

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Histo-pathological Analysis of Salivary Gland Lesions with Ki-67 Immunoprofile.

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ABSTRACT

To evaluate the incidence, age and sex distribution of salivary gland lesions and to analyse the histopathological type and Ki-67 immunohistochemistry expressions in salivary gland tumors. Surgical specimens were fixed in 10% neutral buffered formalin. Sections of 5 μ thickness were cut and stained with Hematoxylin and Eosin stains. Immunohistochemistry of Ki-67 was done in salivary gland tumors. The mean age for benign tumors, malignant tumors and non neoplastic lesions were 39, 49.05 and 33.33 yrs respectively with female predominance. The average Ki-67 labeling indexes for benign tumors were 1% and for malignant tumors were 17%. Pleomorphic adenoma was the most common benign tumor and adenoid cystic carcinoma was the most common malignant tumor. There was significantly higher frequency of Ki-67 positive cells in malignant salivary gland tumors compared to benign tumors.

Keywords: Salivary glands, benign, malignant, Ki-67 immunohistochemistry.

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INTRODUCTION

Salivary gland neoplasms are remarkable for their histological diversity and pose a practical challenge to the pathologists because of their complex classification and the rarity of several varieties.

Salivary gland neoplasms are relatively rare, accounting for approximately 3% to 10% of all head and neck tumors. About 80% of all salivary gland tumors have been located in the parotid gland, 5-10% in the submandibular gland, less than 1% in the sublingual gland and 10-15% in the minor salivary glands. Between 74%-80% of major salivary gland tumors are benign, pleomorphic adenoma being the commonest [65%]. Malignancy most commonly seen in the minor salivary gland. They are usually asymptomatic. However, a number of clinical predictors suggest malignancy, such as rapid growth, pain, fascial nerve involvement and cervical adenopathy.

Salivary glands are not subjected to incisional biopsy or core needle biopsy because of possible risk of causing fistula or tumor implantation through the disrupted capsule. The aim of the present study is to evaluate the incidence of non neoplastic and neoplastic lesions of the salivary glands, to study the age and sex distribution of various neoplasms occurring in the different salivary glands and to analyse the histopathological type and Ki-67 immunohistochemistry expressions in salivary gland tumors.

MATERIALS AND METHODS

This study includes 83 cases of salivary gland lesions over a period of three years in Thanjavur medical college hospital, Thanjavur. Clinicopathological data of salivary gland lesions were reviewed including age, sex, lesion site and histopathological types. All the surgical specimens were fixed in 10% neutral buffered formalin. All the suspicious areas were grossly sectioned of 2-3 mm in thickness and processed in the automatic tissue processor.

Sections of 5 μ thickness were cut and stained with Hematoxylin and Eosin. Special stains were used as and when necessary. Histological classifications of these tumors were done as per classification criteria of WHO.

Immunohistochemistry of Ki-67 was done on deparaffinized 5 μ sections after antigen retrieval by heat using microwave oven. The most stained areas in each section were chosen, and the minimum of 1,000 tumor cells were counted under light microscopic fields [X400]. The percentage of positive tumor cell nuclei was recorded as labeling index [LI].

RESULTS

In the three year study period 13,593 general biopsy materials were received in Thanjavur medical college and Hospital, Thanjavur. Among these, 83 cases were salivary gland lesions. The average incidence of salivary gland lesions were 0.61%.

Among the 83 cases, 18 cases were non neoplastic lesions. Among the 65 neoplastic lesions, 43 were benign tumor. Out of the 43 benign tumors there were 35 cases of Pleomorphic adenoma, 4 cases of Warthin’s tumor, 3 cases of Basal cell adenoma and one case of Cystadenoma. Among the neoplastic lesions incidence of benign tumors were 66.15% and it comprised 51.81% of total salivary gland lesions.

Malignant tumors were diagnosed in 22 cases. Among the 22 cases 7 cases were adenoid cystic carcinoma followed by 6 cases of mucoepidermoid carcinoma 5 were carcinoma ex pleomorphic adenoma. Salivary duct carcinoma, Adenocarcinoma NOS, Epithelial Myoepithelial carcinoma, Basal cell adenocarcinoma constitutes about 1 case each. Among the neoplastic lesions the incidence of malignant tumors were 33.85% and it contributes to 26.51% of the total salivary gland lesions and incidence of non neoplastic lesions were 21.68% of total salivary gland lesions during the study period.

