

## Biphasic cell patterns of salivary gland tumors in a tertiary care hospital

Aneesha Asok Kumar<sup>1</sup>, Rakshitha H.B<sup>2\*</sup>, Nanda Kishore Alva<sup>3</sup>, Sharon Roshin Reginald<sup>4</sup>

<sup>1,2,4</sup>Assistant Professor, <sup>3</sup>Professor, <sup>2-4</sup>Dept. of Pathology, <sup>1</sup>MES Medical College Perinthalmanna, Kolathur, Kerala <sup>2</sup>Adichunchanagiri Institute of Medical Sciences, Karnataka, <sup>3</sup>MS Ramaiah Medical College, Bangalore, Karnataka, <sup>4</sup>DM Wayanad Institute of Medical Sciences, Kerala, India

**\*Corresponding Author: Rakshitha H.B**

Email: dr.rakshatha05@yahoo.in

### Abstract

**Introduction:** Salivary gland tumors are relatively uncommon neoplasms of the head and neck region. These tumors mainly involve major salivary glands. Around 90% of the salivary gland tumors occur in the parotid glands followed by sub-mandibular glands. Tumour in the sublingual glands are uncommon. Malignant tumors generally occur in smaller salivary glands. Some of the salivary gland tumors show complex architecture and morphologic overlap. Some tumors show dual luminal-abluminal cell differentiation. Detailed histomorphologic analysis is required for proper diagnosis of these tumors which is very important for proper treatment and outcome.

**Objective:** To study the biphasic cell patterns in salivary gland tumors of complex architecture.

**Materials and Methods:** This study was conducted for a period of 4 years from January 2010 to December 2014 on the surgically resected salivary gland tumor specimens in the pathology department of M.S Ramaiah hospital in Bangalore, Karnataka.

Standard protocol was followed for surgical grossing of the salivary gland specimens sent to the pathology department. Paraffin sections of 5µm thickness were prepared and stained by haematoxylin and eosin (H & E) for histopathological study.

For all retrospective cases, histopathology reports, slides and paraffin blocks were retrieved and additional sections were made. A total of 66 cases were studied.

**Results:** Out of total 66 cases studied, benign salivary gland tumors were 38 cases (57%) and malignant tumors were 28 cases (43%). Salivary gland tumors were commonly seen between 40- 60 years. Female preponderance was seen in all tumors except warthin tumor. The most common benign tumor was pleomorphic adenoma and the common malignant tumor was mucoepidermoid carcinoma. Common site for these tumors was parotid gland with notable exception being adenoid cystic carcinoma, which showed predilection for the minor salivary glands. Most of the tumor studied showed dual cell differentiation with morphologic overlap. These tumors had myoepithelial cells with mixed cytomorphology and had mixed architectural pattern.

**Conclusion:** Knowledge about the salivary gland tumors with dual luminal-abluminal differentiation will help in making a proper diagnosis.

**Keywords:** Salivary gland neoplasms, Luminal-abluminal differentiation, Benign tumors, Malignant tumors.

### Introduction

Salivary gland tumors are uncommon neoplasms of the head and neck region. These tumors comprise approximately 1% of all neoplasms in the whole body. Malignant salivary gland neoplasms account for 3% to 6% of all head and neck cancers.<sup>1</sup>

Approximately 90% of the salivary gland tumors occur in the parotid gland followed by sub mandibular gland. Majority of these neoplasms are benign in nature. Malignant tumors commonly occur in smaller salivary glands whereas benign tumors are common in major salivary glands.

Salivary gland shows two tiered organization comprising luminal (acinar and ductal cells) and abluminal cells. Luminal cells are acinar and ductal cells. Abluminal cells are myoepithelial and basal cells. The secretory acini and intercalated ducts are wrapped by myoepithelial cells. Striated ducts and subsequent conducting portions are supported by basal cells.<sup>2</sup>

Most of salivary gland tumors arise from or differentiate towards the same cell lines i.e. epithelial (acinar and ductal), myoepithelial, and basal. This results in a considerable morphologic overlap. Also these cells can undergo metaplastic changes (i.e. oncocytic, sebaceous, squamous, clear, chondroid) which results in more difficulty in diagnosis.<sup>3</sup>

Salivary neoplasms often show more than one growth pattern and significant morphologic variability may exist within a single tumor and between different tumors.<sup>4</sup>

It is important to understand the basic cytoarchitectural features of each tumor type in particular whether the tumor shows dual luminal-abluminal cell differentiation, so that diagnosis can be made out logically through analysis of the cellular components, cell arrangement and extracellular components.

