



A HISTOCHEMICAL STUDY OF MUCIN EXPRESSION IN SALIVARY GLAND TUMOURS IN TERTIARY CARE HOSPITAL IN INDIAN POPULATION

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ABSTRACT Neoplasms of the salivary glands account for 2% to 7% of head and neck neoplasms but less than 1% (approximately 0.3%) of malignancies from all body sites. Among them, pleomorphic adenoma (PA), mucoepidermoid carcinoma (MEC), and adenoid cystic carcinoma (AdCC) are the most common subtypes. Present study aims to investigate expression of mucins in the salivary gland tumour using special staining techniques in tertiary care hospital. The primary objectives of the present study are to prepare a database to identify the most common salivary gland tumour, to identify the frequency of salivary gland tumours in different age and sex groups, to identify the type of mucin in salivary gland tumours by using different staining techniques, and to compare mucin expression between benign and malignant salivary gland tumours. Acidic mucin was the most frequently expressed type (60%), followed by mixed (25%) and neutral mucin (15%). Most common tumour was PA followed by MEC and AdCC. In conclusion, histochemical evaluation of mucin offers a cost-effective and reliable adjunct in differentiating benign from malignant salivary gland tumours. Mucicarmine and Alcian Blue demonstrated high diagnostic specificity for benign lesions, particularly PA. The study emphasizes the value of integrating special stains into routine diagnostic protocols and provides a regional dataset on the histopathological spectrum of SGTs in a tertiary care hospital.

KEYWORDS : Mucin, Salivary Gland Tumour, Special Stain, Pleomorphic Adenoma

INTRODUCTION

Salivary glands are exocrine glands that produce saliva. These are divided into major (parotid, submandibular and sublingual salivary glands) and minor (lingual, labial, buccal, molar and palatine glands) [1]. These glands support vital functions such as lubrication, digestion, and tissue repair, contributing to the overall health of the oral and systemic environments [1], [2].

Mucins can be classified into two types, *secreted mucins* and *membrane bounded mucins*. Histochemically mucins are classified into *neutral mucins* and *acidic mucin* (acidic mucin includes sialomucin and sulphomucins) [3]. The aberrant expression of acidic mucin has been linked to the progression of salivary gland malignancies. This altered expression contributes to tumor microenvironment modulation, facilitating immune evasion and proliferation[4], [5].

Salivary gland tumors (SGTs) are accounting for less than 3% of all head and neck neoplasms, they present significant diagnostic challenges due to their complex and overlapping histopathological features. [6]. Despite advances, gaps remain in understanding mucin expression and its clinical significance in salivary gland neoplasms. There is also no study has examined salivary gland origin as a confounding factor in mucin expression of similar tumors. The present study was conducted with an aim to investigate expression of mucins in the SGTs using special staining techniques in tertiary care hospital.

METHODS AND MATERIAL

This cross-sectional observational study was conducted in the Department of Anatomy, AIIMS Gorakhpur, in collaboration with Departments of ENT and Pathology, AIIMS Gorakhpur from February 2024 to March 2025. The study included patients diagnosed with SGTs through fine needle aspiration cytology (FNAC) and undergoing surgical excision. Patients with incomplete medical records or those receiving chemotherapy or radiotherapy were excluded. The sample size was 20 patients, based on a reported prevalence of 6.1%, a precision level of 10%, and a confidence level of 95%. The study enrolled subjects diagnosed with Pleomorphic Adenoma (PA),

Mucoepidermoid Carcinoma (MEC), and Adenoid Cystic Carcinoma (AdCC). Relevant clinical and demographic data were recorded.

A total of 20 tumor specimens (16 PA, 2 MEC, and 2 AdCC), primarily from parotid and submandibular glands, were resected and preserved in 10% formalin. Tissue samples were processed and sections were stained with hematoxylin and eosin (H&E) for general histological evaluation. Special stains were used for mucin detection, including Periodic Acid Schiff-D (PAS-D) for neutral mucins (magenta color), Alcian Blue for acidic mucins (blue color), Mucicarmine for epithelial acidic mucins (pink color), and a combined PAS-D-Alcian Blue stain to differentiate between neutral and acidic mucins.

Ethical clearance was obtained from the institutional ethical committee to carry out this study. All norms related to the use of human resources in teaching and research were followed strictly as per the institutional guidelines. We also declare that the manuscript has been prepared in accordance with the Helsinki Declaration

RESULTS

The different demographic data and gross parameters were recorded and presented in **Table-1**.

The age of the patients ranged from 20 to 91 years, with a mean age of 37.90 years with ± 16.43 standard deviation. The maximum number of patients ranged between age groups 21-40 years of age with 45% cases. The frequency of SGTs was found to be more common in females (65%) as compared to males (35%).

