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Cytohistopathological Study of Salivary Gland Lesions with Emphasis on Diagnotic Pitfalls of Fna.

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KEYWORDS

Salivary gland tumours, Fine needle aspiration cytology(FNAC), pleomorphic adenoma, Cytology-histopathology correlation, Diagnostic Pitfalls.

ABSTRACT:

Introduction: Salivary gland tumours account for 2-6.5% of all the neoplasms of the head and neck. Fine needle aspiration cytology (FNAC) is being increasingly used for the diagnosis of salivary gland tumours.

Objectives: The main aim of this study was to evaluate cytohistological correlation of salivary gland tumors and to identify the causes of diagnostic discrepancies and pitfalls of FNA in correlation with their histopathology.

Methods: A total of 95 FNACs were done on salivary gland tumours from January 2023 to January 2025 at the Department of Pathology, Mysore Medical College and Research Institute, Mysore. These cases were followed up with histopathology. Formalin fixed (10%), surgically resected specimens were received, they were processed and slides were prepared. The stained cytological and histopathological slides were studied, analyzed and correlated.

Results: The cytomorphological features were studied and analyzed and the following lesions were observed: Sialadenosis (8), Pleomorphic adenomas (35), Basal cell adenomas (15), Warthin's tumours (15), Myoepithelioma (8), Cystic lesions (5), Mucoepidermoid carcinomas (3), Adenoid cystic carcinomas (6). Histopathological correlation was done. Out of these, 9 cases were true positives, 5 were false negatives, 81 were true negatives and there were no false positives.

Conclusions: FNAC demonstrated an overall sensitivity of 81.8%, specificity of 100%, and diagnostic accuracy of 94% for distinguishing benign from malignant cases. This study documents the pitfalls of FNAC in salivary gland lesions and underscores key considerations to enhance accuracy and minimize errors in cytological interpretation.

1. Introduction

Salivary gland lesions comprise 2-6.5% of all head and neck neoplasms in adults [1]. Generally salivary gland neoplasms are common in adults with benign tumors occurring usually in the fourth decade and malignant ones in sixth decade. In sex distribution, overall incidence of salivary gland neoplasms shows high preponderance among females [2].

The common presentation of salivary gland tumors is an enlarged mass, which is usually accessible for fine-needle aspiration cytology (FNAC) [1]. Superficial location of tumours, easy accessibility, and high diagnostic accuracy make FNAC a popular method for their evaluation. However, limited cellularity and morphological heterogeneity of lesions can pose diagnostic challenges. Thus, cytopathologic evaluation may be challenging and can be complicated by frequent pitfalls due to various reasons. Histopathological

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diagnosis remains gold standard for diagnosing these neoplasms.

The primary aim of this study was to evaluate the cytohistological correlation of salivary gland tumors and identify the causes of diagnostic discrepancies in FNA.

2. Objectives

The primary objective of this study was to evaluate the cyto-histopathological correlation of salivary gland lesions, with a particular emphasis on determining the diagnostic accuracy of fine-needle aspiration cytology (FNAC) in comparison with final histopathological diagnosis. Additionally, the study aimed to identify the underlying causes of diagnostic discrepancies and to analyze the common pitfalls encountered in FNAC interpretation when correlated with histopathological findings.

3. Methods

This study was conducted from January 2023 to January 2025 at the Department of Pathology, Mysore Medical College and Research Institute, Mysore. It included 95 cases of salivary gland lesions diagnosed through FNAC. After taking the informed consent, the aspiration was done following a thorough clinical examination. The cytological findings were then correlated with histopathology.

The nodule of interest was palpated and fixed with the thumb and the index finger of one hand. Under aseptic precautions, a 10 cc syringe with a 22-25 gauge needle was introduced into the nodule. The aspirated material was smeared onto clean glass slides.

The ethanol fixed smears were stained with Hematoxylin and Eosin(H&E) stain. In cases of fluid aspiration, slides were prepared from the centrifuged sediment.

Formalin fixed (10%), surgically resected specimens were received in the Department of Pathology, processed and stained with hematoxylin and eosin for histopathological examination. The stained cytological and histopathological slides were studied, analyzed and correlated.

