Section: Pathology

Original Research Article

HISTOMORPHLOGICAL PATTERNS OF SALIVARY GLAND TUMOURS AND ITS CORRELATION WITH KI67 IMMUNOPROFILE

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Abstract

Background: Thirty different histological variants are identified among salivary gland tumors and it comprises about 3-6% of all tumors of head and neck region. The objectives of this study include 1. Analysis of histomorphological patterns of various tumors of the salivary glands and 2. Ki67immunoreactivity in various histological types. **Materials and Methods:** Haematoxylin and Eosin stained histopathological slides were examined by the authors and categorized based on latest WHO classification 2022. Ki 67 Immunohistochemistry for assessment of proliferation index was done and correlation was arrived. **Result:** Out of the 50 cases studied, 78% were benign and 22% were malignant. Pleomorphic adenoma was the most frequent benign tumor (74%) and Mucoepidermoid carcinoma was most frequent malignant tumor (36%) diagnosed during our study period. In the present study the normal salivary gland was negative for Ki 67 expressionand the labeling index in pleomorphic adenoma was 0.9%. Among the malignant tumors, it was highest in salivary duct carcinoma (18%), and was negative in adenoid cystic carcinoma. **Conclusion:** From the present study it is evident that histomorphological examination is the mainstay for the diagnosis and categorization of salivary gland tumors. Immunohistochemistry Ki 67 assesses the proliferation potential of the tumor and is also the best prognostic indicator.

INTRODUCTION

Salivary gland tumors [SGT] are uncommon and show diverse morphological patterns. The incidence of salivary gland tumorranges from 0.4-13.5/100,000 people.[1] Salivary gland tumors most commonly involve parotid glands.[2] Malignant tumors are common in minor salivary glands.[3] The benign tumor most often occurs in the 3rd to 4th decade of life.[4] The malignant tumor tends to occur in somewhat later age groups. In general, women are more affected than men except for Warthin tumors and high-grade tumors. Most common benign tumor is pleomorphic adenoma (65% of all tumors). The most common salivary gland carcinoma is mucoepidermoid carcinoma. The tumors of salivary glands are classified clinically into low grade and high grade.[5] Low grade tumors are basal cell adenocarcinoma, cystadenocarcinoma, acinic cell carcinoma, clear cell carcinoma, epithelial myoepithelial carcinoma, and polymorphous low-grade adenocarcinoma. High grade tumors are salivary duct carcinoma, carcinoma ex pleomorphic adenoma, undifferentiated carcinoma and adenoid cystic carcinoma. Histopathological features remain the mainstay for diagnosing salivary gland neoplasm. The Ki-67 protein is expressed in the nucleus of cell during all the phases of the cell cycle except in G0. Therefore it is the sensitive biomarker of cellular proliferation than mitoses, and it is considered as a useful tool in diagnosing the aggressiveness of malignant neoplasm. The expression of Ki-67 is correlated with histological grade, mitotic activity and clinical behavior of tumor.[6]

MATERIALS AND METHODS

This is a prospective study carried out in the department of pathology, Madurai Medical College, Madurai for the period of three years. Specimens received from Government Rajaji Hospital, Madurai were processed and histopathological examination and immunohistochemical analysis was done by our authors.

Inclusion Criteria

Salivary gland tumor specimens belonging to all ages and both sexes were included in our study.

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Exclusion Criteria

All other tumors and inflammatory lesions of the salivary gland were excluded from the study.

Method

The surgical specimens were received in 10% buffered neutral formalin. Gross examination was done and processed either in Toto or as small bits of 2-3 mm thick in the usual way. Sections of 5-micron thickness were cut and stained with routine Haematoxylin and eosin stain. The slides were reported by our expert team of histopathologists. Immunohistochemical examination for ki67 was made. Ki Labeling index was derived by counting nuclear and nucleolar staining in 500 cells.

