

Immunohistochemical Expression of BCL-2 in Adenoid Cystic Carcinoma of Salivary Gland Tumors

BCL-2 protein in Adenoid cystic carcinoma

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ABSTRACT

Objective: To determine expression of BCL-2 protein in Adenoid cystic carcinoma of salivary glands.

Study Design: Descriptive study.

Place and Duration of Study: This study was conducted at the Departments of Surgery, Lahore General Hospital, Mayo Hospital, and de'Montmorency college of Dentistry, Lahore from February 2017 to August 2017.

Materials and Methods: Thirty five cases of Adenoid cystic carcinoma (ADCC), of salivary glands were selected. Slides were prepared by routine hematoxylin and eosin (H & E) staining, as well as by Immunohistochemistry (IHC) for BCL-2. Grading of ADCC was done as low, intermediate and high grades on H&E sections. Scoring of BCL-2 expression was determined on BCL-2 immunohistochemical stained slides. Data was entered into SPSS version 21 and descriptive statistics were determined.

Results: In this study most common age group affected was 41-60 years age (40%), cases of ADCC were more common in female as compared to male (54%) Expression of BCL-2 was strongly positive in all cases of ADCC. In major salivary gland parotid glands was the most common site while in minor salivary glands most common site was palate. Majority cases reported as excisional biopsy (54.3%) with size 2-5cm (68.8%). Histopathologically 19 cases (54%) were categorized as high grade tumor. All cases showed expression of BCL-2 irrespective of the grade of the tumor.

Conclusion: BCL-2 protein is expressed in Adenoid cystic carcinoma. Its expression is helpful in grading small biopsies, predicting behavior, and planning target therapy of Adenoid cystic carcinoma

Key Words: BCL-2, salivary gland tumors, immunohistochemistry, Adenoid cystic carcinoma. Immunohistochemistry,

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INTRODUCTION

The World Health Organization describes ADCC as a basaloid tumor containing both epithelial and myoepithelial cells¹. It is the second most common malignant salivary gland tumor², and approximately 1% of all head and neck region malignancies³. It accounts for 10% of all salivary gland neoplasms⁴. Its frequency is much lower in major salivary glands as compared to minor salivary glands⁵. In the oral cavity, palate is the most common site (39.9%) and tongue is the second most common (19.8%).

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Among the major salivary glands, submandibular gland is the most common site followed by parotid gland, 15-30% and 2-15 % respectively⁶. Slow growth rate, perineural invasion and delayed onset of distant metastasis are the typical features of ADCC. It is ultimately fatal due to distant metastasis and late recurrence¹.

Accurate diagnosis depends upon the histological evaluation by precise method for malignant salivary gland tumors⁷. The histopathological diagnosis of these tumors is usually made through the assessment of histological architecture, cellular structure and differentiation, component of tumor stroma, growth pattern of the tumor borders, and along with the clinical information⁸. There are three growth patterns: the cribriform or glandular type, the tubular type and the solid type.⁹ Tumor is Graded as Low Grade (Tubular pattern), Intermediate Grade (cribriform pattern with < 30% solid component), and High Grade (>30% solid component).¹⁰ Perineural invasions also observed in this pattern which is a characteristic feature of ADCC¹¹.

MATERIALS AND METHODS

This is a descriptive study in which thirty five cases of ADCC of salivary glands were selected from Departments of Surgery, Lahore General Hospital, Mayo Hospital, and de'Montmorency college of Dentistry, Lahore from February 2017 to August 2017. Slides were prepared by routine hematoxylin and eosin (H&E) staining, as well as by Immunohistochemistry (IHC) for BCL-2. Grading of ADCC was done as low, intermediate and high grade. Scoring of BCL-2 expression was determined on BCL-2 immunohistochemical stained slides. BCL-2 immunoreactivity was divided into four groups as follows: Score Zero (0): Negative [When neoplastic cells stained less than 5%], score one (1): + weak positive (WP) [When neoplastic cells stained 5-19%], score two (2): ++ moderate positive [When neoplastic cells stained 20-50%] score three (3): +++ strong positive (SP) [When neoplastic cells stained more than 50%]. Observations were made on the basis of intensity of cytoplasmic staining. The intensity was graded in all

the cases with 0, 1, 2 and 3 to represent negative, weak positive, moderate positive and strong positive staining respectively. Care was taken to decrease the subjectivity by ensuring a) two observations per field area of slide and b) by intra-lesional comparison with a positive control³¹. Data was entered into SPSS version 21 and descriptive statistics were determined.