The peak incidence of benign salivary gland lesions was 3rd-4th decades, for malignant tumors it was 5th-6th decade, and for non neoplastic lesions it was 2nd-3rd decade. The mean age for benign tumors was 39 yrs, malignant tumors it was 49.05 yrs and for non neoplastic lesions it was 33.33 yrs [Table-1].

Table 1: FREQUENCY OF AGE DISTRIBUTION OF SALIVARY GLAND LESIONS

AGE IN YRS	BENIGN	MALIGNANT	NON NEOPLASTIC	TOTAL	% OF TOTAL
1-10	-	-	2	2	2.41%
11-20	5	1	2	8	9.64%
21-30	9	3	7	19	22.89%
31-40	12	4	1	17	20.48%
41-50	6	3	3	12	14.46%
51-60	7	5	1	13	15.66%
61-70	3	4	2	9	10.85%
71-80	1	2	-	3	3.61%

Among the 83 cases of salivary gland lesions 43 cases were female [51.81%] and 40 cases were male [48.19%]. There is slight female preponderance with male to female ratio of 1: 1.07[Table-2].

Table 2: FREQUENCY OF SEX DISTRIBUTION OF SALIVARY GLAND LESIONS

SEX	BENIGN	MALIGNANT	NON NEOPLASTIC	TOTAL	% OF TOTAL
FEMALE	25	12	6	43	51.81%
MALE	18	10	12	40	48.19%

Among the neoplastic lesions 37 cases were female [56.92%] and 28 cases were male [43.08%] with male to female ratio of 1:1.32. [Table-3].

Table 3: FREQUENCY OF SEX DISTRIBUTION OF SALIVARY GLAND TUMORS

SEX	BENIGN	MALIGNANT	TOTAL	% OF TOTAL
FEMALE	25	12	37	56.92%
MALE	18	10	28	43.08%

Among the 83 cases of salivary gland lesions, 54 cases were in the parotid with an incidence of 65.06%, 23 cases were in the submandibular gland with an incidence of 27.71% and 6 cases in minor salivary gland accounting for 7.23% [Table-4].

Table 4: FREQUENCY OF SITE DISTRIBUTION OF SALIVARY GLAND LESIONS

SITE	BENIGN	MALIGNANT	NON NEOPLASTIC	TOTAL	% OF TOTAL
PAROTID	33	14	7	54	65.06%
SUBMANDIBULAR	8	4	11	23	27.71%
MINOR SALIVARY GLANDS	2	4	-	6	7.23%

Pleomorphic adenoma was the most common tumor in the parotid gland with an incidence of 46.29% whereas chronic sialadenitis was the most common lesion in the submandibular gland constituting about 47.82%.

Among the 65 cases of neoplastic lesions, 47 cases were in parotid [72.31%], 12 cases were in submandibular [18.46%] and 6 cases were in minor salivary glands [9.23%] [Table-5].

Table 5: FREQUENCY OF SITE DISTRIBUTION OF SALIVARY GLAND TUMORS

TUMOR SITE	BENIGN	MALIGNANT	TOTAL	% OF TOTAL
PAROTID	33	14	47	72.31%
SUBMANDIBULAR	8	4	12	18.46%
MINOR SALIVARY GLANDS	2	4	6	9.23%

Among the 47 cases in the parotid, pleomorphic adenoma was the most common tumor accounting for 53.19%, followed by mucoepidermoid carcinoma and carcinoma ex pleomorphic adenoma accounting for 10.64% each. In the minor salivary gland, adenoid cystic carcinoma was the most common tumor that constitutes about 66.64% followed by pleomorphic adenoma accounting for 33.33%.

Of the 83 cases of salivary gland lesions during the study period histopathological diagnosis of 65 neoplastic lesions were made, of which 43 cases were benign [66.15%] and 22 cases were malignant [33.85%] [Table-6].

Table 6: HISTOPATHOLOGICAL DIAGNOSIS

SALIVARY NEOPLASMS	NO OF CASES	% OF TOTAL
BENIGN	43	66.15%
MALIGNANT	22	33.85%

The most common benign tumor in our study was pleomorphic adenoma with an incidence of 53.85% of the total neoplasms, and 81.39% of benign salivary gland tumors.

