Section: Pathology

Original Research Article

VALIDATION OF FINE NEEDLE ASPIRATION CYTOLOGY USING MILAN SYSTEM IN PATIENTS PRESENTING WITH SALIVARY GLAND LESIONS AND COMPARISON OF HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY FINDINGS-A RECORD-BASED STUDY IN A TERTIARY CARE HOSPITAL, MANDYA

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ABSTRACT

Background: Salivary gland lesions account for 2% to 6% of head and neck lesions, with benign tumours being more common than malignant ones. Neoplastic lesions are typically managed surgically, while non-neoplastic lesions are treated conservatively. Fine Needle Aspiration Cytology (FNAC) has become an essential tool in diagnosing salivary gland lesions due to its simplicity, cost-effectiveness, and high accuracy in distinguishing benign from malignant lesions. The accuracy of FNAC in diagnosing these lesions ranges from 81% to 100%, with sensitivity between 86% and 100% and specificity between 90% and 100%. To standardize reporting, the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) was introduced in 2015 to assess malignancy risk and guide clinical management. Despite the utility of FNAC, histopathological examination remains the gold standard, often supplemented by immunohistochemistry and other techniques. This present study aims to interpret the various salivary gland lesions already underwent in our tertiary care centre by FNAC. This study aims to analyse salivary gland lesions diagnosed by FNAC at a tertiary care centre, correlating FNAC findings with histopathology and immunohistochemistry, and utilizing the MSRSGC for categorization.

Materials and Methods: The retrospective study, conducted at MIMS Mandya from January 2023 to June 2024, reviewed 90 FNAC cases.

Results: The most frequently involved gland was the parotid (61%), with pleomorphic adenoma being the most common benign neoplastic lesion. The MSRSGC categorization revealed 52(57.7%) non-neoplastic, 34 (37.7%) benign, and 4 (4.4 %) malignant cases. Histopathological correlation in 21 cases confirmed 5(24%) non-neoplastic, 13(62%) benign, and 3(14%) malignant lesions. The study showed 100% specificity and 75% sensitivity for FNAC, with a diagnostic accuracy of 95.2%. Conclusion: The study confirms FNAC's role as an effective diagnostic tool, especially when correlated with histopathological and immunohistochemical findings.

Conclusion: In conclusion, with adequate proper sampling and evaluation by cytopathologists, the majority of salivary gland lesions can be identified through FNAC. It remains the first and most accurate diagnostic tool in majority of the salivary gland lesions. It plays an important role in preoperative evaluation and categorization of various salivary gland lesions for proper management. Even though it has high sensitivity and specificity the ancillary techniques such as

biopsy and IHC might be helpful in difficult cases. The FNAC smears may also be used for special stains and immunocytochemistry.
Keywords: FNAC, MSRSGC, Histopathology and IHC.

INTRODUCTION

Salivary gland lesions constitute approximately around 2% to 6% of all head and neck lesions. Among them benign salivary gland lesions are more common than malignant lesions.[1] About 65% to 80% of lesions arise from the parotid gland, 10% arise from the submandibular gland and other 10% arise from the minor salivary glands including sublingual glands. Most of the benign tumour arise from the parotid gland.[2] Neoplastic lesions are usually managed by surgery while non-neoplastic lesions are managed mostly by conservative treatment. So, Fine Needle Aspiration Cytology provides a proper guidance in the management of salivary gland lesions.[3] Fine Needle Aspiration Cytology has become widely accepted, rapid, simple, cost-effective, time consuming and safe tool in early diagnosis of salivary gland lesions. According to various studies conducted by many authors, the accuracy of FNAC in differentiating benign from malignant salivary gland lesions ranges from 81% to 100%.[4,5] FNAC has overall sensitivity ranges from 86% to 100 % and the specificity ranges from 90% to 100% in diagnosing salivary gland neoplasm. Since there was no proper reporting system or standardization or categorization of FNA cytological findings, the international consortium of experts came with a new reporting system for salivary gland cytology specimens.[4,5] Thus, the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) was started in September 2015 in Milan and was published in 2018. It was developed in order to assess the risk of malignancy and for proper clinical management.[6,7] However, histopathological examination remains the gold standard for diagnosing salivary gland lesions along with ancillary techniques like immunohistochemistry, special stains, tumour markers and cytogenetics.[8]

This present study aims to interpret the various spectrum of salivary gland lesions undergoing in our tertiary care centre, MIMS, by FNAC using Milan system categorization which properly categorize the non-neoplastic, benign and malignant lesions and reduces the risk of mis-interpretation of results in future settings. It also helps in correlation of cytological and histopathological findings and their correlation with age, gender and anatomical site. FNAC remains an excellent first-line tool in providing an early diagnosis and there by avoids the need of unnecessary surgical intervention. It also provides proper therapeutic management for the patient. It also helps to assess the sensitivity, specificity, diagnostic accuracy and efficacy of FNAC.

Objectives

- To evaluate the validity of FNAC in diagnosing salivary gland lesions based on Milan system categorization with comparison to histopathology findings among the patients attending a tertiary care hospital, Mandya.
- To evaluate the validity of FNAC in diagnosing salivary gland lesions with comparison to immunohistochemistry findings among the study population.