RESULTS

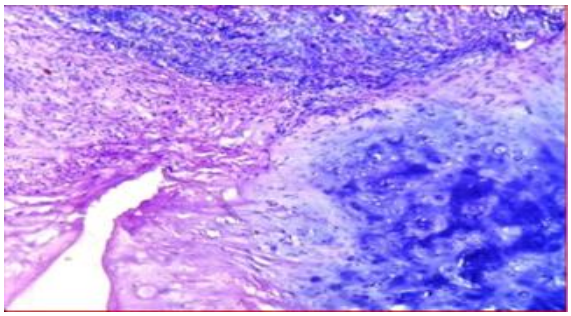

Figure 1: Pleomorphic adenoma: Epithelial cells with ductal differentiation and chondromyxoid area. H & E. 100X (721/16).

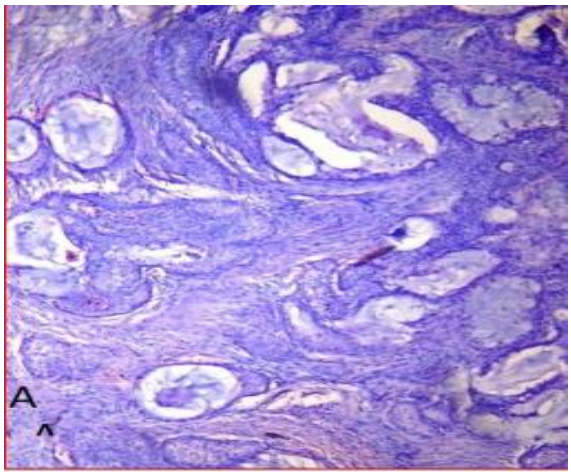

Figure 2: A.Low grade mucoepidermoid carcinoma: Solid area and cystic spaces filled with mucin. H&E.100X(1716/16).

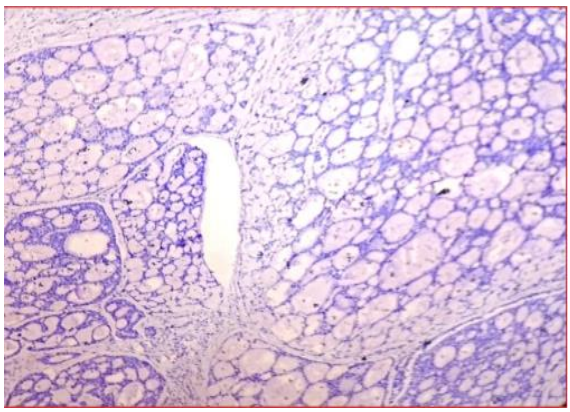

Figure 3: Adenoid cystic carcinoma showing cribriform pattern of arrangement of cells with angulated nuclei. H&E. 100X. (1485/15)

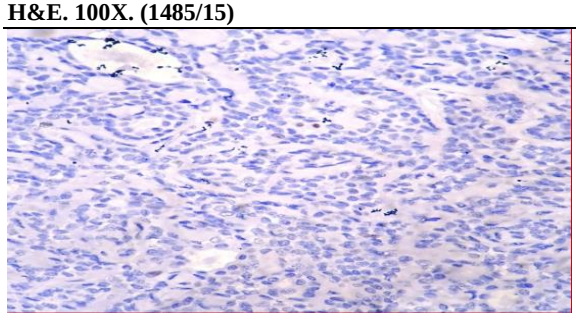

Figure 4: Pleomorphic adenoma showing ki 67 labelling index of 0.9%. IHC 400X. (1416/16)

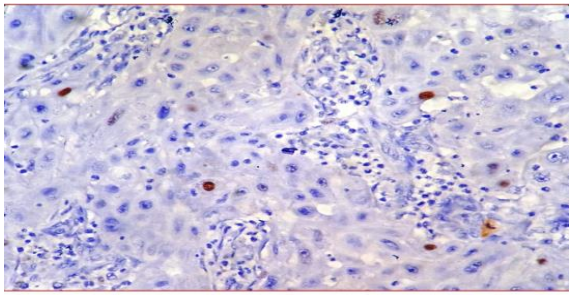

Figure 5: High grade mucoepidermoid carcinoma showing Ki 67 labelling index of 6%. IHC 400X. (860/15)