4. Results

During the study period, 95 cases of salivary gland swellings were aspirated and diagnosed by FNAC, followed by histopathological correlation. The age of patients ranged from 19 to 83 years, with the majority falling within the 41–50-year range. 51 (53.6%) patients were male, and 44 (46.3%) were female, resulting in a male-to-female ratio of 1.1:1. Gender distribution revealed no significant gender predisposition in this study.

The tumors were predominantly located in the parotid gland (80 cases, 84.2%) and, to a lesser extent, in the submandibular gland (15 cases, 15.7%). There were 86 (90.5%) benign lesions and 9 (9.4%) malignant tumors. The most commonly involved gland was the parotid gland.

35 cases (36.8%) were pleomorphic adenomas (PA), 15(15.7%) were Basal cell adenoma, 8 (8.4%) were Myoepithelioma, 15(15.7%) were Warthin's tumours (WT), 3(3.1%) were mucoepidermoid carcinomas (MEC), 6(6.3%) were adenoid cystic carcinomas(ACC). 5 cases were reported as cystic lesion and other 8 cases as sialadenosis (chart1).

Pleomorphic adenoma (PA) was the most common benign tumor, while adenoid cystic carcinoma (ACC) was the most frequently observed malignant tumor.

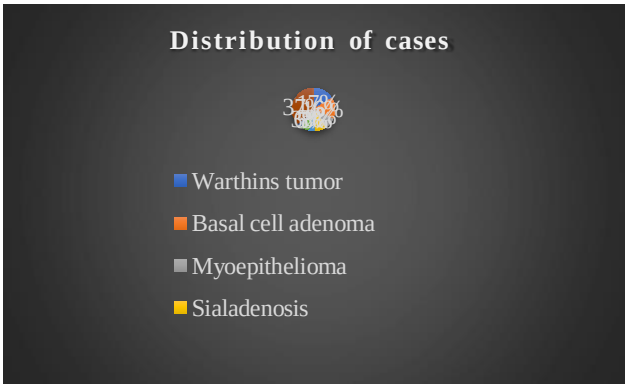

Chart1: Distribution of cases

Table 1: Age Distribution of Patients with Benign and Malignant Lesions

Age Group (years)	Benign Neoplasms	Malignant Neoplasms	Total Patients
19–30	8	1	9
31–40	20	1	21
41–50	45	2	47
51–60	10	4	14
61–70	1	1	2
71–80	1	0	1
81–90	1	0	1
Total			95

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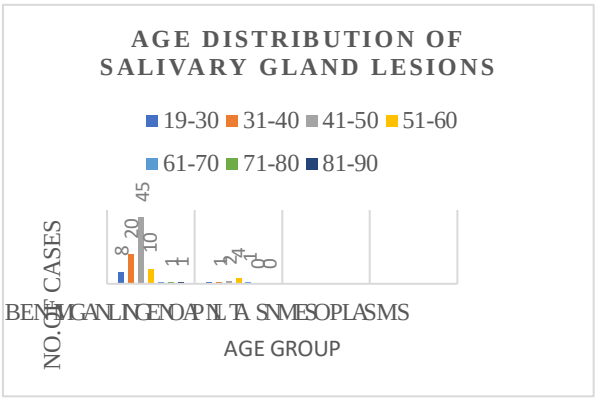

Chart 2: Age Distribution of salivary gland lesions

A total of 95 patients were analyzed for the presence of benign and malignant neoplasms across various age groups ranging from 19 to 90 years. The distribution of cases was detailed in Table 1.

Benign neoplasms were predominant, accounting for 90.5% (n=86) of all neoplasm cases, whereas malignant neoplasms represented only 9.5% (n=9). The highest number of benign neoplasms was observed in the 41–50 age group (n=45), followed by the 31–40 age group (n=20). A sharp decline was noted in patients aged above 60 years.

Malignant neoplasms were more evenly distributed, though the overall frequency remained low. The peak incidence occurred in the 51–60 age group (n=4), followed by 41–50 (n=2).

The combined highest frequency of neoplasm cases, both benign and malignant, was observed in the 41–50 age group (n=47). In contrast, the elderly population (71 years and older) accounted for only 2.1% (n=2) of all cases.

