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Tumors of salivary glands: an institutional study

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Abstract

***Background & Aims*:** This research investigates the clinicopathological features of salivary gland tumors by employing a range of markers, such as pancytokeratin (CKAE1/CKAE3), α-smooth muscle actin (SMA), carcinoembryonic antigen (CEA), and p63.

***Materials & Methods*:** The research investigated formalin-fixed paraffin-embedded (FFPE) tissue slides stained with hematoxylin and eosin (H & E) from a total of 32 cases of salivary gland tumors.

***Results*:** Lesions were detected in individuals ranging from 11 to 79 years of age, with a mean age of 45.5 years. Among the cases analyzed, 19 (59.4%) were categorized as benign neoplasms, which included 12 instances of pleomorphic adenoma (PA), 4 instances of basal cell adenoma (BCA), 2 instances of myoepithelioma, and 1 instance of Warthin's tumor. Conversely, 13 cases (40.6%) were identified as malignant neoplasms, consisting of 7 cases of mucoepidermoid carcinoma, 4 cases of adenoid cystic carcinoma (ACC), 1 case of carcinoma ex pleomorphic adenoma (Ca ex PA), and 1 case of squamous cell carcinoma (SCC). Immunohistochemical analysis demonstrated p63 positivity in PA, BCA, ACC, and SCC. Furthermore, positivity for cytokeratin was found in BCA, ACC, PA, and SCC. SMA positivity was observed in both BCA and ACC.

***Conclusion*:** The predominant benign and malignant tumors of the salivary glands are PA and mucoepidermoid carcinoma (MEC). Immunohistochemical staining of both epithelial and myoepithelial cells serves not only to validate the diagnosis but also assists in formulating the most appropriate treatment strategy.

***Keywords*:** α-smooth muscle actin, Carcinoembryonic antigen, Salivary gland tumors, Squamous cell carcinoma

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Introduction

Salivary gland neoplasms account for 3-10% of all tumors identified in the head and neck region (1). A variety of bioactive proteins have been discovered in saliva (2-4). The salivary glands play a crucial role in the production of immunoregulatory and anti-inflammatory agents (5-7).

These glands are classified into three pairs: the parotid, submandibular, and sublingual glands, in addition to numerous minor glands that are distributed throughout the area. The minor salivary glands are particularly numerous, with estimates suggesting there are between 600 and 1,000 clusters located within the oral submucosa, excluding the anterior hard palate and gingiva (8, 9). They are also present in the submucosal layers of the paranasal sinuses, pharynx, larynx, trachea, bronchi, lips, palate, and oropharynx. Moreover, heterotopic minor salivary glands can form in unusual locations such as lymph nodes, the thyroid gland capsule, facial bones, and the pituitary gland. Approximately 12% of all salivary gland tumors originate from the lachrymal and mucous glands situated on the inner surface of the upper lip, palate, base, and margins of the tongue, nasopharynx, larynx, and accessory nasal sinuses (10).

Salivary gland neoplasms are relatively rare, representing less than 1% of all cancers, with a significant proportion of these tumors being benign (11, 12). These neoplasms can arise in both major and minor salivary glands. The parotid glands are involved in 80% of cases, the submandibular glands in 10%, while the remaining cases are attributed to the sublingual and various minor salivary glands (13, 14). Tumors in the major salivary glands are frequently benign, whereas those in the minor salivary glands are more likely to be malignant. The malignancy rate for tumors in the parotid gland ranges from 20% to 25%, increases to 40% for submandibular gland tumors, and exceeds 90% for sublingual gland tumors (15,16). Additionally, benign salivary gland tumors are more commonly found in females than in males (17). The parotid gland is the most prevalent location for salivary gland tumors, accounting for 70% of all cases (18, 19). Within this category, PA is the most common benign tumor, representing 80% of occurrences, followed by Warthin's tumor (20). In most studies, mucoepidermoid carcinoma (MEC) and adenoid cystic carcinoma (ACC) are identified as the most frequently encountered malignant tumors.

Although hematoxylin-eosin (H&E) staining is the conventional technique for the identification of salivary gland tumors, the use of immunohistochemistry (IHC) can improve diagnostic accuracy, despite certain limitations in its effectiveness.