RESULTS

In this study most common age group affected was 41-60 years age (40%), cases of ADCC were more common in female as compared to male (54%) Expression of BCL-2 was strongly positive in all cases of ADCC. In major salivary gland parotid glands was the most common site while in minor salivary glands most common site was palate. Majority cases reported as excisional biopsy (54.3%) with size 2-5cm (68.8%). Histopathologically 19 cases (54%) were categorized as high grade tumor. All cases showed expression of BCL-2 irrespective of the grade of the tumor.

Table No.1: Different Immunostains and their expression in Adenoid Cystic Carcinoma reported in different studies

Expression of different Immunostains in ADCC			
Author name	Marker	Tumor	Remarks
Zhang et al., 2018 ¹²	Cathepsin D	ADCCs	74.1% expressed
Kintawati et el, 2017 ¹³	Ki67	ADCCs	As grade is increasing expression of Ki-67 is also increasing.
Iyogun et el., 2017 ¹⁴	Ki67, SMA	ADCCs	Both markers expressed strong positive expression (75% cases)
Fujii et el.,2017 ¹⁵	Ki67,MYB, MYC	ADCCs	High Ki-67 index: 24.2% cases MYB expression: 51.5% MYC expression: 63.3%
Bu et el.,2015 ¹⁶	Ki67,Cyclin D1, CD147, Slug,Survivin	ADCCs	strong expression of ki67in all growth patterns
Al-Azzawi, 2013 ¹⁷	Ki67, p53	ADCCs	Ki67 40 %; p53 aberration 73.3%.
Salehinejad et al., 2011 ¹⁸	HER2/Neu	ADCCs	46 % over expression of HER2/neu; significant in grades of ADCC.
West et al., 2011 ¹⁹	Myb	ADCCs	Myb can use to differentiate ADCC from its histology mimics.
Edwards et al., 2003 ²⁰	C-KIT	ADCCs, PLGAs	No role in differentiating between ADCC and PLGA, MA
Penner et al., 2002. ²¹	C-kit, Galectin-3	ADCCs	C-kit is 100 % in ADCC; Galectin -3 in ADCC is 88.8 %
Tsai et. el,2018 ²²	BCL-2 (BLM-s)	ADCCs	BCL-2 (BLM-s) shows a strong positive expression (nuclear staining) in ADCC
Zhu et el. 2018 ²³	BCL-2	ADCCs	31 out of 60 cases (51.67%) were positive for BCL-2
Jiang, 2014 ²⁴	BCL-2	ADCCs	60% positive expression in ADCC
Meer et al., 2011. ²⁵	BCL-2	ADCCs; PLGAs	High expression in the solid and cribriform patterns of ADCC
Xie et al., 2010 ²⁶	BCL-2	ADCC	Prognostic role in ADCC.
Al-Rawi et al., 2010 ²⁷	BCL-2	PA,MEC, ADCC	High expression was observed with greater size, higher grades and greater degree of invasion.
Carlinfante et al.,2005 ²⁸	BCL-2	ADCC	High expression of BCL-2 90%.
Norberg-Spaak et al., 2000 ²⁹	BCL-2	ADCC	No significant association was seen between BCL-2 and grades of ADCC
Soini et al., 1998 ³⁰	BCL-2	Salivary glands tumors (SGTs)	More expression of BCL-2 in Benign than malignant (SGTs).