These findings suggest a notable age-related trend, with benign neoplasms peaking in middle age and malignant neoplasms occurring slightly later, with a modest increase in the sixth decade of life.

Among 35 cases of pleomorphic adenomas diagnosed by FNAC, 33 cases were concordantly diagnosed as pleomorphic adenomas in histopathology. The remaining 2 cases were diagnosed as adenoid cystic carcinoma on histopathology.

Out of the 15 cases of basal cell adenoma diagnosed by FNAC, 13 cases were confirmed as basal cell adenoma in histopathology, while 2 cases were diagnosed as myoepithelioma.

8 cases that were diagnosed as sialadenosis on FNAC, one case was discordantly diagnosed as myoepithelioma in histopathology.

Additionally, 2 out of 5 cases diagnosed as a cystic lesion in FNAC was mistakenly diagnosed. It came to be myoepithelioma and mucoepidermoid carcinoma in histopathology.

All the 8 cases of myoepithelioma diagnosed by FNAC were concordantly diagnosed as myoepithelioma in histopathology.

Out of the 15 cases of Warthin's tumor diagnosed by FNAC, 2 cases were later identified as low-grade mucoepidermoid carcinoma and one case as Lymphoepithelial cyst while remaining 12 cases were confirmed as same in histopathology.

All 6 cases of adenoid cystic carcinoma and 3 cases of mucoepidermoid carcinoma diagnosed by FNAC, were concordantly diagnosed as same in histopathology.

Table 2: Cyto-Histopathological correlation.

Table 3: Diagnostic Concordance and Discrepancy of FNAC with Histopathology.

HISTOPATHOLOGY	**CYTOLOGY**							
	Pleomorphic adenoma	**Warthins tumor**	**Basal cell adenoma**	**Myoepithelioma**	**Sialadenosis**	**Cystic lesion**	**ACC**	**Mucoepidermoid carcinoma**
Pleomorphic adenoma	33							
Warthins tumor		12						
Basal cell adenoma			13					
Myoepithelioma			**2**	8	**1**	**1**		
Sialadenosis					7			
Benign cystic lesion						3		
ACC	**2**						6	
Mucoepidermoid carcinoma		**2**				**1**		3
Lymphoepithelial cyst		1						
Total	35	15	15	8	8	5	6	3

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Sl. no	Histopathological Diagnosis	Total cases	Correct Diagnosis on FNAC	Discordant diagnosis on FNAC	Discrepancy (%)
1	**Pleomorphic Adenoma**	35	33	2	5.7%
2	**Warthins tumor**	15	12	3	20%
3	**Basal cell adenoma**	15	13	2	13%
4	**Myoepithelioma**	8	8	0	0%
5	**Sialadenosis**	8	7	1	12%
6	**Benign cystic lesion**	5	3	2	40%
7	**ACC**	6	6	0	0
8	**Mucoepidermoid carcinoma**	3	3	0	0

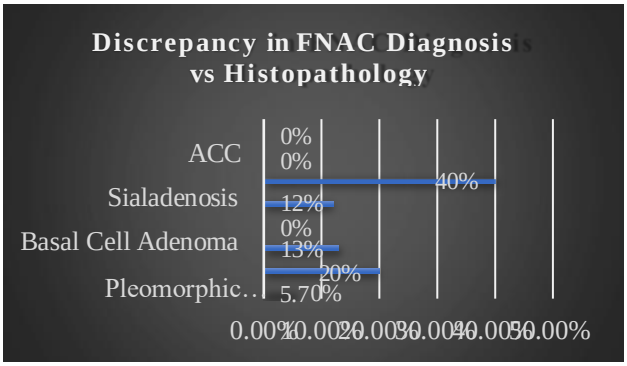

Chart 3: Discrepancy in FNAC Diagnosis vs Histopathology

Table 4: Comparison Table of FNAC with Histopathology

FNAC diagnosed cases	Histopathology confirmed cases			
		Benign	Malignant	Total
	Benign	TN=81	FN=5	86
	Malignant	FP=0	TP=9	9
	Total	81	11	95

Table 5: Validity Parameters for FNAC

Sensitivity	81.8%
Specificity	100%
PPV	100%
NPV	94%
Diagnostic Accuracy	94%

Discussion

Fine Needle Aspiration Cytology (FNAC) remains a cornerstone in the initial evaluation of salivary gland lesions due to its minimally invasive nature, cost-effectiveness, and rapid turnaround time. However, its diagnostic accuracy is known to vary across different lesion types. The present study aimed to assess the diagnostic performance of FNAC in comparison with histopathology and to highlight the patterns and rates of diagnostic discrepancies with a special emphasis on cytomorphological overlaps.