The parotid gland was observed to be the most frequently occurred sites for SGTs with 85% of cases, followed by submandibular gland (15%). No cases of SGT in sublingual gland were found.

70% cases of major SGT were observed to be on left side which was more common as compared to right side (30%).

On the basis of occupation of the patient, 60% of cases of major SGTs were found to be homemakers followed by businessmen (15%), students (15%) and only 5% were seen in teacher and retired persons.

Table-1: General Parameters Of Patients Of Major Salivary Gland Tumour

S. No.	Diagnosis	Type	Size			Shape	Consistency	Adhesion
			General (cm)	USG (mm)	MRI (cm)			
1	PA	benign	4x5	40x36	4x2.4x3.8	Ovoid	Firm	Mobile
2	PA	benign	2x3	26x20	3.5x2.8x2.7	Ovoid	firm	Mobile
3	PA	benign	3x2	21x11	1.7x2.1x2	Globular	Firm	Mobile
4	PA	benign	3x3	30x16	multiple	Ovoid	Firm	Fluctuant
5	PA	benign	1x1	12x9	1.1x2.2x1.7	Ovoid	Firm	Mobile
6	Adcc	malignant	3x2	30x20	2x2.4x3	Ovoid	firm	mobile
7	PA	benign	4x3	33x22	3x2.5x2.1	Globular	Firm to hard	Mobile
8	PA	benign	3x3	29x18	3.4x1.8x3	Ovoid	Firm	Non-mobile
9	PA	benign	1x1	22x15	1.3x2.2x2.3	Ovoid	Firm	Mobile
10	PA	benign	2x2	24x18	2x2.3x3	Globular	Firm	Non-mobile
11	PA	benign	3x2	17x15	2x1.5x2	Ovoid	Firm	Mobile
12	PA	benign	4x2	39x22	3.8x3.1x2.5	Ovoid	Firm	Mobile
13	PA	benign	3x2	19x14	2.1x1.5x1.7	Globular	Firm to hard	Mobile
14	PA	benign	2x2	40x21	3.0x2.9x3.4	Ovoid	Firm to hard	Non-mobile
15	PA	benign	2x2	26x18	3x2.5x2	Globular	Hard	Non-mobile
16	PA	benign	3x2	28x20	5.1x3.6x2.5	Ovoid	firm to hard	Mobile
17	PA	benign	5x4	34x25	3.3x2.9x2.4	Ovoid	Firm	Mobile
18	MEC	malignant	4x3	34x24	4.6x2.1x2.8	Ovoid	hard	Mobile
19	Adcc	malignant	3x3	33x17	4.1x2.4x2.2	Irregular	Firm to hard	Mobile
20	MEC	malignant	2x2	25x22	2x2.4x3.1	Irregular	Hard	Mobile

Acidic mucin expression was observed in 60% cases, indicating a predominant presence of acidic mucin. **Mixed mucin expression** was found in 25% cases, showing a combination of both acidic and neutral mucins (**Figure-1**). **Neutral mucin expression** was found in 15% cases, indicating presence of neutral mucins. (**Table-2**)

These findings highlight that **acidic mucin** is the most frequently expressed mucin found among SGTs.

Table-2: Distribution Of Different Mucin Expression

Mucin Dominant	Frequency	Percent
Acidic	12	60.0%
Mixed	5	25.0%
Neutral	3	15.0%

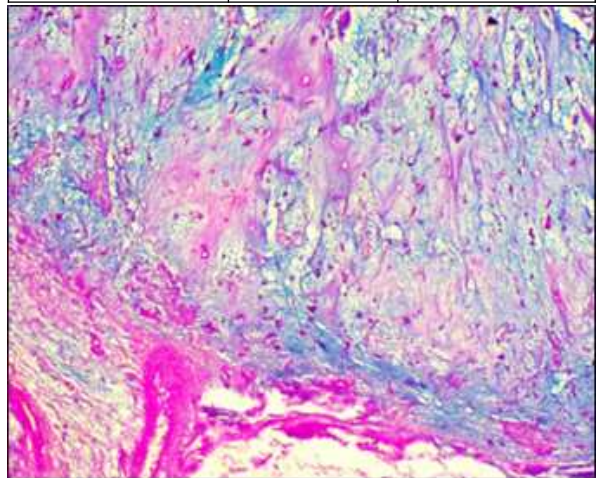


Figure-1: Pleomorphic adenoma: Photomicrograph on PAS-D-AB stain, 20X shows mixed neutral and acidic mucinous areas involving ~70-75% areas.