Table No.2: Clinicopathological Characteristic of Adenoid cystic Carcinoma in Number (Frequency) and Percentage

Clinicopathological characteristics of ADCC	Number (f)	%age
Age		
20- 40	10	28.6
41-60	14	40.0
61-80	11	31.4
Total	35	100.0
Gender		
Male	16	45.7
Female	19	54.3
Total	35	100.0
Hospital		
Mayo hospital	15	42.9
Lahore General	9	25.7
de'Montmorency College of Dentistry/ PDH,	11	31.4
Total	35	100.0
Site		
Parotid Gland	13	37.1
Submandibular Gland	3	8.6
Sublingual Gland	2	5.7
Minor salivary gland on palate	10	28.6
Minor salivary gland on labial mucosa	2	5.7
Minor salivary gland on Buccal mucosa	5	14.3
Total	35	100.0
Laterality		
Right	10	28.6
Left	25	71.4
Total	35	100.0
Specimens		
Incisional	12	34.3
Excisional	19	54.3
Resection	4	11.4
Total	35	100.0
Size		
<1cm maximum diameter	1	2.9
1cm to 2 cm maximum diameter	4	11.4
2.1-5cm	24	68.6
> 5 cm in maximum diameter	6	17.1
Total	35	100.0
Mass		
Solid	35	100
Grade		
Low	4	11.4
Intermediate	12	34.3
High	19	54.3
Total	35	100.0
Expression of BCL-2		
+++ strong positive [staining in >50% of neoplastic cells]	35	100.0
Grades and +++ strong positive BCL-2 expression		
Low grade	4	11.42
Intermediate grade	12	34.28
High grade	19	54.28
Total	35	100

Table No.3: Comparison of BCL-2 Expression in ADCC with Different Studies

Sr. No	Authors Names & Years	Current Study
1	Jiang et al., 2014²⁴	
	ADCC (n)	35
	BCL-2 expression	ADCC 60% All cases of ADCC Showed expression 100 %
3	Manjunatha et al., 2011³²	
	ADCC (n)	21
	BCL-2 expression	All cases expressed with varying intensity: Mild 7 (33.3%), Moderate 6 (28.5%), SP 8(38%) Strong positivity in all pattern of ADCC
4	Meer et al., 2011²⁵	
	ADCC (n)	29
	BCL-2 expression	High positivity in solid and cribriform pattern Strong positivity in all pattern of ADCC
5	Xie et al., 2010²⁶	
	ADCC (n)	31
6	Al-Rawi et al., 2010²⁷	
	ADCC (n)	22
	BCL-2 expression	90 % 100 %
7	Carlinfante et al., 2005²⁸	
	ADCC (n)	21
	BCL-2 expression	ADCC expressed 90% ADCC expressed 100 %
8	Norberg-Spaak et al., 2000²⁹	
	ADCC (n)	31
	BCL-2 expression	Weak, intermediate positive and strong positive cases were found All cases were strong positive
9	Soini et al., 1998³⁰	
	BCL-2 expression	However all cases of ADCC did not show strong positive expression Strong positivity in all pattern of ADCC

ADCC: Adenoid cystic carcinoma, MEC: mucoepidermoid carcinoma, PLGA: polymorphous Low grade adenocarcinoma, BSGT: Benign Salivary Gland tumors, MSGT: Malignant salivary Glands Tumors, SP: Strong positive, IP: Intermediate Positive, WP: Weak positive

DISCUSSION

A study was published in 2014 by Jiang et al. (2014)²⁴ aiming to determine the expression of BCL-2 in ADCC. Expression of BCL-2 was 60% in ADCC in a total sample of 35 cases. In the current study all cases of ADCC expressed positivity of BCL-2 as strong positive

while in Jiang's²⁴ study it was only 60 %. Manjunatha et al., (2011)³² determined expression of BCL-2 in both benign and malignant SGTs as 57% and 78% respectively. In their study as well as in the current study all cases of ADCC were consistently positive for BCL-2. Carlinfante et al., (2005)²⁸ reported a high expression of BCL-2 (90%) in ADCC. Current study showed similar but somewhat higher expression of BCL-2.