Warthin’s tumor constituted about 6.15% of the total neoplastic lesions and 9.30% of benign tumors. Basal cell adenoma was about 4.61% of total neoplastic lesions and 6.98% of benign tumors. Cystadenoma constituted about 1.54% of the neoplastic lesions and 2.33% of benign tumors. The most common malignant tumor in our study was adenoid cystic carcinoma accounting for 10.77% of the total neoplasms and 31.81% of malignant tumors. The next common tumor was mucoepidermoid carcinoma that constituted about 9.23% of all neoplasms and 27.27% of malignant tumors. The carcinoma ex pleomorphic adenoma accounted for about 7.69% of all neoplasms and 22.72% of malignant tumors. The other cases namely, Salivary duct carcinoma, Adenocarcinoma NOS, Epithelial Myoepithelial carcinoma, Basal cell adenocarcinoma with one case each constituted about 1.54% of the all neoplasms and 4.55% of malignant tumors [Table-7].

Table 7: DISTRIBUTION OF VARIOUS SALIVARY GLAND TUMORS

TUMORS	ICD-O	NO OF CASES	% OF TOTAL
PLEOMORPHIC ADENOMA	8940/0	35	53.85%
WARTHIN’S TUMOR	8561/0	4	6.15%
BASAL CELL ADENOMA	8147/0	3	4.61%
CYSTADENOMA	8440/0	1	1.54%
ADENOIDCYSTIC CARCINOMA	8200/3	7	10.77%
MUCOEPIDERMOID CARCINOMA	8430/3	6	9.23%
CARCINOMA EX PLEOMORPHIC ADENOMA	8941/3	5	7.69%
SALIVARY DUCT CARCINOMA	8500/3	1	1.54%
ADENOCARCINOMA NOS	8140/3	1	1.54%
EPITHELIAL-MYOEPITHELIAL CARCINOMA	8562/3	1	1.54%
BASAL CELL ADENOCARCINOMA	8147/3	1	1.54%

Pleomorphic adenoma showed peak incidence in 3rd-4th decade with mean age of 39.05 years with female preponderance [62.86%]. Out of the 35 cases of, 25 were in parotid [71.43%], 8 were in submandibular [22.02%] and 2 in minor salivary glands [5.72%]. Out of 4 cases of Warthin’s tumor three were males with mean age of 49.75 years. All four cases occurred in the parotid gland. All three cases of basal cell adenoma were in the parotid gland.

Adenoid cystic carcinoma shows female preponderance [57.14%] with mean age of 44.85 years. Of the seven cases, three were in submandibular [42.85%] and four were in minor salivary glands [57.14%]. Mucoepidermoid carcinoma shows female preponderance [53.33%] with mean age of 48.33 years. Out of the six cases five were in parotid gland [83.33%] and one in submandibular gland [16.67%]. Of the 18 cases of non neoplastic lesions, 15 were chronic sialadenitis, 2 were chronic sclerosing sialadenitis and one case was Kimura’s disease. Non neoplastic lesions showed peak incidence in 2nd-3rd decade. Among the 18 cases, 7 were in parotid gland [38.89%] and 11 were in submandibular gland [61.11%].

Of the four cases of benign salivary gland tumors, one case of pleomorphic adenoma and Warthin’s tumor showed Ki-67 labeling index of 1% and 3% respectively. One case each of pleomorphic adenoma and basal cell adenoma was negative. Average Ki-67 labeling index of benign tumors were 1% [Table-8].

Table 8: FREQUENCY OF Ki-67 LABELING INDEX IN BENIGN SALIVARY GLAND TUMORS

BENIGN TUMORS	Ki-67 LABELING INDEX %
Pleomorphic adenoma	
Case -1	1%
Case -2	Negative
Warthin’s tumor	3%
Basal cell adenoma	Negative
Average Ki-67 Labeling index %	1%

Among the three cases of mucoepidermoid carcinoma two low grade carcinomas were negative and one high grade showed 50% Ki-67 labeling index. Of the three cases of adenoid cystic carcinoma two cases showed Ki-67 labelind index of 1% each and one case was 5%. Epithelial Myoepithelial carcinoma, Basal cell adenocarcinoma and Salivary duct carcinoma showed Ki-67 labeling index of 3%, 60% and 30% respectively. Average Ki-67 labeling index of malignant tumors were 17% [Table-9],[Table10].

