evaluate clinicopathological distribution and comparison of immunohistochemical (IHC) expression of GATA 3 between in adenoid cystic carcinoma and pleomorphic adenoma of salivary glands because few studies involved in IHC staining of salivary gland tumors

## Methods

This retrospective analysis of open surgical specimens (excisional biopsy) from salivary gland that were done in Al-Hilla Surgical Teaching Hospital, and consisting of 150 cases that divided into three groups: normal salivary gland tissues (n=50), adenoid cystic carcinoma (n=50), and pleomorphic adenoma (n=50).

Clinical data (age, sex, previous diseases), physical examination, and investigations are done for all patients. In addition, the all patients were explained about aim and usage of their surgical specimen for this study.

These 150 cases of paraffin embedding blocks of salivary tissue were stained by ordinary stained (hematoxylin and eosin staining), reexamined by two separated histopathologist to ensure definitive diagnosis of ACC and PA according to WHO Classification of Head and Neck Tumors (4th Edition)<sup>(14)</sup>.

Then, manual IHC staining of GATA 3 to paraffin embedding blocks of salivary tissue were done, according to Bio SB company (Mouse monoclonal antibodies with nuclear staining and BSB2670 catalog no. USA), supported result of this manual procedure by positive (stained with GATA3 score +3) and negative control of breast cancer, which performed with each IHC procedure

Interpretation of GATA 3 IHC staining were depending on nuclear staining of salivary tissue, using intensity grading as weak, moderate, and strong staining, and extension of staining distribution (percentage of nuclear staining of cells) were divided as diffuse ( $\geq 50\%$ ) or focal ( $< 50\%$ )<sup>(15)</sup>.

The SPSS program (version type 22) was used. Continuous documents were estimated as mean $\pm$ SD and categorical as numbers and percentages. Pearson's chi square ( $X^2$ ) and Fisher's Exact Test were showing the significant difference between categorical groups where  $P$  value  $< 0.05$  was significant.

## Results

A total 150 patients were included in this study, were divided into 50 patients for each normal salivary gland tissues, PA, and ACC.

Age groups is younger for normal salivary gland tissues ( $36.36 \pm 8.02$ ) than PA ( $56.94 \pm 11.73$ ), and ACC ( $57.42 \pm 14.41$ ). Sex distribution was slightly increase in male in comparison to female in normal salivary gland tissues (male 56% : female 44%) and PA (male 52% : female 48%) while they were the inverse distribution for and ACC (male 48% : female 52%) as illustrated in Table 1.

**Table 1 : Clinicopathological basic characteristic in normal and Salivary gland tumors**

Characteristics	Sex		Age (years)/ mean±SD	Total N.
	Male	Female		
Normal salivary gland tissues	28 (56%)	22 (44%)	36.36±8.02	50
Pleomorphic adenoma	26 (52%)	24 (48%)	56.94±11.73	50
Adenoid cystic carcinoma	24 (48%)	26 (52%)	57.42±14.41	50

GATA 3 immunohistochemical staining was showing more positivity in normal salivary gland tissues (43/50 patients) than PA (27/50 patients) and ACC (14/50 patients). This positivity in GATA 3 IHC staining were more in male sex, regarding 55.8% in normal salivary gland tissues than ACC 50% and PA 51.9% . There was no association between GATA 3 IHC staining and sex distribution ( $P$  value =0.638, 0.37, and 0.86 )as in table 2.

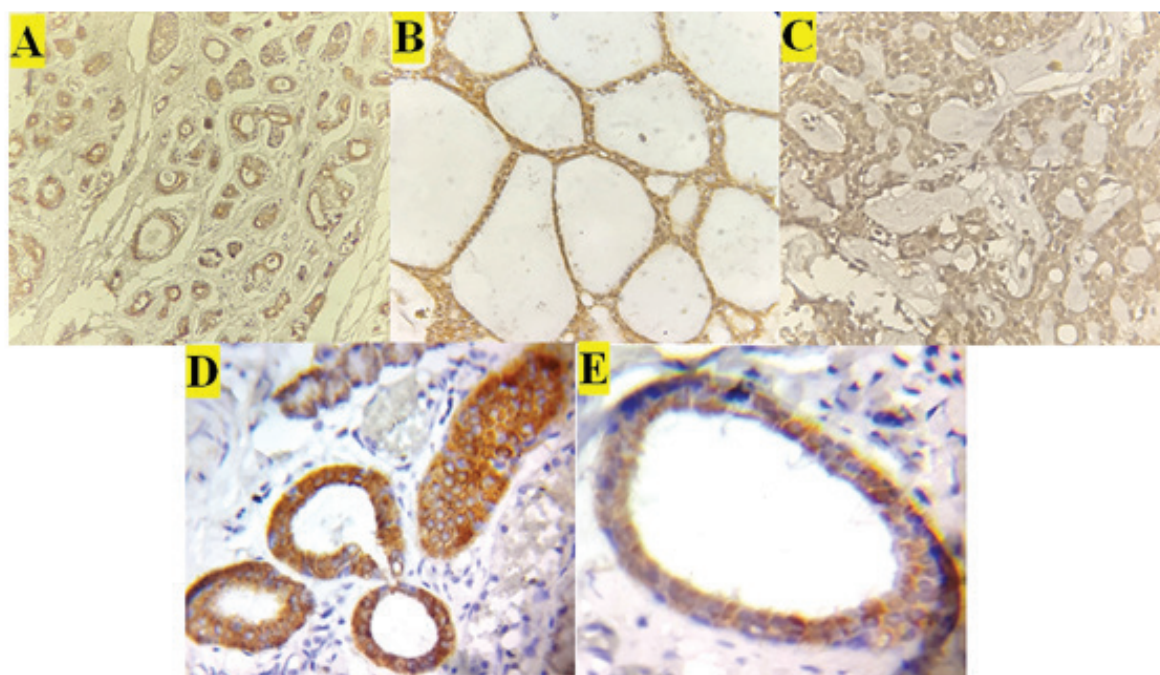
**Table 2 : Compare association of GATA 3 immunohistochemical staining between with normal and Salivary gland tumors according to sex groups.**