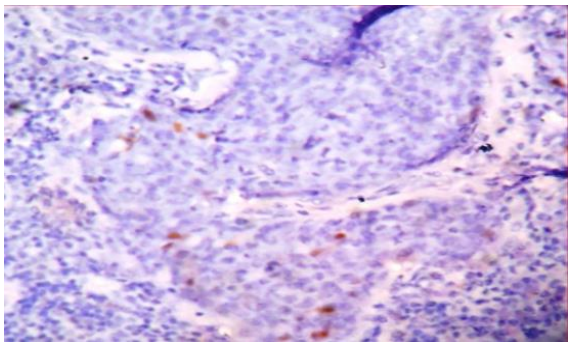

Figure 6: Salivary duct carcinoma showing ki 67 labelling index of 18%. IHC 400X. (2118/16)

In our study sample, peak incidence of salivary gland neoplasm was noted in the age group of 31- 40 years (30%) and lowest incidence in those who were above 70 years of age, youngest patient being 11 years old and the oldest 90 years old. 54% were female and 46% were male, with a Male: Female ratio of 1:1.17

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[Table 1]. 86% of the caseshad their tumor in the parotid region, and 14% had Sub mandibular lesions. Majority, that is 78% were diagnosed as having a benign tumor and remaining were malignant [Table. 2]. Among the benign tumors pleomorphic adenoma was the most frequent tumor. Among the malignant salivary gland tumors, Mucoepidermoid carcinoma was most frequent.

Immunohistochemistry [IHC] was done to analyze the Ki67 immune profile which assesses the proliferation potential of the tumor. In the present study the normal salivary gland was negative for Ki 67 expression and the labeling index in pleomorphic adenoma was 0.9%. Among the malignant tumors, it was highest in salivary duct carcinoma (18%), and was negative in adenoid cystic carcinoma.

Table 1: Gender Distribution

	Male	Female	Total
Benign	17	22	39
	34%	44%	78%
Malignant	6	5	11
	12%	10%	22%

Table 2: incidence of salivary gland tumours

Lesion	Frequency	Percentage
Benign	39	78%
Malignant	11	22%
Total	50	100%

Table 3: distribution of salivary gland tumours

Tumour	Panchal Upsana et al	Present Study
Pleomorphic adenoma	63.3%	58%
Warthin tumour	3.33%	8%
Basal cell adenoma	1.70%	8%
Mucoepidermoid carcinoma	5%	8%
Adenoid cystic carcinoma	2.50%	4%
Carcinoma ex pleomorphic adenoma	2.50%	2%
Myoepithelial carcinoma	1.70%	2%

Table 4: K i 67 labelling index of salivary gland tumours

Tumour	Ki 67 labelling index%
Pleomorphic adenoma	0.9%
Mucoepidermoid carcinoma	
Low grade	1.7%
High garde	6%
Salivary duct carcinoma	18%
Myoepithelial carcinoma	13.3%
Adenoid cystic carcinoma	0

DISCUSSION

This present study was done on fifty cases. An attempt has been made to analyze the histopathological patterns, anatomical site distribution, age distribution and gender distribution. The salivary gland lesions in present study show the incidence rate of 1.32% whereas in Bobati SS et al's study it was 0.6%,and 2% in the Western world.[7] The peak age incidence in our study was fourth decade, but in the study done by Syed et al peak age incidence was third decade.[8] The incidence was slightly more in females and the female male ratio was 1.17:1 in our study. This is similar to Singh et al's study which showed the ratio as 1.4:1.[9] In our study, the incidence was more in the parotid region (86%) followed by submandibular (14%) salivary glands. This correlates with Fatima S et al's study.[10] [Table 3] gives the distribution of salivary gland tumors. In the present study benign tumors outnumber the malignant tumors. This incidence goes in hand with the study done by Mihashi et al (79.1%,20.9%).[11]