MATERIALS AND METHODS

This is a retrospective record based 18months study done at the Department of Pathology, MIMS, Mandya, Karnataka during March 2023 to August 2024. The study period was for six months from September 2024 to February 2025 following the approval obtained by the Institutional Ethics Committee, bearing the number MIMS/IEC/2025/1006. The air-dried smears were stained using Romanowsky stain and the wet smears were stained using Hematoxylin and Eosin (H&E) stain. For histopathological examination, 4 µ to 5 µ thick tissue sections were taken and stained using H & E stain. The slides were then analyzed under the microscope.

Study Population: Records of all patients who had already underwent FNAC and Biopsy in tertiary care centre, Mandya from March 2023 to August 2024 were included in the study.

Sampling Method: Consecutive enrolment.

Inclusion Criteria:

- All cases of FNAC for salivary gland lesions.
- All cases of histopathological examination of salivary gland lesions who underwent surgery following FNAC.

Exclusion Criteria:

All cases already under treatment for salivary gland lesions.

Method of Data Collection (study tools): All cases of FNAC and histopathology for salivary gland lesions were retrieved from the registers maintained at the department of Pathology. The findings were analyzed using descriptive statistics.

Analysis: All the collected data were entered in an excel sheet and the data was statistically analysed using Statistical Package for the Social sciences software (IBM- SPSS trial version).

- Descriptive statistics like mean, Standard deviation for quantitative data (age), percentage and proportion for categorized data (gender and sites).
- The diagnostic validity of FNAC and histopathology parameters in terms of sensitivity, specificity and diagnostic accuracy were evaluated.

Slide review and categorization: Following review of all the FNA slides, each case was categorized

according to The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC).

The categories in the Milan system are:

- Non-diagnostic (ND)
- Non-neoplastic (NN)
- Atypia of Undetermined Significance (AUS)
- Neoplasm
  - A. Neoplasm: Benign (BN)
  - B. Neoplasm: Salivary Gland Neoplasm of Undetermined Significance (SUMP)
  - Suspicious for Malignancy (SM)
  - Malignant neoplasm (MN)

RESULTS

A total of 90 cases were presented with salivary gland lesions on FNAC out of which 21 cases had histopathological correlation. The majority of the cases affected were between 40 to 50 years. [Table 1] shows the age distribution of the patients. 56.6% of the affected patients were male with the parotid being the most commonly involved gland (61%).

[Table 2] shows the sex distribution among various salivary gland lesions. [Table 3] shows the location wise distribution of lesions. [Table 4] shows the FNAC diagnosis of various salivary gland lesions.

The most commonly involved benign neoplastic lesion was pleomorphic adenoma (21cases, 23%). Among the non-neoplastic lesions, the chronic sialadenitis (34.4%) was frequently noted. According to MSRSGC categorization, 3 cases (3.3%) were non-diagnostic, 49 cases (54.4%) were non-neoplastic, 34 cases (37.7%) were benign, 4 cases (4.4 %) were malignant on FNAC. [Table 5] shows FNAC and Histopathological correlation by using MSRSGC.

Out of 21 cases on histopathological correlation, 5 cases (24%) were non-neoplastic, 13 cases (62%) were benign, 3 cases (14%) were malignant and 8 cases had IHC correlation.

According to MSRSGC categorization, FNAC and histopathological correlation, out of 21 cases, 3 cases (14.2 %) were category I, 2 cases (9.5%) were category II, 1case (4.7 %) were category III, 13 cases (62.1%) were category IV and 2 cases (9.5%) were category VI.

Out of 3 cases of category I, benign cystic lesions turned out to be salivary duct cyst and lymphoepithelial cyst. One case of pleomorphic adenoma on FNAC turned out to be canalicular adenoma on histopathological examination.

In the present study, the specificity and the sensitivity were found to be 100 % and 75 % respectively. The positive predictive value of salivary gland cytology was 75 % and diagnostic accuracy was 95.2%. Table 6 and 7 shows the diagnostic accuracy of FNAC.

[Figure 1 to 9] describes the FNA, Histopathology and IHC images of various salivary gland lesions.

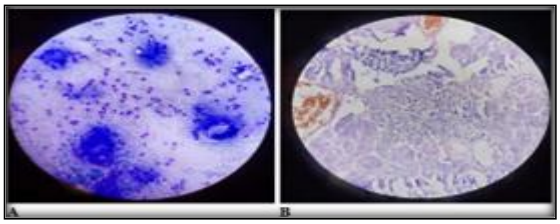

Figure 1: A and B: FNA shows benign epithelial cell clusters in a background of lymphocytes leading to a diagnosis of sialadenitis. (MGG,10x). Histopathology shows serous acini with interstitial inflammatory infiltrate of lymphocytes. (H&E,10x).

