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Cyto-Histo Cross Talk: Our Experience with Salivary Gland Neoplasms from a Peripheral Tertiary Care Centre of Eastern India with Reference to the Second Edition of Milan System of Reporting Salivary Gland Cytopathology

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ABSTRACT
Only 3% of all neoplasms in the body are salivary gland neoplasms, yet around 10% of head and neck neoplasms. Most are benign, while 20% to 30% are malignant. A prospective analysis was conducted on 52 patients who underwent FNAC followed by postoperative histopathological examination. Clinical data, demographic characteristics, FNAC findings were classified according to the second edition of Milan System of Reporting Salivary Gland Cytopathology and compared with histopathology findings to determine the level of diagnostic concordance. Most of the participants were between 41 to 60 years of age with a female predilection. (55.8%). Parotid gland was the most common location (41 out of 52 cases). Majority of cases were benign neoplasms, 73.1%, while only 26.9% were malignant. Pleomorphic salivary adenoma (PSA) was the most common benign neoplasm while mucoepidermoid carcinoma (MEC) was the most common malignant neoplasm. In the MSRSGC, the Risk of malignancy (ROM) was ND, 50%(n=2); NN, 40% (n = 5); AUS (no cases); BN, 3.45 % (n = 29); SUMP, 100% (n = 1); SM, 100%(n=2); and M, 100% (n = 13). The sensitivity, specificity, PPV, NPV and diagnostic accuracy of FNAC were calculated to be 73.68%, 100%, 100%, 86.49% and 90.20% respectively. One of the most significant preoperative diagnostic methods for salivary gland swellings in the past has been FNAC. Reporting salivary gland FNAC using the second edition of MSRSGC could boost FNAC reliability, enhance ROM assessment, improve patient treatment.
Keywords: *Diagnostic accuracy, Mucoepidermoid carcinoma, Pleomorphic salivary adenoma, Milan system of reporting salivary gland cytopathology*

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INTRODUCTION

Despite being relatively uncommon, salivary gland neoplasms can take many different benign and malignant histological subtypes. It makes up roughly 10% of head and neck neoplasms, but only 3% of all neoplasms in the body.[1]

Most salivary gland tumours are benign by nature. Less than 1% of all malignant neoplasms of head and neck are salivary gland cancers, which are also rare. According to histological analysis, mucoepidermoid carcinoma was the most prevalent malignancy (39.7%). Benign salivary gland neoplasms were mostly reported in relatively younger individuals. Most of the tumors were arising in the parotid gland (75%), followed by submandibular gland (16%), minor salivary glands (16%) and lastly sublingual gland (1%). A vast majority of benign tumors were found to be pleomorphic salivary adenoma, predominantly of parotid gland origin. On the contrary, most malignant neoplasms were mucoepidermoid carcinoma, mostly arising from submandibular and minor salivary glands.[2,3]

Historically, fine needle aspiration cytology (FNAC) has been the main preoperative diagnostic tool in evaluating salivary gland tumours. This study aims to compare and correlate cytology and histopathology findings.

The goal of FNAC is to quickly offer the most accurate first assessment on which management decisions can be made. It is utilized in conjunction with clinical and radiological data.[4]

Another major drawback is the varied terminology of reporting salivary gland FNAC. To address this, The

American Society of Cytopathology and International Academy of Cytology has developed a tiered international classification scheme called the "Milan System for Reporting Salivary Gland Cytopathology" (MSRSGC). This aims to reduce the ambiguity and make interpretation easier.[5]
To our knowledge, the data regarding sensitivity, specificity, and diagnostic accuracy of FNAC for salivary gland neoplasms is both limited and controversial, especially in the Indian subcontinent. Furthermore, the recent introduction of second edition of MSRSGC and 5th edition of WHO classification of Head and Neck tumours in the year 2022, both aim to standardize the reporting of salivary gland neoplasms as well as familiarize us with a few new histopathological entities.
The present study has been designed to compare the findings of FNAC and histopathology of salivary gland neoplasms. It also aims to study salivary gland neoplasms with respect to demographic characteristics such as age, sex, site, location, laterality and morphological spectrum.