In these tumors, careful search and detailed morphologic analysis, are required to identify dual cell differentiation.<sup>5,6</sup>

### Objective

To study the biphasic cell patterns in salivary gland tumors of complex architecture.

### Materials and Methods

This study was done for 4 years from January 2010 to December 2014 in the Pathology department of M.S Ramaiah Hospital on the surgically resected salivary gland tumor specimens.

Standard protocol was followed for surgical grossing of the salivary gland specimens in every case. Paraffin sections

of 5µm thickness were prepared and stained by haematoxylin and eosin (H & E) for histopathological study.

For the retrospective cases, the histopathology reports, slides and paraffin blocks were retrieved and additional sections were made.

A total of 66 cases were studied.

Ethical clearance was obtained for the study.

### Inclusion Criteria

All epithelial origin, major and minor salivary gland tumors

### Exclusion Criteria

1. All inflammatory and cystic lesions of salivary glands.
2. All mesenchymal origin salivary gland tumors.
3. Metastasis in salivary glands.

## Results

### Distribution of Benign Tumor in Patients

Out of 66 cases, 38 cases (57%) were benign tumors. Most common benign salivary gland tumor was pleomorphic adenoma (Fig. 1) which constituted 29 cases (44%) and warthin tumor (Fig. 2) constituted 9 cases (13%).

### Distribution of Malignant Tumor in Patients

There were 28 cases (43%) of malignant salivary gland tumors. Most common malignant tumor was mucoepidermoid carcinoma (Fig. 3) which constituted 19 cases (29%) followed by 7 cases of Adenoid cystic carcinoma (11%) and 2 cases of acinic cell carcinoma (3%).

### Age and Gender Distribution in the Patients

Most of the patients with salivary gland tumors were between 41-60 years (Table 1). All salivary gland tumors were common in women except warthin tumor which was common in males (Table 2).

### Location of the Tumor

Most common location of salivary gland tumor was parotid (72.7%) except in case of Adenoid Cystic Carcinoma which was more common in minor salivary glands.

### Cytomorphology of Myoepithelial Cells in Benign Salivary Gland Neoplasms

Pleomorphic adenoma had myoepithelial cells in mixed morphology with commonest patterns being epithelial and spindle shape. Warthin tumor had bilayered epithelium, an outer basaloid and a luminal oncocytoid epithelial layer (Table 3).

### Cytomorphology of Myoepithelial Cells in Malignant Salivary Gland Neoplasms

Malignant salivary gland tumors also showed myoepithelial cells with mixed morphology. In Acinic cell carcinoma myoepithelial / basal cells were not appreciated (Table 4).

### Architectural Patterns in Benign Salivary Gland Neoplasms

In Pleomorphic adenoma main architectural pattern was mixed type with the most common pattern being reticular. Other patterns seen were myxoid, solid, cribriform, microcystic and mixed patterns (Table 5).

### Architectural Patterns in Malignant Salivary Gland Neoplasms

Most salivary gland tumors showed mixed architectural pattern. In mucoepidermoid carcinoma microcystic and solid patterns were predominant, whereas in adenoid cystic carcinoma and Acinic cell carcinoma solid pattern was predominant (Table 6).

### Types of Matrix in Different Salivary Gland Neoplasms

Most of the Warthin tumors had a rich lymphoid stroma. Majority of pleomorphic adenomas had myxoid matrix and adenoid cystic carcinomas had hyalinized matrix. Adenoid cystic carcinomas had predominantly hyalinised matrix (Table 7).

## Discussion

### Pleomorphic Adenoma

Pleomorphic Adenoma (Fig. 1) was the most common salivary gland tumor and representing 76.3% of benign and 43.9% of total salivary gland neoplasms.

Depending on the proportion of both epithelial and mesenchymal-like tissues pleomorphic adenoma were sub-classified into stroma-rich in 14 cases (48.2%), cell-rich in 05 (17.2%) and classic in 10 cases (34.4%) (Table 8). It has been suggested that recurrence is more frequent for stroma-rich tumor, which has a higher chance of spillage of mucoid stroma during operation. Highly cellular tumors, on the other hand, may be more prone to malignant change. Dardick et al<sup>7</sup> states that majority of classic tumors have 8q12 rearrangement whereas myxoid pleomorphic adenomas demonstrate normal karyotype. 12q13-15 rearrangement is also more common in cellular pleomorphic adenomas.