Parameters	Sex	GATA 3 immunohistochemical staining		P value
		Positive	Negative	
Normal salivary gland tissues	male	24(55.8%)	4(57.1%)	^0.638
	female	19( 44.2%)	3(42.9%)	
	Total	43(100%)	7(100%)	
Pleomorphic adenoma	male	14(51.9%)	12(52.2%)	^0.37
	female	13(48.1%)	11(47.8%)	
	Total	27(100%)	23(100%)	
Adenoid cystic carcinoma	male	7(50%)	17(47.2%)	*0.86
	female	7(50%)	19( 52.8%)	
	Total	14(100%)	36(100%)	
*Chi-square Test				
^Fisher exact test				

Age group 31-40, 40-50 and 61-70 years were showing more positive GATA 3 IHC staining in normal salivary gland tissues, PA, and ACC respectively. Also, there was insignificant association between GATA 3 IHC staining and age groups distribution ( $P$  value =0.253, 0.222, and 0.822 ) as in table 3.

**Table 3 : Compare association of GATA 3 immunohistochemical staining between with normal and Salivary gland tumors according to age groups.**

Parameters	Age groups	GATA 3 immunohistochemical staining		*P value
		Positive	Negative	
Inflammatory salivary gland tissues	10-20	6(14%)	0(0%)	0.253
	21-30	3(7%)	0(0%)	
	31-40	24(55.8%)	7(100%)	
	41-50	10(23.2%)	0(0%)	
	Total No.	43(100%)	7(100%)	
Pleomorphic adenoma	40-50	14(51.9%)	6(26.1%)	0.222
	51-60	4(14.8%)	8(34.8%)	
	61-70	7(25.9%)	7(30.4%)	
	71-80	2(7.4%)	2(8.7%)	
	Total No.	27(100%)	23(100%)	
Adenoid cystic carcinoma	30-40	2(14.3%)	6(16.7%)	0.822
	41-50	3(21.4%)	8(22.2%)	
	51-60	2(14.3%)	8(22.2%)	
	61-70	6(42.9%)	8(22.2%)	
	71-80	1(7.1%)	4(11.1%)	
	81-90	0(0%)	2(5.6%)	
	Total No.	14(100%)	36(100%)	
*Chi-square Test				



**Figure 1: GATA 3 immunohistochemical staining in the Salivary gland tumors : (A) Adenoid cystic carcinoma is diffuse and moderate immunoreactivity staining at 10X ; (B) Adenoid cystic carcinoma is diffuse and strong immunoreactivity staining at 40X; (C) Pleomorphic adenoma is showing diffuse and moderate immunoreactivity staining at 40X ; (D-E) Normal salivary gland is showing strong and moderate immunoreactivity staining respectively at 40X.**

## Discussion

In basis of clinical data, surgical operation for salivary glands are done for 150 cases, diagnosis are PA, ACC, and normal salivary gland. Sex distributions are 52% male (N= 78), 48% female (N=72), and age mean is 50.24 years old. Similar study, Fonseca et al<sup>(16)</sup> include 659 salivary gland tumor that show 54% females, 45% males, and mean age 57.6 years old.

GATA3 IHC staining have helpful for diagnosis, distinguish subtypes of SGT, and although it is related as marker for breast and urothelial cancer but its positivity give hint for searching for SGT<sup>(15)</sup>.

Among 150 patients, 43(86%) patients, 27(54%) patients, and 14 (28%) patients are confirmed to be GATA3 positive cases for normal, PA, and ACC respectively. Schwartz et al<sup>(15)</sup> research demonstrate a wide types of salivary tumors, resulting 13/34 (38%)

and 9/41 (22%) for PA and ACC respectively. Also, Adkins et al<sup>(17)</sup> is assessed GATA3 staining in ACC 45% (10/25).

In comparing to studies above, our results, there are a positive GATA3 staining cases in SGT, regarding ACC has 28% positive GATA3 and this result lies between results of two studies above, but PA has high positive GATA3 cases in comparing to Schwartz et al and may related to usage of tissue microarray and this technique has restriction points for tissue evaluation such as its small size, loss of core tumor, and presence of cancer heterogeneity<sup>(18-20)</sup>.

The association between sex groups and GATA3 staining is studies. Among PA and normal disease, shows males groups is more positivity than females with equal percentage in ACC. There are no significant difference between sex groups and GATA3 staining.



Also, comparison between age groups with GATA3 staining is involved, resulting age groups 31-40, 40-50, 61-70 years, are more positive for normal, PA, and ACC respectively, with no association between age groups with GATA3 staining for all three diseases.

In conclusion, GATA3 IHC is good marker and can be used for searching salivary gland tumor origin, assisting in diagnosis SGT subtypes but GATA3 IHC staining has no association to specific sex or age groups in SGT. Usage of surgical operations for histopathological specimens preparation are better and give good efficiency for IHC markers staining.

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