Malignancies existed in 9.4% cases, benign neoplasms in 90.5% cases. The rate of malignant lesions was consistent with the expected rate of malignant disease. Parotid gland was most frequently involved salivary gland followed by submandibular gland. This finding was consistent with other previous studies[3].

Among the 95 cases of salivary gland lesions in the present study, 85 were concordantly diagnosed. 10 cases were discordantly diagnosed. In these cases, the FNAC slides were reviewed.

In the present study, the highest rate of diagnostic discrepancy was observed in cystic lesions, followed by Warthins tumor reflecting the inherent challenges in accurately interpreting these entities on FNAC.

Pleomorphic Adenoma (PA)

PA is the most common salivary gland tumour, accounting for 37% of all the salivary gland lesions. These tumours occur in the middle aged individuals. The reliability of FNAC in diagnosing PA has been reported as 90-97%[4]. In this study, FNAC accurately diagnosed 94.2% of pleomorphic adenomas, consistent with previous studies and showed discrepancy of 5.7%. Two cases were diagnosed as ACC in histopathology.

Pleomorphic adenoma (PA) typically presents with abundant chondromyxoid matrix and a dual population of epithelial and myoepithelial cells, features that usually

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facilitate an accurate cytologic diagnosis. However, diagnostic challenges arise when the aspirate is predominantly cellular with scant matrix, leading to potential misinterpretation as ACC or myoepithelioma. A critical overlap occurs between PA and adenoid cystic carcinoma (ACC), as both tumors can exhibit similar cytological components, including epithelial cells embedded within hyaline or mucoid material [9]. In PA, the hyaline or fibrillary matrix can sometimes mimic the characteristic hyaline globules of ACC, while variants of ACC, such as the tubular and solid forms, may show a paucity of large hyaline globules, thereby resembling PA [9]. Furthermore, focal mild atypia, seen in up to 20% of PA cases, can exacerbate diagnostic uncertainty. The absence of Diff-Quik-stained smears, which are superior to H&E stains in highlighting matrix material and hyaline globules, can further compound the risk of misdiagnosis [11], as noted in our misinterpreted case. (Fig1a,b)