The prototypic histologic appearance of pleomorphic adenoma is tumor islands appearing to melt into the sea of chondromyxoid stroma. Mesenchymal-like elements of pleomorphic adenoma including chondroid and myxoid tissues are related to neoplastic myoepithelial cells migrating into the stroma. In the present study, the predominance of the myxoid and chondroid stroma was clear. Hyalinization of the stroma was present in 3 cases (10.3%) (Table 9). Prominent zones of hyalinization have been related to an aggressive behavior or malignant transformation of pleomorphic adenoma, however hyalinization as an isolated fact is not sufficient to predict this progression.<sup>8</sup>

Metaplasia within the stromal elements of benign mixed tumors is also a common finding. Stromal adipose was found in only 2 pleomorphic adenomas. Extensive stromal lipid metaplasia has also been described earlier in the literature, with fat comprising up to 90% of the tumor in some case reports.

Cutaneous adnexal differentiation is well-recognized in benign mixed tumors occurring in cutaneous sites. When

cutaneous adnexal differentiation occurs in salivary gland pleomorphic adenomas, it can present a diagnostic pitfall that must not be misinterpreted as carcinoma at biopsy, fine needle aspiration, or frozen section.<sup>9</sup> But in this study we didn't find any pleomorphic adenoma with cutaneous adnexal differentiation.

In our study epithelioid and spindle cells were the most frequent cell type (Table 10). As the occurrence of plasmacytoid cells is restricted to pleomorphic adenoma and myoepithelioma, their identification is of great diagnostic value. In this study we found 41.4% of pleomorphic adenoma with plasmacytoid morphology. Ellis et al suggests that these cells appear to be in transition from one form to the other form. Interestingly, recent studies shows that plasmacytoid cells in pleomorphic adenoma originate from luminal cells rather than myoepithelial cells.<sup>10</sup>

Extensive squamous metaplasia and keratin pearl formation could be mistaken for malignant tumors such as mucoepidermoid carcinoma and squamous cell carcinoma. Squamous metaplasia was found only in 1 out of 29 pleomorphic adenomas. Acellular keratin with basaloid and squamous epithelial cells without the characteristic matrix material that is normally diagnostic of a pleomorphic adenoma pose a diagnostic challenge.

Fine needle aspiration related changes like haemorrhage was noted in 2 cases. Focal areas of marked atypia and/or bizarre tumor cells were not noted in any of the pleomorphic adenomas. If found, atypical cytomorphology could be attributed to previous manipulation by biopsy or fine needle aspiration, especially within infarcted areas and necrotic tissue.<sup>9</sup>

### Warthin Tumor

Nine cases of warthin tumor (Fig. 2) were encountered with all of them presenting in parotid glands of elderly males. Warthin tumor had bilayered epithelium, an outer basaloid and a luminal oncocytoid epithelial layer.

### Mucoepidermoid Carcinoma

In our study we had all grades of mucoepidermoid carcinoma (Fig. 3). Out of 19, 8 mucoepidermoid carcinomas were predominantly cystic and solid in four cases. Using the AFIP criteria (Ellis and Auclair 1996),<sup>11</sup> tumors could be classified into low (eight cases), intermediate (seven cases), and high (four cases) grade.

**Table 1:** Age distribution in patients studied

Age in years	No. of patients	%
<20	4	6.1
20-30	9	13.6
31-40	15	22.7
41-50	18	27.3
51-60	12	18.2
61-70	7	10.6
>70	1	1.5
Total	66	100.0

### Adenoid Cystic Carcinoma

Adenoid Cystic Carcinoma (Fig. 4) constituted 10.6% of all tumors and 25% of malignant ones. The microscopic appearance of the tumor is heterogeneous, consisting of varying amounts of 3 distinct growth patterns, however, the cytology of the tumor cells themselves is relatively uniform. Three growth patterns for adenoid cystic carcinoma have been described: cribriform, tubular, and solid. In our study 6 out of 7(85.7%) showed cribriform pattern and 5(71.4%) showed solid pattern.

The percentages of each pattern form the basis of the grading system composed by Szanto et al.<sup>12</sup>

Grade I tumors contain only the tubular or cribriform growth pattern, grade II tumors contain cribriform or tubular growth with less than 30% solid component, and grade III tumors contain more than 30% solid component. Although the prognostic significance of this grading system has been questioned, the presence of a solid component has been a consistent predictor of poor prognosis in several series.<sup>13</sup>

In the present study out of 5 adenoid cystic carcinoma with solid pattern, only 2 had solid pattern more than 30% So only 2 adenoid cystic carcinomas were of Grade III and 3 cases were of Grade II.