MATERIAL AND METHODS
This was a prospective study conducted at the Pathology department of our medical college after obtaining approval from the institutional ethics committee of our institution vide Memo No BMC/I.E.C/202; dated 17th July 2023. During the study period (July 2023 to January 2025) we encountered a total of 52 cases with salivary gland swellings who were subjected to FNAC and subsequently underwent histopathological examination. These 52 patients were our study population. Patient particulars, FNAC findings and histopathology reports were collected and compiled.
Inclusion Criteria:
Patients presenting to our FNAC clinic with clinically palpable swellings in the region of major (parotid, submandibular and sublingual glands) and minor (e.g.: palate, buccal mucosa, gums, parapharyngeal space etc.) salivary glands, with or without other clinical features suggestive of salivary gland pathology.
Exclusion criteria: Only cases lacking definitive final histological confirmation—the gold standard—were removed from the research cohort. As long as the patient had surgical resection, FNAC specimens with low cellularity or concealing materials (Milan Category I) were purposefully kept in the final statistical analysis (n=52). This method guaranteed optimal exploitation of the institutional cohort while preventing data loss.
FNAC Technique:
Informed consent was obtained from each patient. Every aseptic and antiseptic care was taken during the procedure. Material was aspirated from the lesion using a disposable needle that was 22-, 23-, or 24-gauge and connected to a 10-milliliter disposable syringe. The process was conducted two to four times from various lesion locations to obtain sufficient sample. After smearing the aspirated material onto sterile glass slides, the smears were promptly fixed in fixative solution for at least twenty minutes. To prepare them for May-Grünwald-Giemsa (MGG) staining, certain smears were allowed to air dry. Every slide was properly labelled.
Staining:
Haematoxylin and Eosin (H&E) was used to stain wet-fixed smears, while May–Grünwald–Giemsa (MGG) stain was used to stain air-dried smears. The slides were saved after being mounted using DPX. A microscopic analysis was then performed to assess the cytomorphological characteristics of the salivary gland lesions.
Reporting:
FNAC reporting was performed by strictly following The Second Edition of the Milan System for Reporting Salivary Gland Cytopathology. The diagnostic categories were[6]:
Category I: Non-Diagnostic
Category II: Non-Neoplastic
Category III: Atypia of Undetermined Significance (AUS)
Category IV: Neoplasm
IVA: Benign
IVB: Salivary gland neoplasm of uncertain malignant potential (SUMP)
Category V: Suspicious for Malignancy
Category VI: Malignant
HPE Technique:
All the fifty-two patients underwent surgery for their salivary gland swelling at the ENT or general surgery department of our medical college. The post-operative specimen was sent to our pathology department. Gross examination was done and formalin fixed paraffin embedded (FFPE) tissue blocks were prepared. Around 3-to-5-micron tissue sections were prepared from the blocks with the help of Leica rotary microtome. The sections were floated on water bath and subsequently put on clean grease free glass slides.
Staining:
Staining of the tissue sections were done with Harris' Haematoxylin and Eosin stain.
Reporting:
HPE reporting was performed using binocular light microscope by two expert histopathologists of our

institution. Both the reporters were blinded to the cytology reports to avoid confirmation bias in the results. WHO Classification of Head and neck tumours, 5th Edition was strictly followed during each reporting.

Statistical analysis:

Statistical analysis was conducted using Jamovi software, version 2.6.23 and MS Excel 2021. For recording sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of FNAC according to MSRSGC, Category IV(SUMP) cases were calculated as benign while Category V (suspicious for malignancy) cases were assumed as malignant. The statistical parameters were defined as follows:

Discrepant cases: Where the diagnosis (morphological subtype) on FNAC is different from the same on HPE irrespective of benign or malignant.

True positive: Where the neoplasm is malignant on both FNAC and HPE.

True negative: Where the neoplasm is benign/non neoplastic on both FNAC and HPE.