Histochemical evaluation of mucin expression was done by using three stains **Periodic Acid-Schiff (PAS-D)**, **Alcian Blue (AB)**, and **Mucicarmine (MC)**. **>50% expression was considered as high and < 50% was considered as low.** A Positive correlation was observed between benign tumours and PAS-D staining. **High PAS-D expression** was found in benign tumours. The above finding was statistically significant. (p value = .025) (**Table-3**) (**Figure-2**).

Positive correlation was observed between SGTs (benign and malignant) and alcian blue (AB) staining. **High AB expression** was seen only in benign tumours whereas low AB expression was observed in both benign and malignant tumours with equal frequencies. The findings was statistically significant (p value =.006) (**Table-3**) (**Figure-3**).

Positive correlation was observed between SGTs (benign and malignant) and mucicarmine (MC) staining. **High MC expression** was seen only in benign tumours whereas low MC expression was observed in both benign and malignant tumours with slightly higher frequencies in malignant cases. The findings was statistically significant (p value =.002) (**Table-3**) (**Figure-4**).

Table-3: Comparison Of Mucin Expression In Benign And Malignant Tumours

Stain		Type						p-value
		benign		Malignant		Total		
		Cou nt	Row N %	Cou nt	Row N %	Cou nt	Row N %	
PAS-D	PAS-D_high	10	100.0 %	0	0.0%	10	100.0 %	.025*, b
	PAS-D_low	6	60.0 %	4	40.0%	10	100.0 %	
	Total	16	80.0 %	4	20.0%	20	100.0 %	
AB	AB_high	12	100.0 %	0	0.0%	12	100.0 %	.006*, b
	AB_low	4	50.0 %	4	50.0%	8	100.0 %	
	Total	16	80.0 %	4	20.0%	20	100.0 %	
MC	MC_high	13	100.0 %	0	0.0%	13	100.0 %	.002*, b
	MC_low	3	42.9 %	4	57.1%	7	100.0 %	
	Total	16	80.0 %	4	20.0%	20	100.0 %	

Results are based on nonempty rows and columns in each innermost subtable.

*. The Chi-square statistic is significant at the .05 level.

b. More than 20% of cells in this subtable have expected cell counts less than 5. Chi-square results may be invalid.

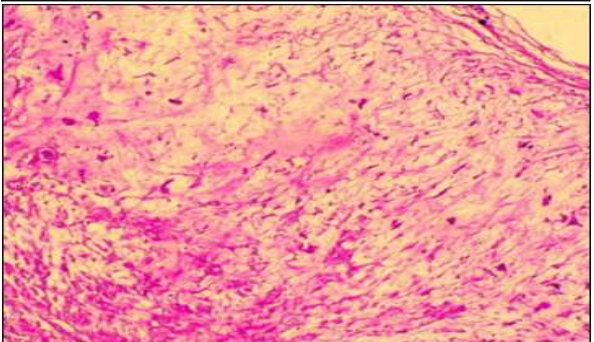


Figure-2: Pleomorphic adenoma: Photomicrograph on PAS-D stain,

20X shows ~50-75% mucinous area with scattered myoepithelial cells

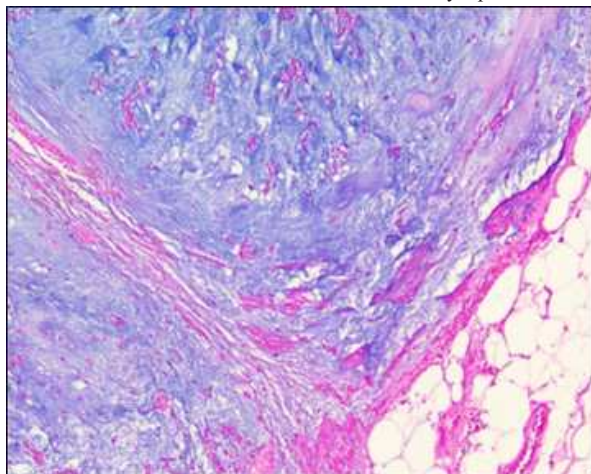


Figure-3: Pleomorphic adenoma: Photomicrograph on Alcian blue stain, 20X shows >70-75% mucinous areas demonstrating extensive deposits

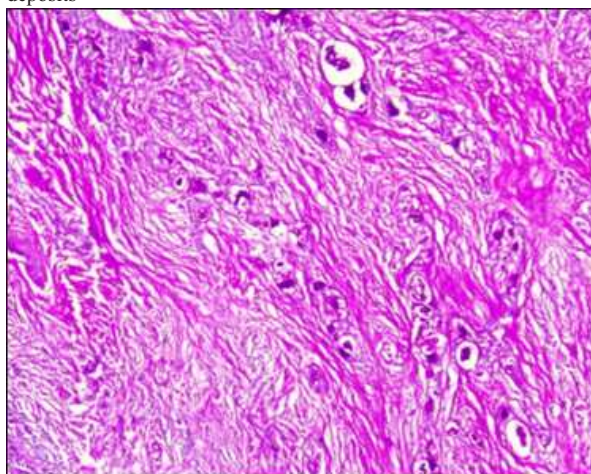


Figure-4: Mucoepidermoid Carcinoma: Photomicrograph on PAS-D, 20X shows ~5% mucoid areas (magenta pink globules).