Careful documentation of perineural invasion during staging is especially important as identification of perineural invasion has been shown to be of greater prognostic significance. In our study 5 out of 7 adenoid cystic carcinoma showed perineural invasion. Almost all the adenoid cystic carcinomas showed desmoplastic stroma.

### Acinic Cell Carcinoma

Differentiation of salivary gland acinic cell carcinoma (Fig. 5) from mucoepidermoid carcinoma can be diagnostically challenging as both may have prominent mucin production.

In contrast to classic acinic cell carcinoma and acinic cell carcinoma-high grade type, more than half of the tumors historically categorized as zymogen granule poor acinic cell carcinoma actually represent Mammary Analogue Secretory Carcinoma (MASC). There are subtle morphologic and immunophenotypic differences between true zymogen granule poor acinic cell carcinoma and MASC. ETV6 testing will separate these two groups more reliably. Although biologically distinct, it is still unclear whether this separation results in significant clinical impact.<sup>14</sup>

**Table 2:** Gender distribution according to tumor type

Gender	Pleomorphic adenoma	Warthin Tumor	Mucoepidermoid Carcinoma	Adenoid Cystic Carcinoma	Acinic Cell Carcinoma
Male	48.3%	88.9%	36.8%	42.9%	0%
Female	51.7%	11.1%	63.2%	57.1%	100%

**Table 3:** Cytomorphology of myoepithelial cells in benign salivary gland neoplasms

	Basaloid	Epitheloid	Clear	Spindle	Plasmacytoid	Mixed	Oncocytic
Pleomorphic adenoma	00	100%	27.6%	79.3%	41.4%	86.2%	4%
Warthin tumor	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen

**Table 4:** Cytomorphology of myoepithelial cells in malignant salivary gland neoplasms

	Basaloid	Epitheloid	Clear	Spindle	Plasmacytoid	Mixed
Mucoepidermoid Carcinoma	0	100%	68.4%	00	68.4%	68.4%
Adenoid cystic Carcinoma	100%	0	0	0	14.3%	14.3%
Acinic cell carcinoma	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen

**Table 5:** Architectural patterns in benign salivary gland neoplasms

	Myxoid	Solid	Reticular	Cribriform	Microcystic	Mixed
Pleomorphic adenoma	75.9%	37.9%	93.1%	6.9%	6.9%	72.4%

**Table 6:** Architectural patterns in malignant salivary gland neoplasms

	Myxoid	Solid	Reticular	Cribriform	Microcystic	Mixed
Mucoepidermoid Carcinoma	0	57.9%	5.3%	15.8%	68.4%	84.2%
Adenoid cystic carcinoma	0	71.4%	14.3%	85.7%	0	71.4%
Acinic cell carcinoma	0	100%	0	0	33%	100%

**Table 7:** Various types of matrix in different salivary gland neoplasms

	Myxoid	Hyalinised	Chondroid	Lipomatous	Mixed	Others
Pleomorphic adenoma	96.6%	0.3%	72.4%	3.4%	79.3%	-
Warthin tumor	Not seen	Not seen	Not seen	Not seen	Not seen	-
Mucoepidermoid carcinoma	Not seen	Not seen	Not seen	Not seen	Not seen	-
Adenoid cystic carcinoma	28.6%	85.7%	0	0	14.3%	-
Acinic cell carcinoma	-	-	-	-	-	-

**Table 8:** Comparison of quantity of matrix in pleomorphic adenoma

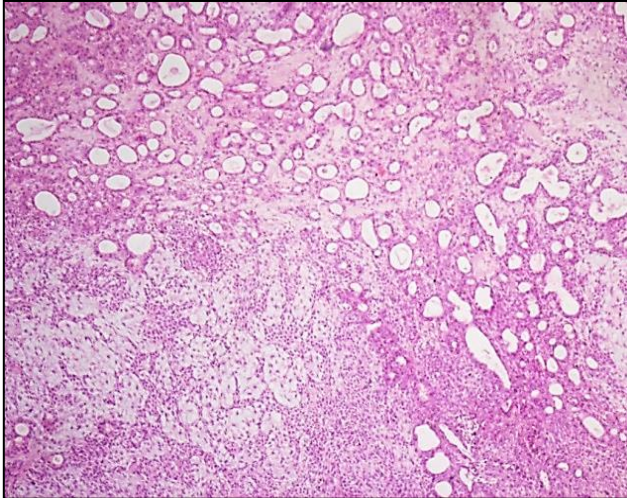
	Ito et al <sup>8</sup>	Current study
Stroma rich	52%	48.2%
Stroma poor	37%	17.2%
Classic	11%	34.4%