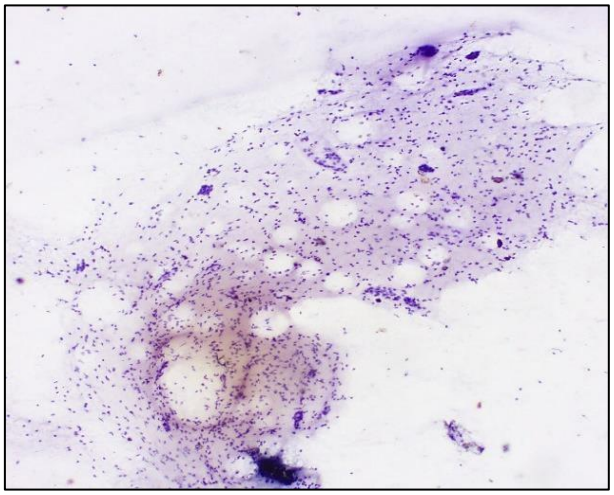

Fig 1a: FNAC misdiagnosed as Pleomorphic adenoma

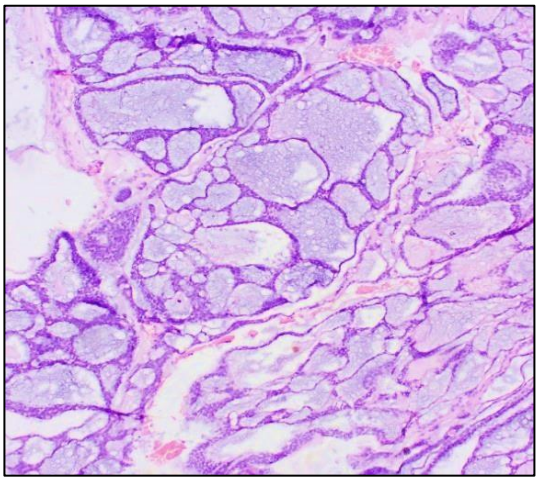

Fig 1b:Histopathologically Adenoid cystic carcinoma

Warthins Tumor

In our study, a total of 15 cases were initially diagnosed as Warthin's tumor based on fine-needle aspiration cytology (FNAC). Of these, 12 cases demonstrated concordant histopathological diagnoses, confirming the initial cytologic impression. However, diagnostic discrepancies were noted in three cases, accounting to 20% of discrepancy. Two cases were subsequently identified as low-grade mucoepidermoid carcinoma, and one case was ultimately diagnosed as a lymphoepithelial cyst on histopathological examination.

Warthin tumor is recognized as the second most common salivary gland neoplasm, characterized cytologically by three key components: sheets of oncocytic epithelial cells, a polymorphous population of mature lymphocytes, and a dirty, granular background. Despite these distinguishing features, preoperative diagnosis of Warthin tumor can pose significant challenges, as its cytologic appearance may overlap with a variety of other salivary gland lesions like low-grade mucoepidermoid carcinoma, squamous cell carcinoma, oncocytic carcinoma, oncocytoma, and lymphoepithelial cysts [16].

Diagnosing low-grade mucoepidermoid carcinoma (MEC) via FNAC poses a significant challenge, primarily because these tumors are often cystic and may yield scant cellularity. Microscopically, it shows three

To mitigate this diagnostic pitfall, pathologists must meticulously assess aspirates for subtle features such as the presence of cylindromatous patterns and small, discrete hyaline globules, which may hint at underlying ACC [13]. Incorporating routine Diff-Quik staining, maintaining a high index of suspicion in matrix-scarce or highly cellular smears, and correlating cytologic findings closely with radiologic and clinical features can significantly enhance diagnostic accuracy. Future practices should emphasize the need for a multi-modal diagnostic approach, including ancillary techniques such as immunocytochemistry. A recent study demonstrated that MYB1 nuclear expression of ACC can differentiate ACC from PA. CD117 and SOX10 are expressed in the majority (94%) of ACC cases in addition to S-100 and myoepithelial markers like SMA and calponin [12,14].

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main types of cells: epidermoid cells, mucous vacuolated cells and intermediate cells, whereas the proportions of these types differ. Moreover, other cell types could be seen in minor or focal proportions as columnar, clear cells and oncocytic cells. In some cases, one type of predominance could be seen [7].

On review of the cytology smears in both cases, the presence of epithelial cell clusters and a few squamoid (epidermoid) cells, initially interpreted as metaplastic squamous cells of Warthins tumor, was noted. Mucin cells were misinterpreted as background cyst macrophages. MEC often shows a cystic architecture with rupture or leakage of mucin into surrounding tissues, leading to a secondary inflammatory response. This inflammation recruits a dense population of lymphocytes, which can become intermixed with tumor cells in aspirates. On FNAC smears, the admixture of neoplastic epithelial cells, squamoid or intermediate cells, mucin cells, and abundant lymphocytes can mimic the classic cytologic appearance of Warthin tumor [15] (Fig 2a,b).

Thus, careful attention must be paid to the morphology of epithelial cells, the presence of true mucin (confirmable with mucin stains), and the overall architectural pattern. Recognition that lymphoid infiltrates can occur as a reactive phenomenon in MEC is essential. Ancillary techniques such as mucicarmine staining for mucin, or molecular testing for MECT1-MAML2 fusion, can aid in distinguishing MEC from benign lymphoid-rich lesions[16].