The anatomy of the salivary gland consists of ducts and acini (ducto-acinar units), which are made up of four distinct cell types: ductal, acinar, myoepithelial, and basal cells. Pan-cytokeratin (CKAE1/CKAE3) is present in all four cell types, while epithelial membrane antigen (EMA) and carcinoembryonic antigen (CEA) are expressed in both ductal and acinar cells. Myoepithelial and basal cells show positivity for CK14 and p63, but are negative for EMA and CEA. Only myoepithelial cells express markers such as smooth muscle actin (SMA), muscle-specific actin (MSA), podoplanin, calponin, and vimentin. The staining pattern for S-100 protein differs among the four cell types.

This study aims to explore the clinicopathological features of salivary gland tumors and their relationship with immunohistochemical markers, including CKAE1/CKAE3, CEA, SMA, and p63.

Materials & Methods

This retrospective study was conducted from April 2019 to April 2022. Case information was acquired from the department's histopathological records from 32 total cases. Inclusion criteria comprised all cases of salivary gland neoplasms. Inflammatory lesions and non-neoplastic cysts were considered as exclusion criteria.

Aspiration was performed using 22-gauge needles and smears were fixed with 95% ethyl alcohol. Formalin-fixed and paraffin-embedded (FFPE) sections were stained with H&E. IHC was performed using markers including CKAE1/CKAE3, CEA, SMA, and p63.

Results

Clinical symptoms included sensations of numbness, muscle weakness, and persistent pain localized to the salivary gland region. Salivary gland lesions were observed in individuals aged between 11 and 79 years, with a mean age of 45.5 years. The cohort consisted of 14 male patients (46.67%) and 16 female patients

(53.33%). A higher incidence of malignant lesions was found among male patients, while female patients presented a greater number of benign lesions.

Among the total cases, 13 (40.6%) were categorized as malignant neoplasms, which comprised 7 cases of mucoepidermoid carcinoma, 4 cases of ACC, one case of carcinoma ex pleomorphic adenoma (Ca ex PA), and one case of squamous cell carcinoma (SCC) (Table 1).

Table 1. Basic characteristic features of salivary gland tumors

	No. of cases	Percentage
Tumors		
Benign	19	59.4%
Malignant	13	40.6%
Benign tumors		
PA	12	63.15%
Basal cell adenoma (BCA)	04	21.06%
Myoepithelioma	02	10.53%
Warthin's tumor	01	5.26%
Malignant tumors		
MEC	07	53.85%
ACC	04	30.77%
Ca ex PA	01	7.6%
SCC	01	7.6%
Age incidence		
< 30 yr	7	23.33%
30-39 yr	5	16.67%
40-49 yr	3	10%
50-59 yr	4	13.33%
60-69 yr	5	16.67%
70-79 yr	6	20%
Sex		
Female	16	53.33%
Male	14	46.67%
Sites		
Parotid gland	18	60.00%
Submandibular gland	7	23.33%
Sublingual salivary gland	3	10.00%
Minor salivary gland	2	6.67%

Table 2 depicts the distribution of salivary gland tumors according to their specific anatomical locations. Table 3 offers a comprehensive overview of the site-specific distribution of malignant salivary gland tumors.

Table 2. Site-wise distribution of benign salivary gland tumors neoplasms

Neoplasms	Parotid	Submandibular	Sublingual	Minor salivary gland	*P-value*
PA	07	03	02	0	0.89*
BCA	04	0	0	0	
Myoepithelioma	02	0	0	0	

Neoplasms	Parotid	Submandibular	Sublingual	Minor salivary gland	*P-value*
Warthin's tumor	01	0	0	0	
Total	14	03	02	00	

*Chi-Square test

Table 3. Site-wise distribution of malignant salivary gland tumors

Malignant	Parotid	Submandibular	Sublingual	Minor salivary gland	*P-value*
MEC	05	01	0	01	0.85*
ACC	02	01	**0**	01	
Ca ex PA	01	0	**0**	0	
SCC	01	0	**0**	0	
Total	09	02	**0**	02	

*Chi-Square test

Figure 1 displays H&E staining results for various tumors, including BCA, ACC, MEC featuring anaplastic epidermal cells and mucus-producing cells, acinic cell carcinoma, and PA, highlighting a variety of histological characteristics. Figure 2 further illustrates PA, Warthin's tumor with papillary structures, spindle-shaped myoepithelial cells, BCA, mucoepidermoid carcinoma, ACC, SCC, and Ca ex PA, emphasizing their diverse features.