Table 9: FREQUENCY OF Ki-67 LABELING INDEX IN MALIGNANT SALIVARY GLAND TUMORS

MALIGNANT TUMORS	Ki-67 LABELING INDEX %
Mucoepidermoid carcinoma	
High grade	50%
Low grade [2 cases]	Negative
Adenoid cystic carcinoma	
Case -1	1%
Case -2	1%
Case -3	5%
Epithelial Myoepithelial carcinoma	3%
Basal cell adenocarcinoma	60%
Salivary duct carcinoma	30%
Average Ki-67 Labeling index %	17%

Table 10: FREQUENCY OF AVERAGE Ki-67 EXPRESSION IN SALIVARY GLAND TUMORS

SERIES	AVERAGE Ki-67 LABELING INDEX %	
	BENIGN TUMORS	MALIGNANT TUMORS
Murakami et al ¹⁰⁴	1%	18.3%
Our study	1%	17%

DISCUSSION

In the present study benign tumors were most common in 3rd-4th decade with a mean age incidence of 39 years and malignant tumors were common in 5th-6th decade with a mean age incidence of 49.05 years. Benign tumors occur at younger age group than malignant tumors [1,2]. In the present study 37 female cases and 28 male cases were seen with a female preponderance [3-8]. In the present study, parotid was the most common site of involvement with an incidence of 72.31% followed by submandibular gland 18.46% and minor salivary glands 9.23% [1,3,9].

Pleomorphic adenoma was the commonest tumor in our study. In the present study 35 cases diagnosed as pleomorphic adenoma in histopathology constituted about 53.85% of total neoplasms and 81.39% of benign tumors [3,5]. In our study, pleomorphic adenoma presents with female preponderance [62.86%] with mean age of 39.05 years and the most common location being parotid [6,7,10,11]. In our study 3 cases had squamous metaplasia that constituted about 8.37% [Figure-1].

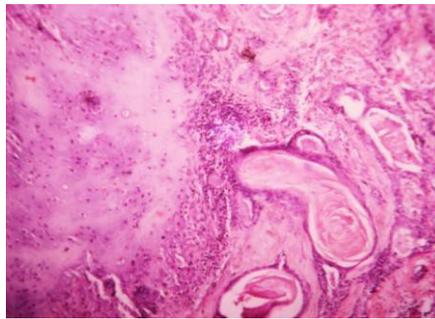


Figure 1: Pleomorphic adenoma showing pseudocartilagenous areas with squamous metaplasia. H & E 10X

J W Eveson [12] stated that areas of squamous metaplasia with or without keratin pearls were seen in PA. The cells are cytologically bland. If mucous cells are seen in conjunction with these areas it would be misdiagnosed as mucoepidermoid carcinoma. But keratin pearl formation is uncommon in mucoepidermoid carcinoma and careful examination of the stromal elements should avoid the confusion.

Warthin's tumor was the second most common benign tumor in our study accounting for 6.15% of all neoplasms and 9.30% of benign tumors [6,13,14]. In our study, all four cases occurred in the parotid, out of which three of them were males and this correlated well with Vargas et al [11] and Sancio Goncalves et al [15]. In our study 3 cases of basal cell adenoma were seen, accounting for 4.16% of all neoplasms and 6.98% of benign tumors [7]. In our study, all three cases occurred in the parotid gland [3]. We reported a case of cystadenoma of 28 years female in the parotid gland. Our case showed multiple cystic spaces with papillary projections lined by mucous secreting columnar cells.

In our study of 65 salivary gland tumors, adenoid cystic carcinoma was the most common tumor that constituted about 31.81% in the age range of 27-65 years with mean age of 44.86 years and it showed female preponderance [57.14%] and the most common location being the minor salivary gland which correlated with the literature [9,11,13]. In our study, mucoepidermoid carcinoma [Figure-2] constituted about 27.27% with parotid predominance which correlated with those reported in the literature [1,5,15,16]. William V Healey et al [16] in their study of 60 mucoepidermoid carcinomas, observed female preponderance with peak incidence of 5th -6th decade as seen in our study.