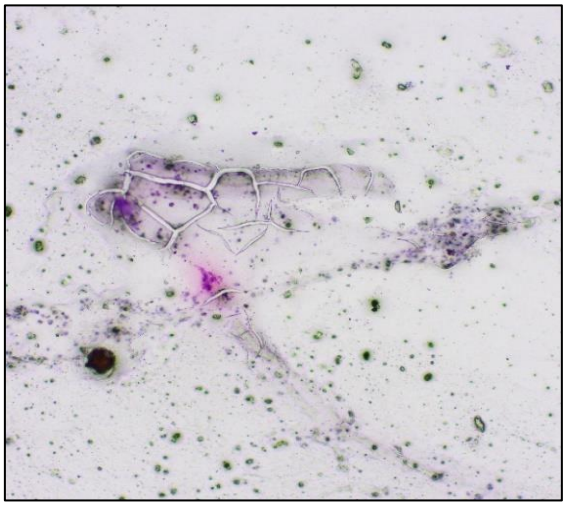

Fig2a: FNAC smears from aspirated fluid sample from salivary gland lesion misdiagnosed as Warthin's tumour

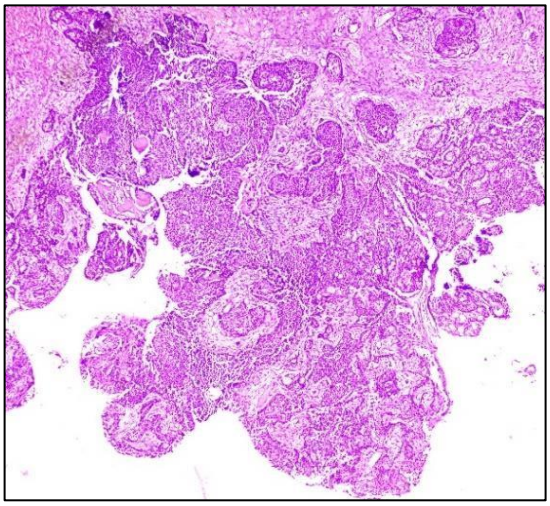

Fig2a: FNAC smears from aspirated fluid sample from salivary gland lesion is diagnosed as Warthin's tumour

Basal Cell Adenoma (BCA)

In present study, a total of 15 cases were initially diagnosed as Basal cell adenoma based on fine-needle aspiration cytology (FNAC). Of these, 13 cases demonstrated concordant histopathological diagnoses, confirming the initial cytologic impression. However, diagnostic discrepancies were noted in two cases, accounting to 13% of discrepancy. Two cases were later diagnosed as Myoepithelioma on histopathological examination.

In fine-needle aspiration cytology (FNAC), basal cell adenoma (BCA) and myoepithelioma share several overlapping cytological features, which often lead to diagnostic confusion. Both tumors are characterized by high cellularity with cohesive clusters of small, uniform basaloid cells exhibiting scant cytoplasm and bland, round to oval nuclei. These similarities are particularly pronounced when myoepithelioma displays predominantly epithelioid cell morphology, closely mimicking BCA. Myoepitheliomas demonstrate spindle-shaped or plasmacytoid cells, features that are uncommon in BCA. The discrepancy likely occurred in

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our case is because FNAC had sampled only the basaloid component of myoepithelioma, suggesting the need for multiple site aspirations (Fig 3a,b).

In ambiguous cases, pathologists must maintain a high index of suspicion and recommend ancillary techniques such as cell block preparation followed by immunocytochemistry. Markers like S100, smooth muscle actin (SMA), calponin, and glial fibrillary acidic protein (GFAP) are typically expressed in myoepithelioma, aiding in the definitive diagnosis. Incorporating these strategies into clinical practice can significantly improve diagnostic accuracy and prevent misinterpretation of myoepitheliomas as BCA.

One FNA slide showed moderate cell yield, these cells were arranged predominantly in cohesive clusters and acinar patterns. These cells had round nucleus with regular nuclear border and fine uniformly distributed chromatin. Based on these cytomorphology, diagnosis of sialadenosis was rendered. On histopathology, this was diagnosed as myoepithelioma. A case of myoepithelioma was missed due to presence of spindle cells that was thought to be fibroblasts in background of chronic inflammatory cells leading to false diagnosis of sialadenosis (Fig 4). Because of varied morphology, inadequate sampling and lack of established cytological criteria, diagnosing myoepithelioma in FNA is difficult [5].