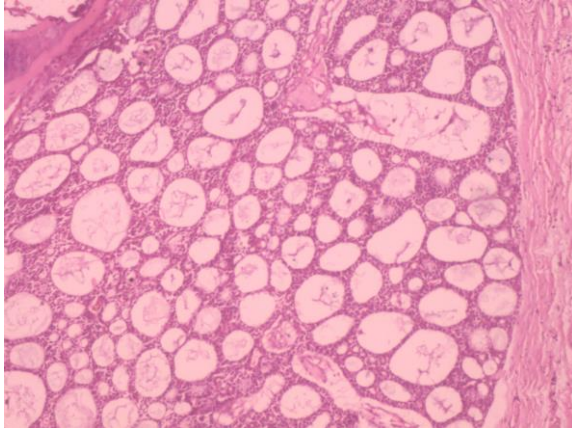


Figure No.1: H & E staining of intermediate grade Adenoid cystic carcinoma showing the cribriform pattern (X100)

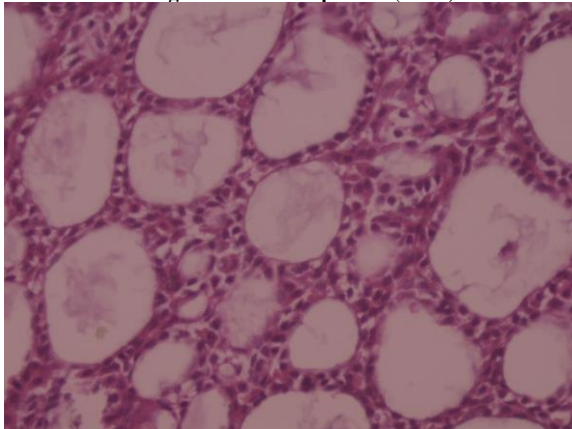


Figure No.2: H & E staining of intermediate grade ADCC showing the cribriform pattern (X400).

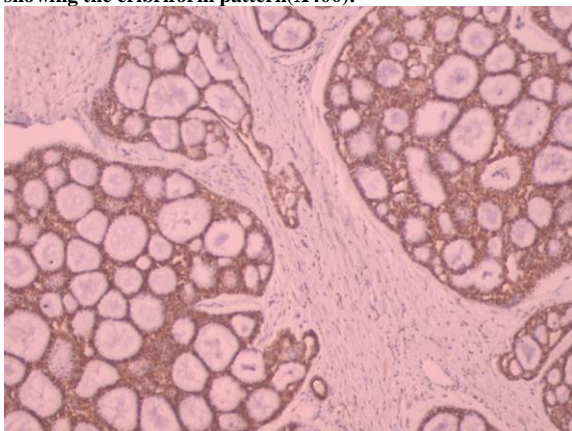


Figure No.3: BCL-2 immunostaining of Intermediate grade ADCC showing strong positive expression (X100).

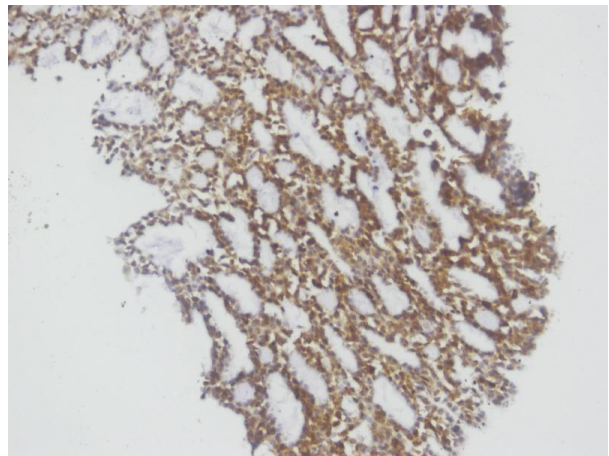


Figure No.4: BCL-2 immunostaining of intermediate grade ADCC showing strong positive expression (X200)