The most frequent salivary gland tumor was the pleomorphic adenoma [Figure 1]. This correlates with the study done by Uma Dhayalet al.[12] Histopathological feature shows both epithelial and mesenchymal components. Epithelial component shows ductal structures formed of inner epithelial and outer myoepithelial cells forming myoepithelial cell melting in the stroma. The other differentiation includes squamous, basaloid, cuboidal, oncocytoid, and mucous. Mesenchymal component included myxoid, hyaline, cartilaginous or osseous differentiation. Chondromyxoid stroma is characteristic. Differential diagnosis (DD) includes Polymorphous low grade adenocarcinoma [PLGA] and carcinoma ex Pleomorphic adenoma.[2]

The second most common benign tumor was Warthin's tumor, and this goes in hand with the odbervation by Panchal Upasana et al.[13] This tumoroccurs exclusively in elderly male patients and in the parotid gland. Microscopically cysts with papillae lined by epithelial tall columnar and basaloid oncocytic cells are seen. The cystic spaces are filled

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with lymphoid stroma and few show lymphoid follicles.

In the present study malignant SGTs consist of 22% of all SGTs. Mucoepidermoid carcinoma [Figure 2] is the most common malignant SGT(8%) and this correlates with the study done by khandekar et al.[14] In Subashraj' sstudy adenoid cystic carcinoma was the most common malignant tumour in their study population.[15] On histopathological examination, it shows varying proportions of mucous, epidermoid and intermediate-type cells with mucin-filled cystic lumens. Mucin is strongly positive for mucicarmine stain. This carcinoma is reported as low, intermediate or high grade tumor based on Armed Forces institute of Pathology Grading system.

Adenoid cystic carcinoma [Figure 3] was the second common malignant SGT (4%). The histopathological patterns of ACC include cribriform (50%), tubular (20%-30%) and solid patterns (10%–15%). Stroma may be hyalinized or collagenous. This tumor has the propensity for perineural invasion, found in >50% of cases in the present study. DDs are PLGA, basaloid squamous cell carcinoma and epithelial-myoepithelial carcinomas. IHC are positive for keratin, CEA, lysozyme, lactoferrin, S-100 protein and CD117 markers.[2,12]

The Ki-67 is a valuable marker of cellular proliferation. The Ki-67 protein ispresent in the nucleus, during inter-phase. It is shifted to the surface of thechromosomes during mitosis. Ki 67 is present in all the phases of cell cycle (G2,S, G1 and mitosis) and is not present in cells which are in resting phase(G0).[16] Ki-67 Immunostaining is very much helpful to know the recurrence rate and prognosis of tumors.[17] Ki index of >15% is associated with aggressiveness of the tumor.[18]

Immunohistochemistry for the labeling index of Ki 67 [Table 4] showed negativity in normal salivary gland and 0.9% of the cells expressed ki 67 in cellular pleomorphic adenoma [Figure 4]. In high grade mucoepidermoid carcinoma [Figure 5] it was 6%. According to to Skalova A et al's,[19] study Ki index helps to differentiate between PLGA and adenoid cystic carcinoma specially in small biopsies. It was higher in adenoid cystic carcinoma compared with PLGA. Ki index cut off for benign salivary gland tumors are less than 5% and for malignant tumors it is more than 5%.[18] Our study correlates with their findings.In this study the expression of Ki 67 was significantly higher in salivary duct carcinoma, mucoepidermoid carcinoma and myoepithelial carcinoma than pleomorphic adenoma. This correlates with the study conducted by Azadeh Andishehet al and sangeetha et al.[20,21]

Ki index has different usage in SGT depending upon the histological types and grades. It is low in benign tumors, higher in high grade tumors. In case of basal cell adenoma it predicts the recurrence. It is also useful to assess the aggressiveness of the tumors.[22]

CONCLUSION

The present study was a single institutional experience with analysis of 50 cases. The age, sex, site distribution and pathologic features encountered in our study were in agreement with the studies reported from India and other parts of the world. In an era of prognostic oriented therapy, Ki 67 proliferation index is an usefulguide to determine the behavior as well as prognosis of the tumour and it should be done in a prospective stydy in a large scale.

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