DISCUSSION

The age distribution in this comprehensive observational study showed a wide range from 20 to 91 years, with a mean age of 37.90 years with ± 16.43 standard deviation. Most of cases was found in the 21–40-year age group. This aligns with findings by F.N. Mahamad Apandi et al., F. Begum et al., N. Taghavi et al., Rameeza & Hemalata, K. Subhashraj who also reported peak incidences among middle-aged adults, particularly in the 4th decade for both benign and malignant lesions [5], [6], [7], [8], [9]. We also observed 0% cases from age ranging between 61–80 years, may reflect sampling limitations or demographic peculiarities in the study population.

Most of the SGTs were found among females (65%) as compared to males (35%). A. S. Almeslet, A. Rameeza et al, S. Bobati et al, M. T. Božinović and N. Taghavi et al have also reported similar findings [10], [8], [11], [12], [7].

In the present study, most frequently involved salivary gland was parotid gland (85%), followed by submandibular gland (15%), and no cases was observed in sublingual gland. This distribution reflects classical findings, as nearly 70–80% of all SGTs are known to occur in the parotid gland [6], [11], [8], [12] [13]. The absence of sublingual tumours may be attributed to their rarity and lower glandular volume.

70% of all the SGTs in our study was found on left side as compared to right side (30%). This finding was not in accordance with findings recorded by Shazima Sheereen et al who documented a **predominantly right-sided distribution** with **73%** of cases on right and only **27%** on left. This divergence could be due to population-specific variations or sample size differences [14].

We recorded SGTs on the basis of occupation of the patient, 60% of

cases of major SGTs were homemakers followed by businessmen (15%), students (15%), teacher (5%) and retired persons (5%). There were not such study has done on the basis of occupation before.

N. Taghavi et al, A. Omhare et al, P. A. Shinde, S. Naag and R. P. Adi in their study found that PA was predominance as the leading salivary gland neoplasm with 45–74% of all SGTs. This was in concordance with the present study, clinically diagnosed cases of pleomorphic adenoma (PA) was found most common tumour with 80% cases, reaffirming its global predominance as the leading salivary gland neoplasm [7] [15] [16] [3].

Acidic mucin is the most frequently expressed mucin found among SGTs. N. Mahamad Apandi et al found the similar findings. They found that sialomucin (acidic mucin) was the most expressed mucin in all SGTS irrespective of origin [5].

High PAS-D mucin expression (>50%) was found in benign tumours. Low PAS-D mucin expression (<50%) was found in both benign and malignant with higher frequency in benign tumour. Similar finding was observed by S Naag et al, Shazima Sheereen et al, Faeza Begum [3], [14], [6]. They found strong positivity of PAS with mucin expression.

High alcian blue (AB) mucin expression was observed only in **benign tumours**, while **low AB mucin expression** was seen in both benign and malignant tumours at equal frequencies. This was statistically significant. Similar finding was observed by N. Mahamad Apandi et al. where they observed greater sialomucin expression in benign SGTs compared to the malignant ones [5]. These findings were similar to the present study.

S. Naag and Adi, N. Mahamad Apandi et al., observed in their study strong mucicarmine (MC) expression in benign (PA). This was not in complete concordance with our study where we observed high MC expression (>50%) in benign tumours (PA), while low expression (<50%) was seen in both benign and malignant with higher expression in malignant tumours, particularly AdCC and MEC. [3], [5]

CONCLUSION

This study provides an overview of SGTs on mucin expression, patient demographics, tumour localization in a tertiary care hospital. The findings reaffirm that pleomorphic adenoma is the most prevalent benign tumour, predominantly affecting the parotid gland, while mucoepidermoid carcinoma (MEC) and adenoid cystic carcinoma (AdCC) remain the most common malignant tumours. High mucin expression across all three stains (PAS-D, AB, MC) was observed in PA, whereas low expression was more common in malignant tumours (MEC and AdCC). Acidic mucin (60%) was most frequently expressed, followed by mixed (25%) and neutral mucin (15%). In conclusion, the integration of routine histology with specialized mucin staining can enhance diagnostic accuracy, guide clinical decision-making, and ultimately improve patient outcomes. Regional studies such as this are essential to enrich the existing knowledge base and support the development of population-specific diagnostic and treatment protocol.

Conflict Of Interest: None

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