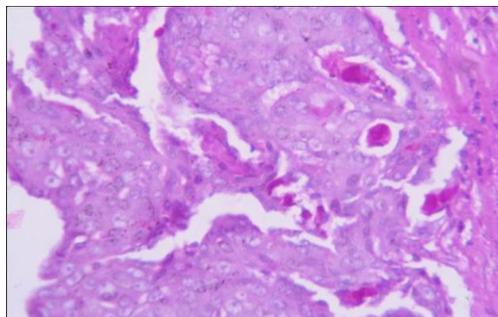


Figure 2: Mucoepidermoid carcinoma high grade showing scattered PAS positive mucous cells in the solid nests. PAS STAIN 40X

Carcinoma ex pleomorphic adenoma constituted about 22.72% which was within the range with those reported in the literature [5,13]. According to Livolsi and Perzin [17] carcinoma ex pleomorphic adenoma occurs in patients more than 50 years of age, as seen in our study. We reported a case of salivary duct carcinoma in a 62 years male in the parotid gland. Our case histopathologically showed cribriform pattern with central comedo necrosis [Figure-3]. According to Ruby Delagado et al [18] salivary duct carcinoma has a male predilection [M: F 4:1] with a mean age of 59 years and most common location being parotid gland, as seen in our study.

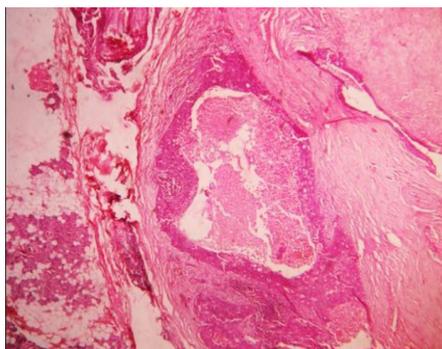


Figure 3: Salivary duct carcinoma showing central comedo like necrosis. H & E 10X

We reported a case of adenocarcinoma NOS in a female patient of 30 years and the location of the tumor was in the parotid which shows glandular pattern in the histopathology

and this correlates with the AFIP series [19]. We reported a case of basal cell adenocarcinoma in a 50 years male in the parotid gland, histopathologically showing solid pattern with infiltrating margins as seen in the literature [20]. We reported a case of Epithelial Myoepithelial carcinoma [Figure-4] in a 72 years male in the parotid gland [21].

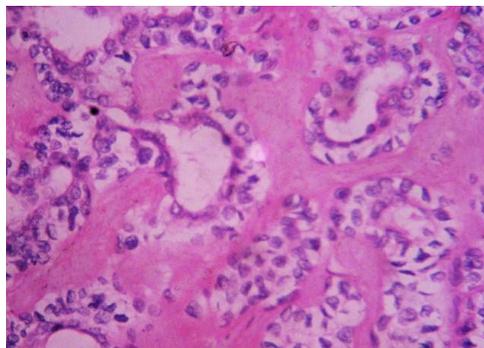


Figure 4: Epithelial myoepithelial carcinoma showing inner layer of ductal cells and outer layer of myoepithelial cells separated by hyalinized stroma. H & E 40X.

We reported a case of Kimura's disease in an 18 year male in the parotid gland. In our study, a case was reported as Kuttner tumor which occurred in the submandibular gland as seen in the literature [22].

Our study average Ki-67 labeling index for benign tumors were 1% and malignant tumors were 17% which correlated with Murakami et al [23] [Table-10]. Anna Kazanceva et al [24] stated that Ki-67 expression in pleomorphic adenoma was in the range of 0.07-4.81% which correlated with our study [0-1%]. Eveson and Nagao [25] stated that in basal cell adenoma and Warthin's tumor Ki-67 labeling index was <5%. In our study basal cell adenoma Ki-67 labeling index was negative and Warthin's tumor Ki-67 labeling index was 3% which correlated with Eveson and Nagao [25]. Amoueian et al [26] observed in his article that Ki-67 expression in adenoid cystic carcinoma was in the range of 0-85% which correlated with our study [0-5%] [Figure-5].



Figure 5: Adenoid cystic carcinoma, Tubular type showing Ki-67 labeling index of 5%.

In our study basal cell adenocarcinoma showed 60% Ki-67 labeling index [Figure-6].

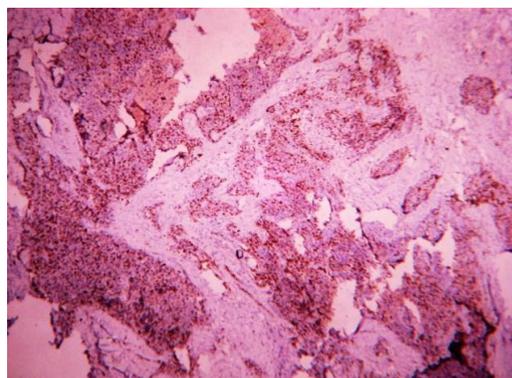


Figure 6 Basal cell adenocarcinoma showing Ki-67 labeling index of 60%.