**Table 9:** Comparison of various types of matrix in pleomorphic adenoma

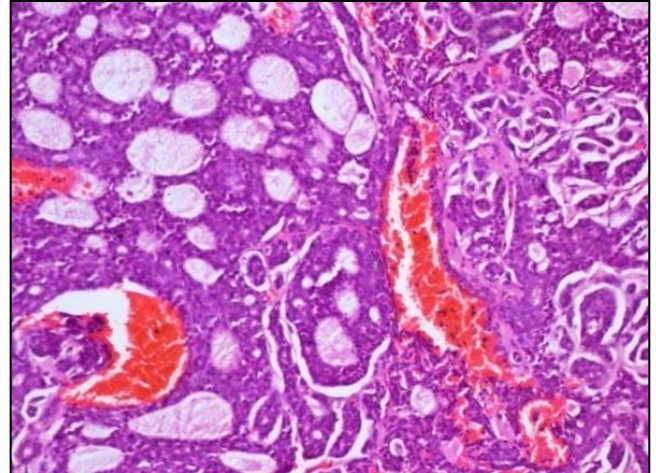
	Ito et al <sup>8</sup>	Present study
Chondroid	82.5%	72.4%
Myxoid	94.5%	96.6%
Hyalinisation	79.9%	10.3%
Lipomatous	-	3.4%
Osseous	2.1%	-

**Table 10:** Comparison of cytomorphology of myoepithelial cells in pleomorphic adenoma

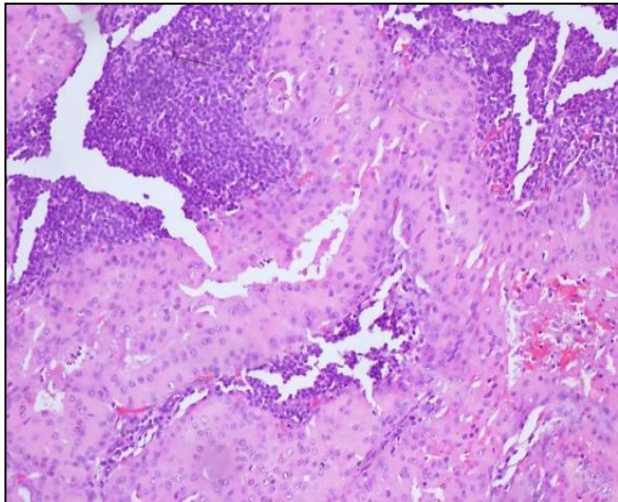
	Ito et al <sup>8</sup>	Current study
Basaloid	49%	00
Epitheloid	85%	72.4%
Clear	66%	27.6%
Spindle	95%	79.3%
Plasmacytoid	100%	41.4%
Oncocytic	10%	4%



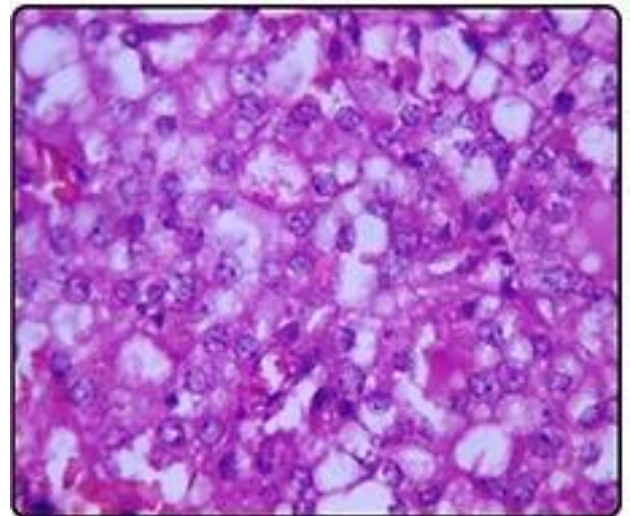
**Fig. 1:** Microscopic appearance of Pleomorphic adenoma showing both epithelial and mesenchymal components. H&E stain (10X).