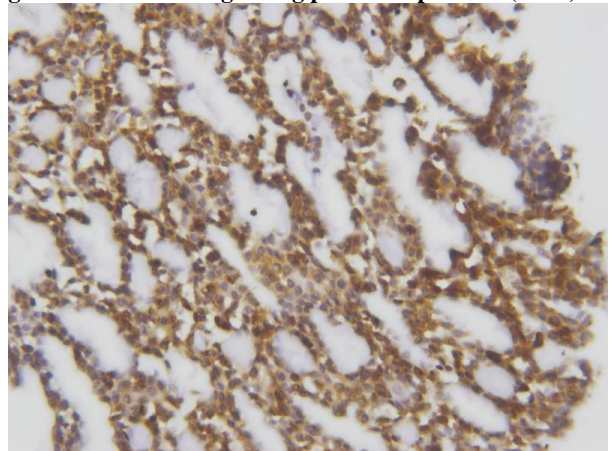


Figure No.5: BCL-2 immunostaining of intermediate grade ADCC showing strong positive expression (X400)

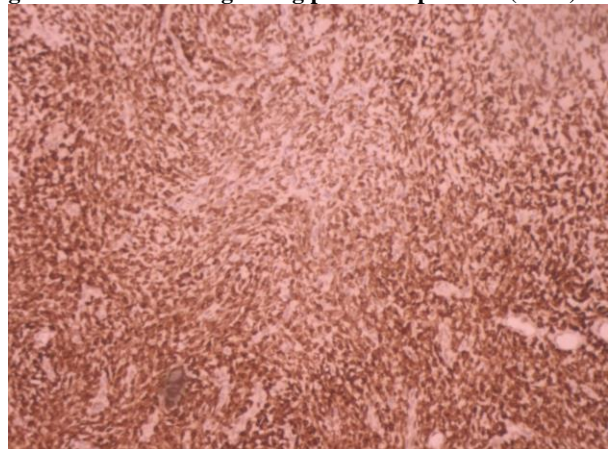


Figure No.6: BCL-2 immunostaining, control in tonsil showing strong positive expression (X100)

All cases of ADCC expressed BCL-2 expression but there was no weak and moderate positive staining group in this study. All cases of ADCC showed strong positive expression of BCL-2 in present study which is in contrast to Soini's³⁰ study where all cases of ADCC did not express strong positive expression. In another study by Norberg-Spaak et al. (2000)²⁹, biological

behavior of ADCC was determined in its three subtypes, solid, cribriform, and tubular, by using BCL-2. However, BCL-2 expression did not show any correlation with grade of ADCC and results were statistically insignificant ($p=0.49$). In our study, results are contrary to Norberg's study, where all types of ADCC were strongly positive for BCL-2 expression.

There were certain limitations of the current study which might have caused the difference in results, such as a limited sample size, owing to the rare nature of the tumor. Similarly, there was an unequal distribution of the numbers and grades of these tumors. The distribution of the tumors was also unequal in terms of the site of tumor. Further studies with larger sample size are recommended to find out the precise role of BCL-2 in ADCC.

CONCLUSION

Diagnosis of ADCC on routine staining (H&E) is difficult in some cases due to different histopathological variants which mimics with variants of other malignant salivary gland tumors such as Polymorphous Low Grade Adenocarcinoma. The BCL-2 protein has shown a strong positive expression in ADCC, regardless of grade. Its definitive role needs to be determined on large sample size. Positive expression of BCL-2 in this tumor can help in predicting the behavior of this tumor. BCL-2 has a definitive role in the carcinogenesis of ADCC of salivary gland tumor. In addition, molecular target therapy against BCL-2 can be planned in future for its better management.