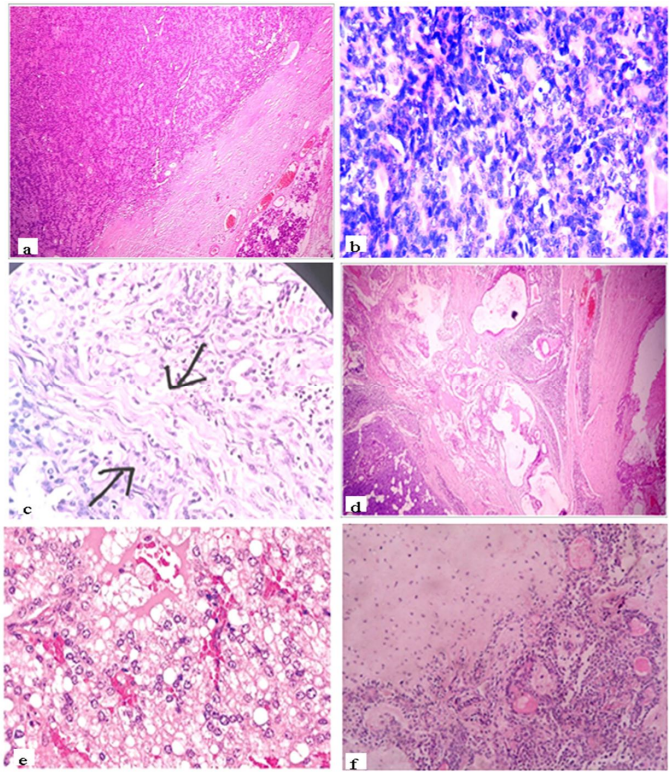

Fig. 1. a. BCA with adjacent normal parotid gland (×100). **b.** BCA with trabecular type (×400). **c.** ACC with perineural invasion (×400). **d.** MEC with epidermal cell anaplasia & mucus cells (×100). **e.** Acinic cell carcinoma (×400). **f.** PA with chondroid differentiation (×100).