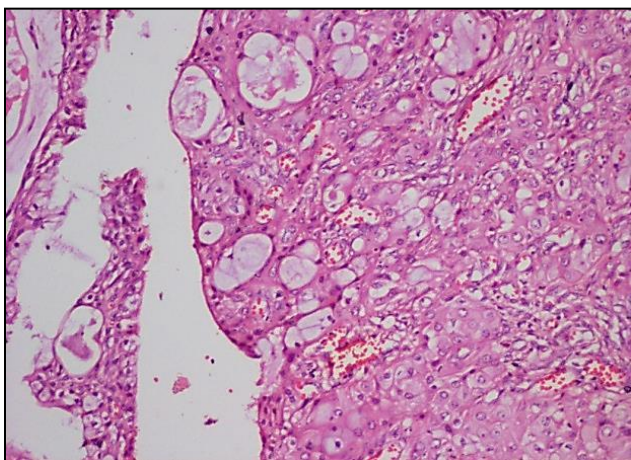
**Fig. 4:** Microscopic appearance of Adenoid cystic carcinoma. H&E stain (10X).



**Fig. 2:** Microscopic appearance of Warthin tumour. H&E stain (10X).



**Fig. 5:** Microscopic appearance of Acinic cell carcinoma. H&E stain (10X).



**Fig. 3:** Microscopic appearance of Mucoepidermoid carcinoma. H&E stain (10X).

### Conclusion

Salivary gland neoplasms show morphologically overlapping features. Proper diagnosis of salivary gland tumor is required for proper treatment and outcome.

Most of the tumors in our study had myoepithelial cells with mixed cytomorphology and had mixed architectural pattern. Proper diagnosis needs consideration of histological findings to differentiate between benign and malignant neoplasms. A clear knowledge about the salivary gland tumors with dual luminal-abluminal differentiation will help to arrive at a definitive diagnosis.

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**Conflict of Interest:** None.

## References

1. Speight PM and Barrett AW. Prognostic factors in malignant tumours of the salivary glands. *Br J Oral Maxillofac Surg* 2009;47:587-93.
2. Cheuk W and Chan JK. Advances in salivary gland pathology. *Histopathology* 2007;51:1-20.
3. Simpson RH. Classification of tumors of the salivary glands. *Histopathology* 1994;24:187-91.
4. Turk AT and Wenig BM. Pitfalls in the Biopsy Diagnosis of Intraoral Minor Salivary Gland Neoplasms: Diagnostic Considerations and Recommended Approach. *Adv Anat Pathol* 2014;21:1-11.
5. Barnes L, Eveson JW, Reichart P. World Health Organization Classification of Tumours: Head and Neck Tumours. IARC Press, Lyon, 2005.
6. Wah Cheuk, John KC. Salivary gland tumors. In: Flechter C.D. ed Diagnostic Histopathology of Tumors, 3rd ed, vol 1; 2007.
7. Dardick I, Byard RW, and Carnegie JA. A review of the proliferative capacity of major salivary glands and the relationship to current concepts of neoplasia in salivary glands. *Oral Surg Oral Med Oral Pathol* 1990;69:53-67.
8. Ito FA, Jorge J, Vargas PA, Lopes MA, Histopathological findings of pleomorphic adenomas of the salivary glands. *Med Oral Pathol Oral Cir Bucal* 2009;14:57-61.
9. Jahangirmezhad M, Moghadam SA., Mokhtari S, & Taravati S. Different Histopathologic Features of Pleomorphic Adenoma in Salivary Glands. *Int J Oral Maxillofac Pathol* 2013;4:7-11.
10. Ogawa Y, Kishino M, Atsumi Y. Plasmacytoid cells in salivary-gland pleomorphic adenomas: evidence of luminal cell differentiation. *Virchows Arch* 2003;443:625-8.
11. Ellis GL and Auclair PL. Tumors of the Salivary Glands. Atlas of tumor pathology, 3rd series, fascicle 17. Washington D.C.: Armed Forces Institute of Pathology. 1996.
12. Szanto PA, Luna MA, Tortoledo ME, White RA. Histologic grading of adenoid cystic carcinoma of the salivary glands. *Cancer* 1984;54:1062-9.
13. Da Cruz Perez DE, de Abreu Alves F, Nobuko Nishimoto I, de Almeida OP, Kowalski LP. Prognostic factors in head and neck adenoid cystic carcinoma. *Oral Oncol* 2006;42:139-46.
14. Chiosea SI, Griffith C, Assaad A, Seethala RR. The profile of acinic cell carcinoma after recognition of mammary analog secretory carcinoma. *Am J Surg Pathol* 2012;36:343-50.

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