Author's Contribution:

Concept & Design of Study:	Faiz Rasul
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Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

- Dalirsani Z, Mohtasham N, Pakfetrat A, Delavarian Z, Ghazi A, Rahimi S, et al. Adenoid Cystic Carcinoma of the Buccal Mucosa with Rare Delayed Frontal Bone Metastasis: A Case Report Dent Mater Tech 2016;5(3):208-12.
- Schwarz S, Müller M, Ettl T, Stockmann P, Zenk J, Agaimy A. Morphological heterogeneity of oral salivary gland carcinomas: a clinic pathologic study of 41 cases with long term follow-up emphasizing the overlapping spectrum of adenoid cystic carcinoma and polymorphous low-grade adenocarcinoma. Int J Clin Exp Pathol 2011; 4(4):336.
- Wiseman SM, Popat SR, Rigual NR, Hicks WL, Orner JB, Wein RO, et al. Adenoid cystic carcinoma of the paranasal sinuses or nasal cavity: a 40-year review of 35 cases. ENT: Ear, Nose & Throat J 2002;81(8).
- Dillon PM, Chakraborty S, Moskaluk CA, Joshi PJ, Thomas CY. Adenoid cystic carcinoma: a review of recent advances, molecular targets, and clinical trials. Head & Neck 2016;38(4):620-7.
- Yaga US, Gollamudi N, Mengji AK, Besta R, Panta P, Prakash B, et al. Adenoid cystic carcinoma of the palate: case report and review of literature. Pan Afri Med J 2016;24(1).
- Bradley PJ. Adenoid cystic carcinoma of the head and neck: a review. Current opinion in otolaryngology & head and Neck Surg 2004;12(2): 127-32.
- Ashraf MJ, Azarpira N, Khademi B, Shaghasemi S, Bagheri N. The value of immunohistochemical markers in pleomorphic adenoma and adenoid cystic carcinoma of the salivary gland. Iranian Red Crescent Med J 2009;11(4):414.
- 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 histochemica et cytochemica 2012;45(5):269-82.
- Stenner M, Klussmann JP. Current update on established and novel biomarkers in salivary gland carcinoma pathology and the molecular pathways involved. Eur Arch Oto-Rhino-Laryngol 2009; 266(3):333-41.
- Jaso J, Malhotra R. Adenoid cystic carcinoma. Archives of Pathol Lab Med 2011;135(4):511-5.
- Almeida LO, Guimarães DM, Martins MD, Martins MA, Warner KA, Nör JE, et al. Unlocking the chromatin of adenoid cystic carcinomas using HDAC inhibitors sensitizes cancer stem cells to cisplatin and induces tumor senescence. Stem cell Res 2017;21:94-105.
- Zhang M, Wu JS, Yang X, Pang X, Li L, Wang SS, et al. Over expression Cathepsin D contributes to perineural invasion of salivary adenoid cystic carcinoma. Frontiers Oncol 2018;8:492.
- Kintawati S, Darjan M, Yohana W. Analysis of Ki-67 expression as clinicopathological parameters in predicting the prognosis of adenoid cystic carcinoma. Dent J (Majalah Kedokteran Gigi) 2017; 50(4):205-10.
- Iyogun CA, Omitola OG. Immunohistochemical differentiation of Adenoidcystic Carcinoma from polymorphous Low-Grade Adenocarcinoma using Ki67 and Alpha-SMA 2017;4:2.
- Fujii K, Murase T, Beppu S, Saida K, Takino H, Masaki A, Ijichi K, et al. MYB, MYBL 1, MYBL