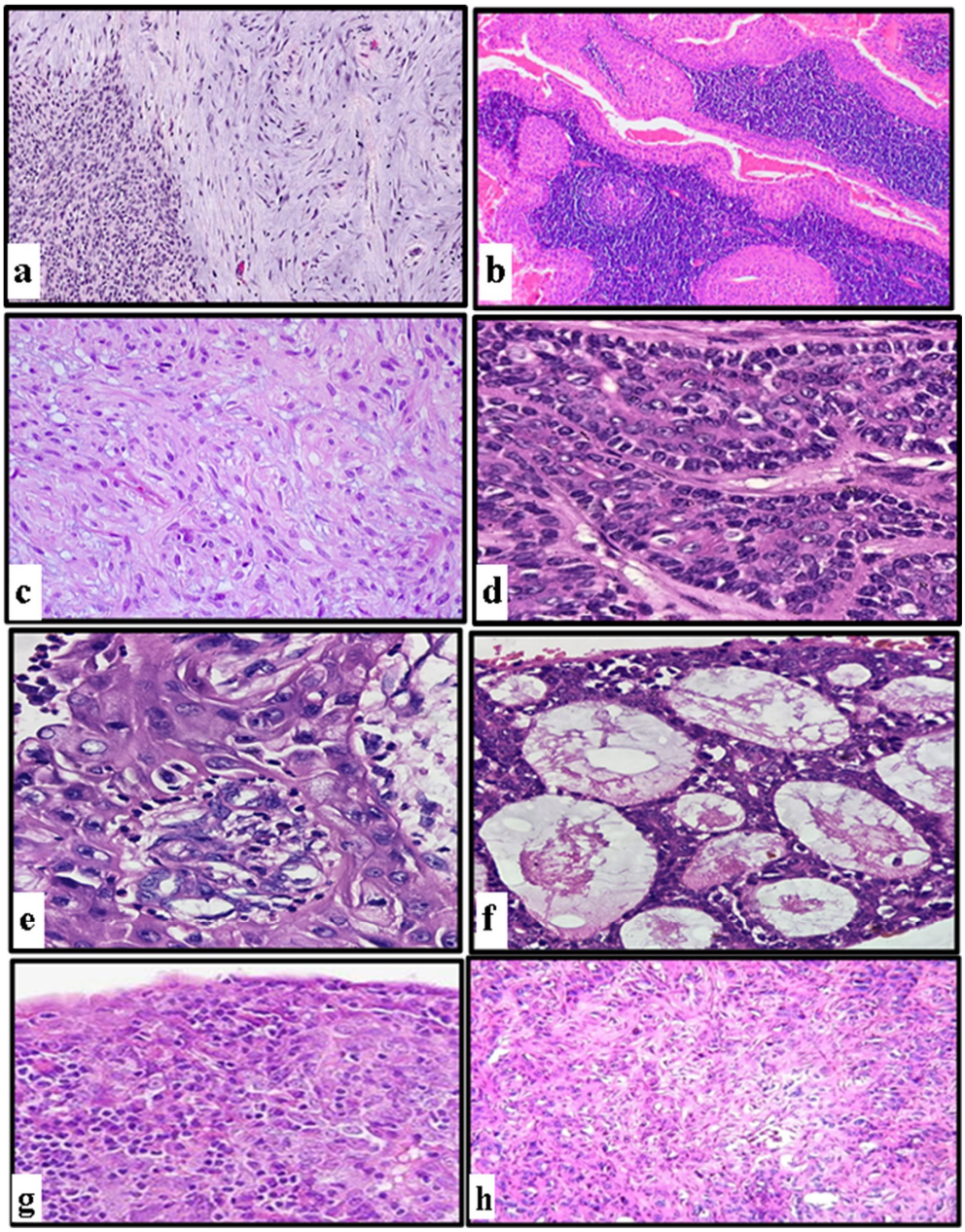

Fig. 2. H & E staining. **a.** PA - Spindle-shaped myoepithelial x-cells in a myxoid background. **b.** Warthin's tumor- Papillary structures lined by bilayered oncocytic epithelial cells, surrounded by a lymphoid stroma. **c.** Myoepithelioma- Spindle shaped myoepithelial cells. **d.** BCA- Solid tumor nests with a peripheral palisading manner. **e.** Mucoepidermoid carcinoma- Showing epidermal, mucin and intermediate cells. **f.** ACC- Cribriform pattern is composed predominantly of myoepithelial cells, admixed with hyalinized or myxoid globules. **g.** SCC- Showing pleomorphic cells. **h.** Ca ex PA- Showing both benign and malignant components. Magnification: ×400.

Figure 3 presents the findings of an immunohistochemical analysis indicating p63 positivity in PA, BCA, ACC, and SCC.

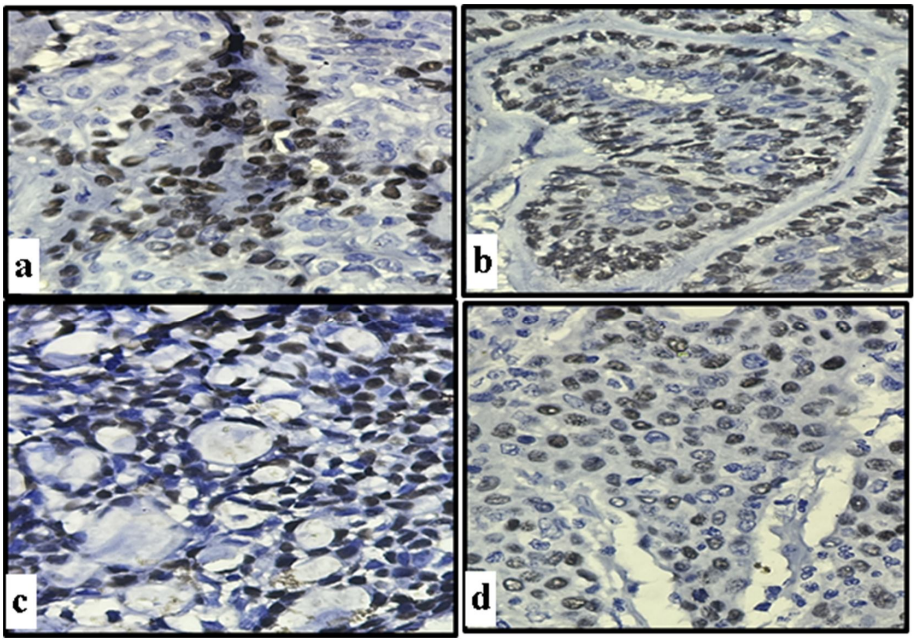

Fig. 3. IHC: p63 Positivity. **a.** PA (Myoepithelial cells + ve for p63& SMA). **b.** BCA (basaloid cells + ve for p63). **c.** ACC (Myoepithelial component is positive for SMA, p63, GFAP & cytokeratins). **d.** SCC (P63 + ve for basaloid type cells).