False positive: Where the neoplasm was malignant on FNAC but came out to be benign/non neoplastic on HPE.

False negative: Where the neoplasm was benign/non neoplastic on FNAC but came out to be malignant on HPE.

Risk of malignancy (ROM) were calculated for each of the MSRSGC categories using this formula:

$$\text{ROM: } \frac{\text{Number of malignant histopathology cases} * 100}{\text{Total cases in each category}}$$

To maintain institutional data completeness and avoid selection bias, cases classified under Milan Category I (Nondiagnostic) were kept in the diagnostic performance evaluations if final surgical histology was obtained (n = 52). For binary diagnostic accuracy evaluations, Category I (Nondiagnostic) cases were assigned to the [benign / malignant] analytical group based on their final cytomorphological categorization parameter (2 × 2 contingency matrix). This illustrates clinical treatment paths that cross-reference tissue-level results with inadequate initial samples.

Cases were categorized as either benign or malignant to assess the definitive binary diagnostic performance of FNAC versus histology. To avoid confusing the distinct clinical sensitivity and specificity thresholds with intermediate risk borderline data, the solitary case classified under Category IVB (SUMP) was removed from the 2*2 contingency matrix computations.

RESULTS

The study focused on 52 unilateral salivary gland cases involving patients between 6 and 66 years old, though the 41–60-year age bracket saw the highest frequency. Women made up most of the cohort at 55.8% (29 patients), and a significant 75% of cases (41) occurred on the left side. The parotid gland was the primary site for cases. Other cases were found in the submandibular (8) and minor salivary glands (3).

Initial preoperative FNAC found 38 benign (73.1%) and 14 malignant (26.9%) cases. When applying the Milan System of Reporting Salivary Gland Cytopathology (MSRSGC), the distribution was quite specific: 2 cases (3.8%) fell into Category I, 5 cases (9.6%) fell into Category II, 30 cases (57.7%)into Category IVA, just one (1.9%) in Category IVB, 2 cases in Category V(3.8%) and 12 (25%) in Category VI, as shown in Table 1. There were no cases attributed to Category III. Pleomorphic salivary adenoma (PSA) appeared as the most frequent FNAC diagnosis overall with 24 cases, while mucoepidermoid carcinoma, and poorly differentiated carcinoma were the most common malignant findings at 5 cases each.

Final histopathological (HPE) results eventually confirmed 32 benign (61.5%) and 20 malignant (38.5%) tumors, with both types appearing most often in the 41-60 age group. PSA stayed the most frequent diagnosis (20 cases), followed by Warthin tumor and mucoepidermoid carcinoma at 6 cases each.

The data revealed five false negatives—including instances where mucoepidermoid and adenoid cystic carcinomas were misidentified as chronic sialadenitis—but zero false positives. Other diagnostic shifts occurred when single cases of Warthin tumor, basal cell adenoma, myoepithelioma, and adenoid cystic carcinoma were incorrectly diagnosed as PSA on cytology, and one case of acinic cell carcinoma was categorized as Salivary gland neoplasm of uncertain malignant potential (SUMP).

The risk of malignancy (ROM) was calculated for each category, except for Category III (AUS) which had no cases attributed to. The values are as follows:

Category I (ND): Out of two cases, one appeared to be chronic sialadenitis while the other was diagnosed as acinic cell carcinoma (ACC) on HPE. ROM was 50% for this category.

Category II (NN): Out of total 5 cases, three were diagnosed as benign (chronic sialadenitis) while rest two were malignant, with one each of mucoepidermoid carcinoma (MEC) and adenoid cystic carcinoma (ADCC). Thus, ROM was calculated to be 40%.

Category IVA (Benign neoplasm): Only one case out of twenty-nine came out to be malignant (adenoid cystic carcinoma) on HPE. ROM came out to be only 3.45%.