- 2 and NFIB gene alterations and MYC over expression in salivary gland adenoid cystic carcinoma. *Histopathol* 2017;71(5):823-34.
16. Bu LL, Deng WW, Huang CF, Liu B, Zhang WF, Sun ZJ. Inhibition of STAT3 reduces proliferation and invasion in salivary gland adenoid cystic carcinoma. *Am J Cancer Res* 2015;5(5):1751.
 17. Al-Azzawi LM. Expression of Ki67 and p53 as proliferation and apoptosis markers in adenoid cystic carcinoma. *J Baghdad Coll Dent* 2013;25(2): 76-9.
 18. Jafarian AH, Salehinejad J, Joushan B, Omidi AA. Immunohistochemical Study of HER2/neu Over expression in Adenoid Cystic Carcinoma of Salivary Glands. *Iranian J Pathol* 2011;6(2):86-92.
 19. West RB, Kong C, Clarke N, Gilks T, Lipsick J, Cao H, et al. MYB expression and translocation in adenoid cystic carcinomas and other salivary gland tumors with clinic-pathologic correlation. *Am J Surgical Pathol* 2011;35(1):92.
 20. Edwards PC, Bhuiya T, Kelsch RD. C-kit expression in the salivary gland neoplasms adenoid cystic carcinoma, polymorphous low-grade adenocarcinoma, and monomorphic adenoma. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiol Endodontol* 2003;95(5):586-93.
 21. Penner CR, Folpe AL, Budnick SD. C-kit expression distinguishes salivary gland adenoid cystic carcinoma from polymorphous low-grade adenocarcinoma. *Modern Pathol* 2002;15(7):687.
 22. Tsai MS, Hsieh MS, Huang HY, Huang PH. Nuclear immunoreactivity of BLM-s, a pro-apoptotic BCL-2 family member, is specifically detected in salivary adenoid cystic carcinoma. *Human Pathol* 2018 Sep 24.
 23. Zhu X, Yu Y, Hou X, Xu J, Tan Z, Nie X, Ling Z, Ge M. Expression of PIM-1 in salivary gland adenoid cystic carcinoma: Association with tumor progression and patients' prognosis. *Oncol Letters* 2018;15(1):1149-56.
 24. Jiang LC, Huang SY, Zhang DS, Zhang SH, Li WG, Zheng PH, et al. Expression of beclin 1 in primary salivary adenoid cystic carcinoma and its relation to Bcl-2 and p53 and prognosis. *Brazilian J Med Biological Res* 2014;47(3):252-8.
 25. Meer S, Singh S, Altini M. C-kit and bcl-2 are not useful markers in differentiating adenoid cystic carcinoma from polymorphous low-grade adenocarcinoma. *ISRN Pathol* 2011.
 26. Xie X, Nordgård S, Clausen OP, Boysen M. Prognostic significance of Bax and Bcl-2 expressions in adenoid cystic carcinoma of major and minor salivary glands of nasal and oral epithelium. *Open Otorhinolaryngol J* 2010;4:20-6.
 27. Al-Rawi, N. H., Omer, H. and Al Kawas, S. Immunohistochemical analysis of P53 and bcl-2 in benign and malignant salivary glands tumors. *J Oral Pathol. Med* 2010 39: 48-55.
 28. Carlinfante G, Lazzaretti M, Ferrari S, Bianchi B, Crafa P. P53, bcl-2 and Ki-67 expression in adenoid cystic carcinoma of the palate. A clinicopathologic study of 21 cases with long-term follow-up. *Pathol Res Pract* 2005;200(11-12): 791-9.
 29. NorbergSpaak L, Dardick I, Ledin T. Adenoid cystic carcinoma: Use of cell proliferation, BCL2 expression, histologic grade, and clinical stage as predictors of clinical outcome. *Head & Neck: Journal for the Sciences and Specialties of the Head and Neck* 2000;22(5):489-97.
 30. Soini Y, Törmänen U, Pääkkö P. Apoptosis is inversely related to bcl-2 but not to bax expression in salivary gland tumours. *Histopathol* 1998;32(1): 28-34.
 31. Mohammed HO, Ahmed JN, AL-Rawi NH. Immunohistochemical Expression of P53 and bcl-2 in benign and malignant salivary glands tumors. *Mustansiria Dent J* 2007;4(1):13-21.
 32. Manjunatha BS, Kumar GS, Raghunath V. Immunohistochemical expression of Bcl-2 in benign and malignant salivary gland tumors. *Med Oral Patol Oral Cir Bucal* 2011;16(4):503-7.