Figure 4 illustrates the immunohistochemical evaluation of cytokeratin positivity in BCA, ACC, PA, and SCC.

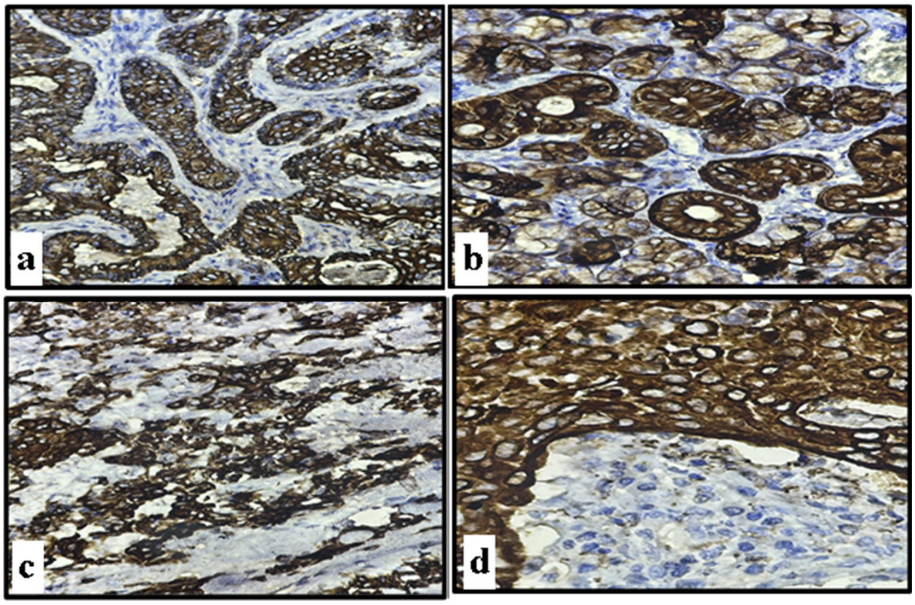

Fig. 4. IHC: Cytokeratin Positivity. **a.** BCA. **b.** ACC. **c.** PA. **d.** SCC

Finally, Figure 5 showcases the immunohistochemical assessment of SMA positivity in BCA and ACC.

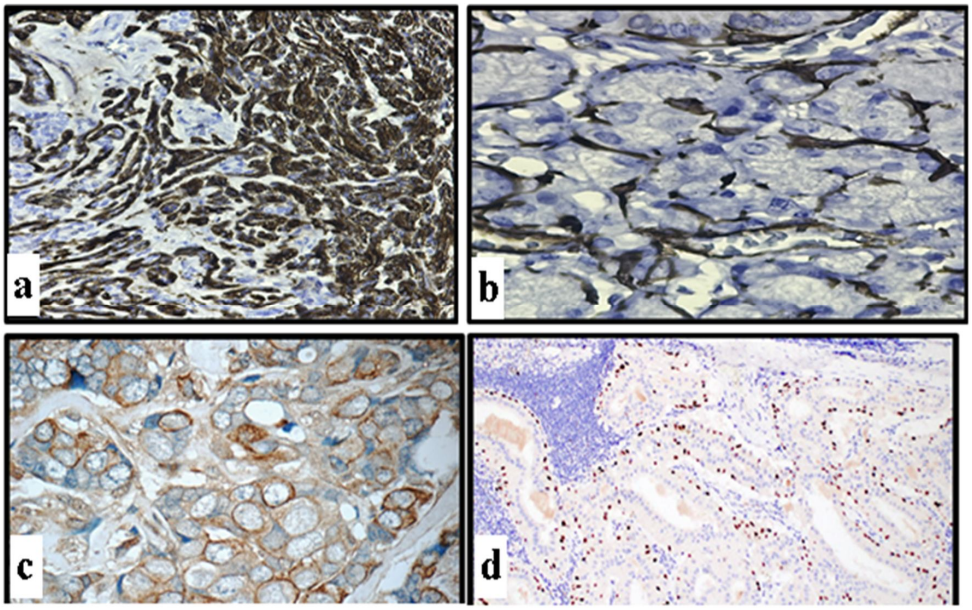

Fig. 5. IHC: SMA positivity. **a.** BCA. **b.** ACC. **c.** Cytokeratin positive in mucoepidermoid carcinoma. **d.** P63 in Warthin's tumor.