Category IVB (SUMP): This category only had a sole case which was malignant on HPE, making ROM for this category 100%.
Category V (Suspicious for malignancy): Only had two cases and both came out to be malignant on HPE. ROM was 100%.
Category VI (Malignant): All the cases (n=13) were malignant on HPE making the ROM value 100%.
2*2 contingency matrix for calculation:

	HPE Malignant	HPE Benign	Total
FNAC Malignant	14(True Positive/TP)	0 (False positive/FP)	14
FNAC Benign	5 (False negative/FN)	32 (True negative/TN)	37
Total	19	32	51

Sensitivity: TP/(TP+FN) = 14/ (14+5) = 73.68%
Specificity: TN/(TN+FP) = 32/ (32+0) = 100%
Positive predictive value (PPV): TP/(TP+FP) = 14/ (14+0) = 100%
Negative predictive value (NPV): TN/ (TN+FN) = 32/ (32+5) = 86.49%,
Diagnostic accuracy: (TP+TN)/ Total cases = (14+32)/51 = 90.20%

Milan Category	**n (%)**	**Malignant Cases**	**ROM (%)**
I	**2 (3.8%)**	**1**	**50.0%**
II	**5 (9.6%)**	**2**	**40.0%**
IVA	**29 (55.8%)**	**1**	**3.45%**
IVB	**1 (1.9%)**	**1**	**100%**
V	**2 (3.8%)**	**2**	**100%**
VI	**13 (25%)**	**13**	**100%**

TABLE 1: Table showing comparison between Milan System 2nd Edition and Histopathology. Risk of malignancy (ROM) has also been compared across different categories.

Study	ND (%)	NN (%)	AUS (%)	BN (%)	SUMP (%)	SM (%)	M (%)
Our Study	50.0*	40.0*	—	3.45	100.0*	100.0*	100.0
Institute Curie[17]	50.0	16.8	—	4.3	50.0	56.1	98.2
Milan 1st Ed [15]	25.0	10.0	20.0	5.0	35.0	60.0	90.0
Milan 2nd Ed[6]	15.0	11.0	30.0	<3.0	35.0	83.0	>98.0
Wang 2022 [12]	11.4	10.9	30.5	2.8	37.7	83.8	97.7
Farahani 2019 [16]	17.0	8.0	34.0	4.0	42.0	58.0	91.0
Jalaly 2020 [18]	16.9	10.5	39.9	2.9	39.4	84.2	97.5
Wei 2017 [19]	25.0	10.2	—	3.4	37.5	58.6	91.9

Table 2: This table provides a compact comparison of the Risk of Malignancy (ROM) across various Milan categories as reported in recent literature and the current study.
*Low no of cases.

DISCUSSION

In our study, there were 9 discrepant cases, 14 true positive, zero false positive, 32 true negative and 5 false negative cases.
Although our cohort demonstrated a left sided laterality predominance (75%), this finding likely reflects a localized referral or procedural variance rather than a biological predisposition, as standard literature indicates no such skewed distribution.
Warthin tumour was the second most common benign neoplasm in our study with a total of 5 cases on histopathology. Unfortunately, two cases of warthin tumor were diagnosed wrongly on cytology, one as oncocytic adenoma and another as Pleomorphic salivary adenoma (PSA).
Mucoepidermoid carcinoma (MEC) was the most common malignant neoplasm in our study followed by adenoid cystic carcinoma (ADCC) and acinic cell carcinoma (ACC).
There were also a few cases of salivary duct carcinoma and carcinoma ex pleomorphic adenoma.
There was a total of eight discrepancies between the results of FNAC and HPE. They are described below along with some discussion into why these discrepancies occurred in our study.
In our study, one case each of basal cell adenoma, myoepithelioma, warthin tumour and adenoid cystic carcinoma was wrongly diagnosed as PSA on cytology. This can be caused by the varied cytological picture of PSA which can range from abundant chondromyxoid stroma with very little cellular component to cellular PSA where the characteristic stroma is scanty.

There was one case each of mucoepidermoid carcinoma and adenoid cystic carcinoma that were wrongly diagnosed as chronic sialadenitis on cytology. This led to erroneous risk of malignancy (ROM) assessment for Milan Category II (Non neoplastic). Both were probably due to insufficient cytology material, abundant amount of stromal material obscuring cellular details or delay in processing. This can be minimized by repeating the procedure, taking samples from different areas of the swelling and avoiding delay in processing or slide staining.