Which correlated with Eveson and Nagao [25]. Vacchi Suzzi et al [27] in his article observed that Ki-67 index in salivary duct carcinoma was in the range of 20% - 70% as seen in our study [30%] [Figure-7].

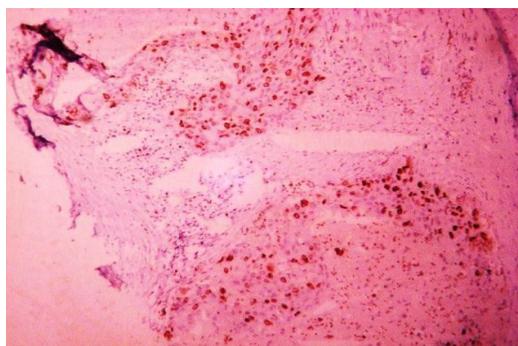


Figure 7: Salivary duct carcinoma showing Ki-67 labeling index of 30%

Michael Jaehne et al [28] in their study of 34 cases of salivary duct carcinoma 18 cases showed Ki-67 index in the range of 26-50% which correlated with our study [30%].

Van Heerden et al [29] observed that in high grade mucoepidermoid carcinoma Ki-67 positivity was in the range of 9.2-68% with mean of 38.3% whereas in intermediate and low grade mucoepidermoid carcinoma range was 0.8-13% and 9-36% respectively. They also stated that lack of Ki-67 staining was seen in all grades of mucoepidermoid carcinoma. In our study, two cases of low grade mucoepidermoid carcinoma showed negative Ki-67 expression. Mitsukuni Okabe et al [30] stated that Ki-67 labeling index of mucoepidermoid carcinoma was in the range of 0.4%-89.1%. In our study high grade mucoepidermoid carcinoma [Figure-8] showed Ki-67 labeling index of 50% which correlated with Van Heerden et al [26] and Mitsukuni et al [27].

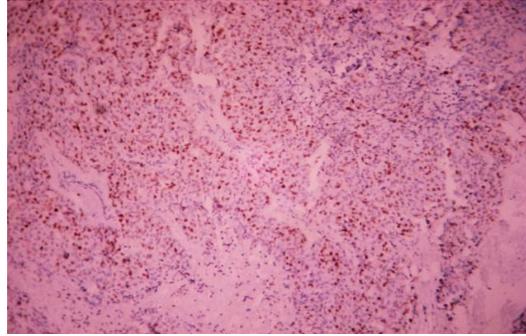


Figure 8: Mucoepidermoid carcinoma high grade showing Ki-67 labeling index of 50%

Douglas R.Gneep [31] stated that in Epithelial Myoepithelial carcinoma Ki-67 proliferative activity was largely restricted to the myoepithelial component with variable expression in the range of 0-50% with the mean of 16.9% which correlated with our study [3%]. High Ki-67 expression is generally associated with aggressive behavior and poor prognosis in salivary gland tumors.

CONCLUSION

Incidence of salivary gland lesions were 0.61% of total general biopsies with mean age for benign tumors were 39 yrs, malignant tumors it were 49.05 yrs and for non neoplastic lesions it were 33.33 yrs. Salivary gland tumors showed female preponderance with male to female ratio of 1:1.32. Majority of tumors were in parotid accounted for 72.31% followed by submandibular gland 18.46% and minor salivary glands constituted about 9.23%. Benign tumors were most commonly occurred in the parotid gland whereas in the minor salivary glands malignant tumors were more common. Inflammatory lesions were commonly seen in the submandibular gland.

The most common benign tumor in our study was pleomorphic adenoma with an incidence of 53.85% of the total neoplasms, and 81.39% of benign salivary gland tumors followed by Warthin's tumor. The most common malignant tumor in our study was adenoid cystic carcinoma accounting for 10.77% of the total neoplasms and 31.81% of malignant tumors followed by mucoepidermoid carcinoma. The average Ki-67 labeling indexes for benign tumors were 1% and for malignant tumors it were 17%. There was significantly higher frequency of Ki-67 positive cells in malignant salivary gland tumors compared to benign tumors.

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