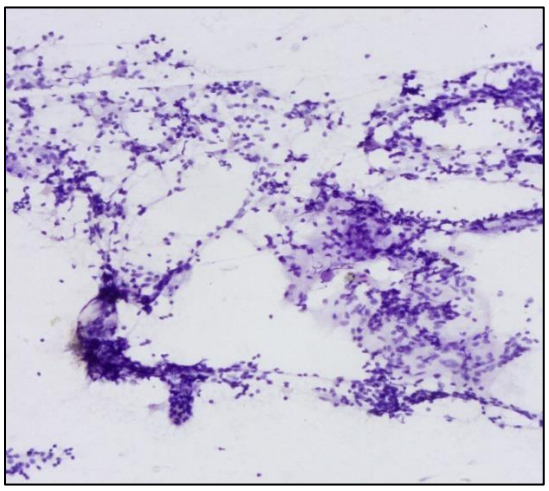

Fig 3a: FNAC, misdiagnosed as Basal cell adenoma

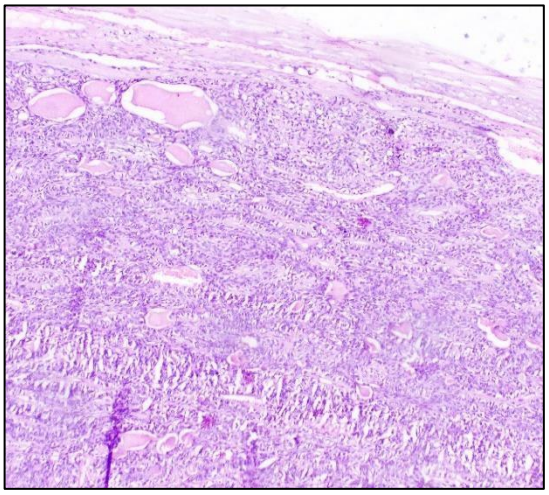

Fig3b: Myoepithelioma

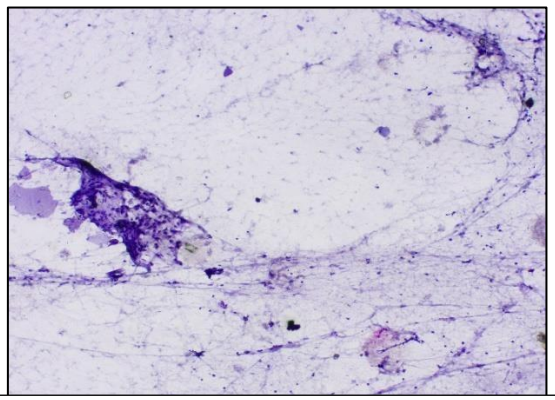

Fig 4: A case of myoepithelioma was missed due to presence of spindle cells that was thought to be fibroblasts in background of chronic inflammatory cells leading to false diagnosis of sialadenosis

Cystic Lesions

Cystic lesions pose major diagnostic discrepancy in our study, contributing to 40%. In two cases, aspiration of cystic fluid led to misdiagnosis, with final histopathology revealing myoepithelioma and mucoepidermoid carcinoma, respectively. Sampling from cystic areas often yields scant or non-representative material, masking the true nature of the tumor. To reduce such errors, repeat aspiration targeting solid areas under imaging guidance or proceeding to core needle biopsy should be considered in cystic salivary gland lesions.

CONCLUSION

This study highlights the diagnostic challenges associated with FNAC in salivary gland tumors, emphasizing its limitations in sensitivity (81.8%) despite

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its high specificity (100%) and overall accuracy (94%). The primary pitfalls contributing to false-negative results included sampling errors, cystic nature of lesions, morphological overlap between benign and malignant tumors.

Pitfalls in diagnosis in FNAC can be due to sampling problems including false negative diagnosis in cystic tumours, small size of lesion, regenerative epithelial hyperplasia and squamous metaplasia in sialadenitis or Warthin's Tumor. Moreover, other sources of diagnostic errors were shown to be due to high celluarity in Pleomorphic Adenoma and overlapping cytological features such as hyaline stromal globules.

An adequate and representative specimen is essential for proper cytological evaluation to reduce errors in diagnosis. Relevant clinical data and radiological findings, along with cooperation between the clinician and cytopathologist are essential in order to use FNAC to its best advantage.

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