One case of adenoid cystic carcinoma was wrongly diagnosed as PSA on cytology. The distinction of PSA from well differentiated ADCC is both clinically important and sometimes very difficult. Both tumors are "biphasic," meaning they contain both epithelial and myoepithelial cells. In a cellular version of PSA, the myoepithelial cells can appear "basaloid"; small, dark, and uniform—which is the hallmark appearance of ACC. Hyaline stromal globules, characteristic of ADCC can also sometimes be seen in PSA.[7] The primary distinguishing factor between adenoid cystic carcinoma and pleomorphic adenoma in our investigation was the quantity of cytoplasm in individual tumor cells. The plasmacytoid morphology is a definitive indicator of pleomorphic adenoma, allowing for the exclusion of adenoid cystic carcinoma.[8]

One case of Warthin tumour (WT) was mistakenly diagnosed as oncocytic adenoma (also known as oncocytoma). Both benign entities are very close mimics of each other. Especially if oncocytes predominate in the smear of Warthin's tumour with scanty lymphoid cells, the distinction becomes extremely difficult. However, they can be separated on the basis that the oncocytic cells of oncocytic adenoma typically form multilayered aggregates rather than flat sheets as seen in WT.[9]

Subsequently, we also found one case of myoepithelioma that was wrongly diagnosed as PSA on cytology. This can occur if the smear contains exclusively myoepithelial cells and scanty or absent ductal cells. One can try to avoid this problem by being very careful when diagnosing a neoplasm as PSA in absence of any ductal cells.[10]

In the study conducted by Rammeh et al [11] on correlation between cytology and histopathology of salivary glands, the sensitivity and specificity were determined to be 77.8% and 100% respectively, which was quite in accordance with our study.[11]

In a meta-analysis conducted by Wang et al (2022), where they also compared FNAC findings with biopsy reports, sensitivity and specificity were 88% and 98.5% respectively, which was not far off from our results.[12]

Majority of the neoplasms in our study were in the major salivary glands with parotid gland taking up most of them (82.2%). Submandibular gland was the next most common site with 13.3% cases, while only 4.4% tumours were in the minor salivary glands of oral cavity. A similar result was also seen in a study conducted by Mckenzie et al (2023) where they also found parotid glands to be the most prevalent site.[13]

Pleomorphic salivary adenoma was the most common benign as well as overall most common neoplasm in our study. Mckenzie et al (2022) also found similar results in their study, where pleomorphic salivary adenoma was the most common benign neoplasm while mucoepidermoid carcinoma was the most common malignant tumours followed by adenoid cystic carcinoma.[13]

Alsenie et al (2022) also reported pleomorphic salivary adenoma as the most prevalent benign tumours in their study followed by Warthin tumour, while mucoepidermoid carcinoma and adenoid cystic carcinoma were the most common malignant neoplasms.[14]

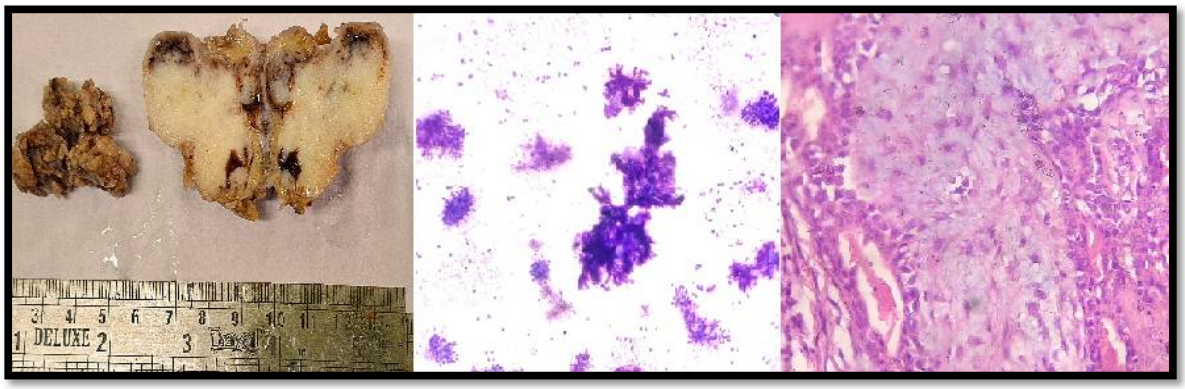
The second edition of MSRSGC has revised the ROM of each of the categories from the first edition. Currently, the ROM has values set as 15% (Category I, ND), 11% (Category II, NN), 30% (Category III, AUS), <3% (Category IVA, Benign), 35% (Category IVB, SUMP), 83% (Category V, suspicious for malignancy) and 98% (Category VI, malignant)[15].