Discussion

Salivary gland tumors occur at an estimated rate of 0.4 to 13 cases per million individuals each year, with 70 to 80 percent of these tumors arising in the parotid gland. Fine needle aspiration cytology (FNAC) plays a crucial role in distinguishing between benign and malignant lesions. In the present study, the mean age of participants was 45.5 years, with ages ranging from 11 to 70 years. Comparable results have been documented in the studies conducted by Shrestha et al. and Bashir et al (21, 22).

Our findings revealed a higher incidence of tumors in females than in males. Specifically, female patients showed a greater prevalence of benign lesions, whereas males were more often diagnosed with malignant tumors. The parotid gland emerged as the most frequently affected site, representing 60% of the cases (18 cases), followed by the submandibular gland at 23.33% (7 cases) and the sublingual salivary gland at 10% (3 cases). Minor salivary glands were implicated in two cases, accounting for 6.67%.

The predominance of parotid gland involvement observed in our study is consistent with the findings of Bashir et al. (22), Cohen et al. (23), and Chatterjee and Panda (24). Among the 32 cases examined, 19 (59.4%) were classified as benign neoplasms, which included 12 cases of PA, 4 cases of BCA, 2 cases of myoepithelioma, and 1 case of Warthin's tumor. In contrast, 13 cases (40.6%) were identified as malignant neoplasms, consisting of 7 cases of mucoepidermoid carcinoma, 4 cases of ACC, 1 case of Ca ex PA, and 1 case of SCC.

According to Bhargava et al. (25), the most common malignant tumor is ACC (14%), followed by MEC (3%) and acinic cell carcinoma (2%). In our analysis, histopathological evaluation indicated that benign tumors were predominantly PAs (59.4%), while malignant tumors were primarily mucoepidermoid carcinomas (40.6%). The higher prevalence of benign neoplasms relative to malignant ones in our study aligns with findings from prior research (26, 27, 28).

In our study, PA was identified as the most common benign tumor, accounting for 63.15% of the cases. This observation is consistent with findings from prior

research. The current results confirm that PA is the leading benign neoplasm, followed by monomorphic adenoma, supporting earlier studies. Furthermore, our analysis indicated that 53.85% of the cases were classified as MEC, aligning with the results reported by Panda and Agarwal (5%), 30. Khazanchi et al. (9.5%), and Soni et al. (8%) (29, 30, 31).

Among the malignant tumors, MEC was found to be the most prevalent, followed by ACC and acinic cell carcinoma. The identification of low-grade MEC presents considerable diagnostic challenges, as it may be incorrectly diagnosed as chronic sialadenitis, Warthin's tumor, mucous retention cysts, or mucous salivary gland adenomatoid hyperplasia. Diagnosis is established through H & E staining, followed by confirmation with IHC.

Immunohistochemical analysis demonstrated that epithelial cells express various markers, including cytokeratins, EMA, and carcinoembryonic antigen (CEA) (32, 33). In contrast, myoepithelial cells do not show epithelial markers but express specific myoepithelial markers such as p63 and SMA. In our research, p63 was used to identify abluminal myoepithelial cells, as it is recognized as the most reliable myoepithelial marker in these tumors (34, 35). The immunohistochemical findings indicated that the clear abluminal cells are of myoepithelial origin, while the luminal cells, characterized by eosinophilic cytoplasm, are derived from ductal cells.

Conclusion

The most commonly observed benign and malignant tumors of the salivary glands are PA and mucoepidermoid carcinoma, respectively. Histopathological analysis is considered the definitive method for diagnosing salivary gland tumors. The use of immunohistochemical staining on epithelial and myoepithelial cells plays a crucial role in verifying the diagnosis, which in turn aids in determining an appropriate treatment strategy.

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Ethical statement

The proposal for this study was approved by the Ethics Committee of SVS Medical College, Mahabubnagar (IEC/SVS/2023/Pathology). The researchers adhered to all the principles recommended by the Helsinki Convention on Ethics in Research. Patients' personal and attributable information were kept confidential.

Data availability

The data that support the findings of this study are available upon request from the corresponding author.

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

None declared.

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