In our study, the ROM for ND (50%), NN (40%) were significantly higher than the Milan system values. However, as these sample sizes were very small and in single digit, the resulting percentages are prone to high variance and should be interpreted with extreme caution when compared to international benchmarks. For Category I (ND), it also implies a strong surgical selection bias, as only patients with high clinical or radiological suspicion for tumors proceeded to formal resection However, as only 2 cases out of 52 (3.8%) were in Category I (ND), our study demonstrated concordance with international quality benchmark (under 10% non-diagnostic rate).[16]

The higher ROM value for SUMP and suspicious for malignancy (SFM) cases can be mostly attributed to very low number of cases in their respective categories. This can be alleviated by conducting a study with a larger sample size in a larger institute for a longer duration.

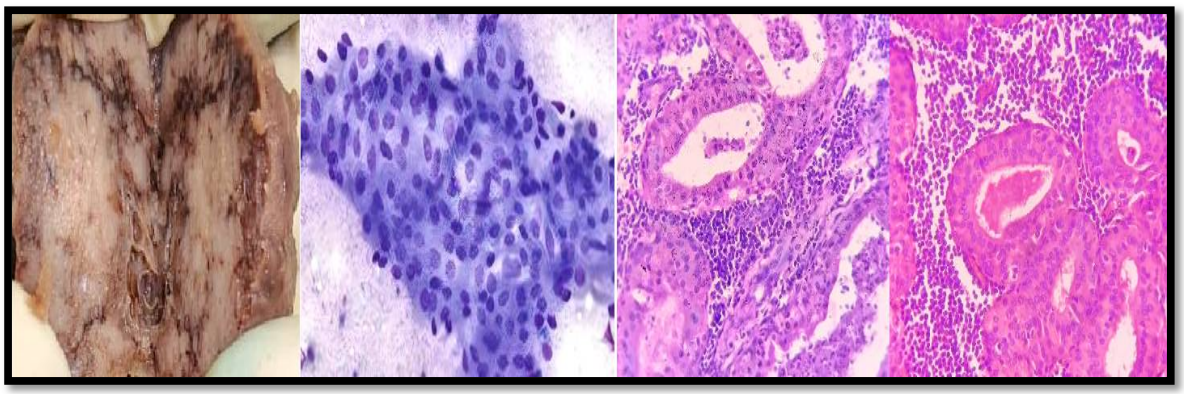
The complete absence of Category III (AUS) cases in our cohort can be hypothesized to stem from an institutional preference toward rendering definitive diagnostic categorizations whenever possible; however, this requires further validation. Table 2 gives a comparative summary regarding ROM value among our study, Milan system and few other significant studies in the last decade. Our study established

FNAC as a reliable preoperative diagnostic tool for salivary gland neoplasms with a high specificity, PPV, NPV, diagnostic accuracy and relatively high sensitivity.



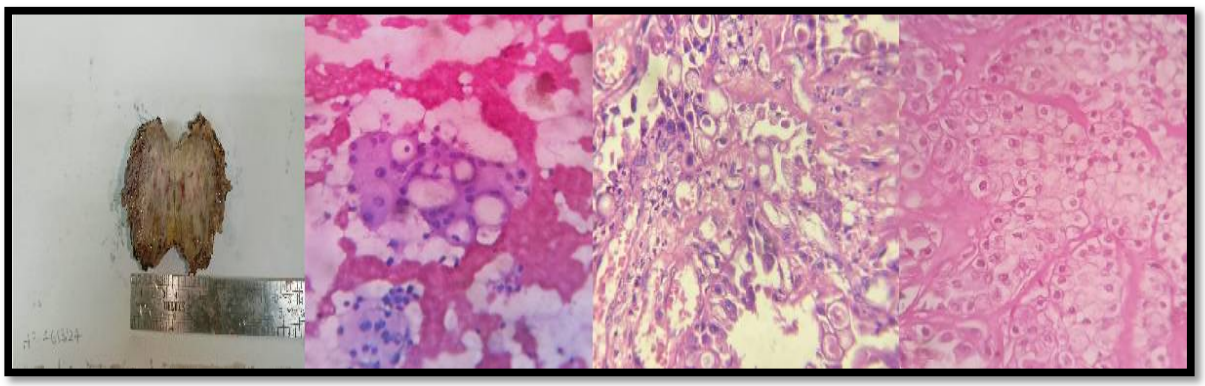
a **b** **c**

FIGURE 1: a) Gross specimen of PSA. b) FNAC smear of PSA showing spindled myoepithelial cells in a purplish fibrillary stroma. (MGG, 100x). c) HPE section of PSA showing biphasic pattern with both ductal structures and myoepithelial cells. The latter blends into the chondromyxoid stroma. (H&E, 400x)



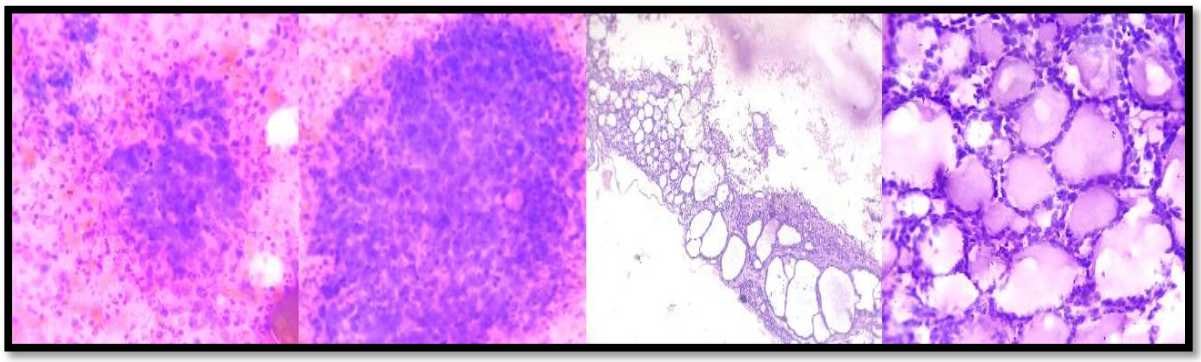
a **b** **c** **d**

FIGURE 2: a) Gross specimen of Warthin tumor showing a variegated mass with areas of hemorrhage. b) FNAC smear from same case showing oncocytic cell clusters mixed with lymphocytes. (MGG, 400x) c) and d) HPE section from same case showing characteristic bilayered oncocytic epithelium with an abundant lymphocyte rich stroma. (H&E, 400x)



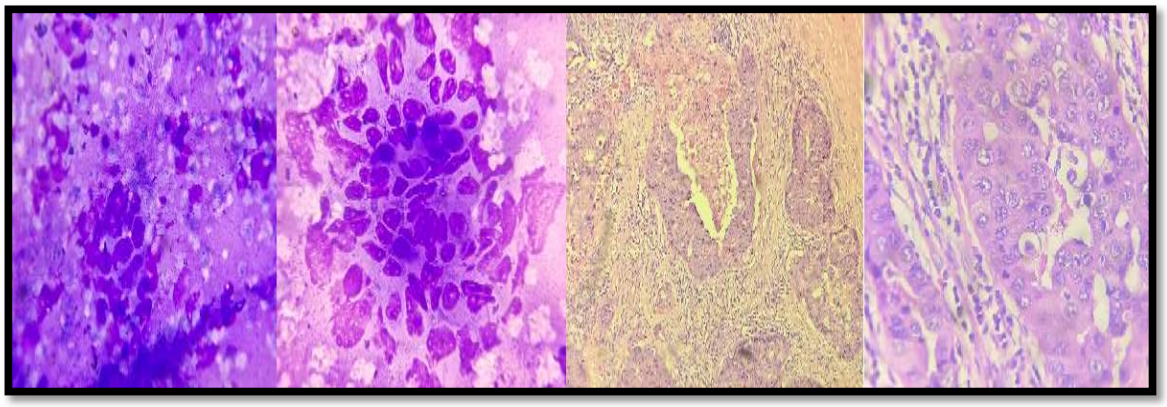
a **b** **c** **d**

FIGURE 3: a) Gross picture of mucoepidermoid carcinoma (MEC) showing a variegated cystic solid tumour with areas of hemorrhage. b): FNAC smear from same case showing a large cluster of mucous and squamoid cells. Another smaller cluster below shows few intermediate cells. (MGG,400x) c) HPE section shows plenty of mucous and intermediate cells. (H&E, 400x). d) Another area showing squamoid or epidermoid cells with abundant eosinophilic cytoplasm. (H&E, 400x)



a **b** **c** **d**

FIGURE 4: a) and b) FNAC smears from adenoid cystic carcinoma (ADCC) showing basaloid tumour cells arranged in clusters with abundant pinkish extracellular hyaline material. (H&E,400x) c) HPE section of same case showing basaloid cells arranged in a cribriform architecture. (H&E, 100x) d) High power view showing cribriform architecture with double layered basaloid cells. (H&E, 400x)



a **b** **c** **d**

FIGURE 5: a) Low power view of salivary duct carcinoma (SDC) showing highly pleomorphic cells in a necrotic and dirty background. (MGG, 100x) b) High power view showing high degree of nuclear pleomorphism, coarse irregularly distributed chromatin and irregular nuclear membrane. (MGG, 400x) c) HPE section from same case showing cells arranged in clusters and trabeculae with large areas of comedo necrosis. (H&E, 100x) d) High power view shows extreme nuclear pleomorphism, irregular nuclear membrane and prominent nucleoli. (H&E, 400x)

CONCLUSION

Despite being relatively rare, salivary gland neoplasms are an important entity that is often hard to subcategorize due to morphological overlap among different entities. Historically, FNAC has been one of the most important preoperative diagnostic tools for salivary gland swellings.

Our study established FNAC as a reliable preoperative diagnostic tool for salivary gland neoplasms with a high specificity, PPV, NPV, diagnostic accuracy and relatively high sensitivity.

We acknowledge that since all the 52 patients underwent both FNAC and surgery, patients who were managed conservatively or lost to follow up were excluded, which may artificially elevate the apparent diagnostic performance.

We also acknowledge that as the study was conducted at a single peripheral rural resource poor tertiary care hospital in eastern India with a relatively small sample size (n = 52), it may limit the broader applicability of the study to more diverse populations.

Conducting a larger study with a study population from multiple different communities and socio-economic status in a larger institution for a longer period might overcome some of these limitations.

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CONFLICT OF INTERESTS
No conflicts of interests were present.

FUNDING:
No funding was received for conducting this study.

ETHICS STATEMENT
This study was conducted at the Pathology department of our medical college after obtaining approval from the institutional ethics committee of our institution vide Memo No BMC/I.E.C/202; dated 17th July 2023.

INFORMED CONSENT
Proper informed consent was obtained from every participant of our study, conforming to the current standards employed in India.

DATA AVAILABILITY
All the mentioned and analyzed data and information can be found in the master chart attached as supplementary material.

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