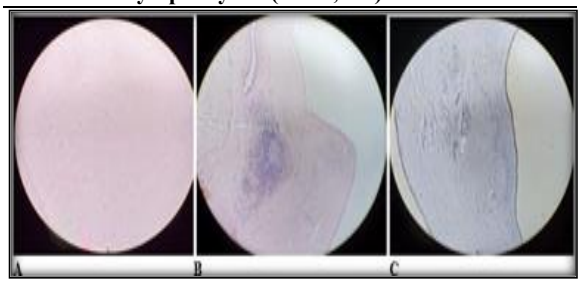

Figure 2: A, B and C: FNA shows lymphocytes in a proteinaceous background leading to a diagnosis of lymphoepithelial cyst. (H&E,10x). Histopathology shows cyst lined by low cuboidal epithelium, sub-epithelium shows polymorphous lymphoid tissue with germinal centres and lymphocytes. (H&E,10x). IHC Staining for AE1 shows positive in epithelial cell lining.

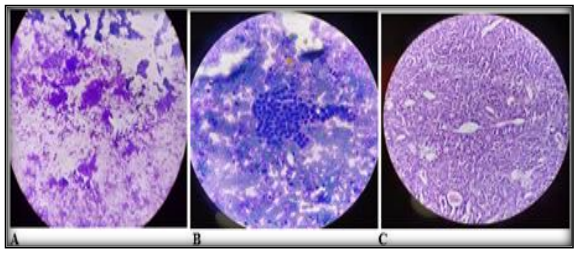

Figure 3: A, B and C: FNA of Basaloid cell adenoma shows benign clusters of basaloid cells with monomorphic round nuclei and scant cytoplasm. (MGG, 10x and 40x). Histopathology shows basaloid cells in tubular and trabecular pattern. (H & E,10x).

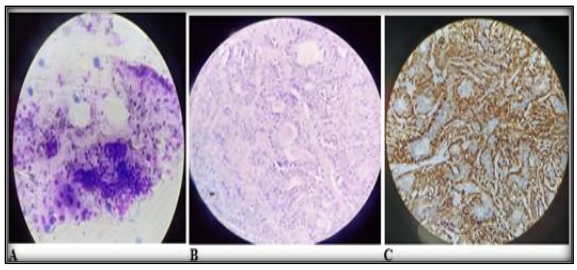

Figure 4: A, B and C: FNA shows benign clusters of epithelial cells with myxoid background diagnosed as pleomorphic adenoma (MGG, 40x). Histopathology shows bilayered anastomosing cords of tumour cells with cystic change diagnosed as Canalicular adenoma. (H & E,40x). IHC shows diffuse S100 positivity.

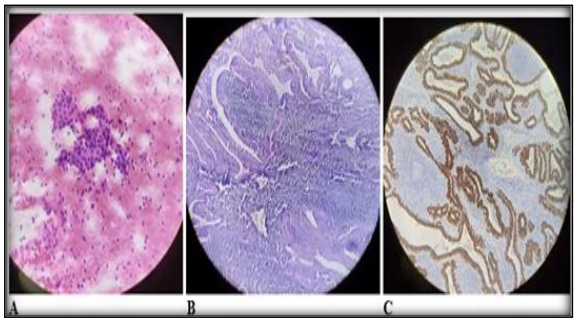

Figure 5: A, B and C: FNA shows benign clusters of epithelial cells with a background of lymphocytes diagnosed as Warthin's tumour (MGG, 40x). Histopathology shows papillary cystic structure lined by bilayered oncocytic epithelial cells surrounded by a lymphoid stroma. (H & E,10x). IHC shows CK7 positive in epithelial cells. (10x)

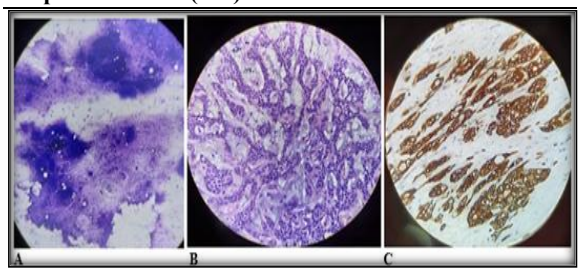

Figure 6: A, B and C: FNA shows benign clusters of epithelial cells with myxoid background diagnosed as pleomorphic adenoma (MGG, 10x). Histopathology shows ductal epithelial cells and myoepithelial cells in tubules and cords with a myxoid stroma. (H & E,40x). IHC shows diffuse strong CK7 positivity for ductal epithelial cells.

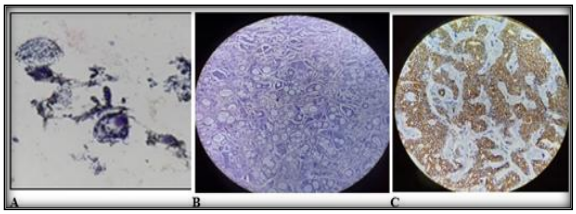

Figure 7: A, B and C: FNA shows clusters of tumour cells with central hyaline material diagnosed as adenoid cystic carcinoma (MGG, 10x). Histopathology shows cribriform to tubular arrangement of bilayered epithelium. (H & E,10x). IHC shows CD117 positivity.

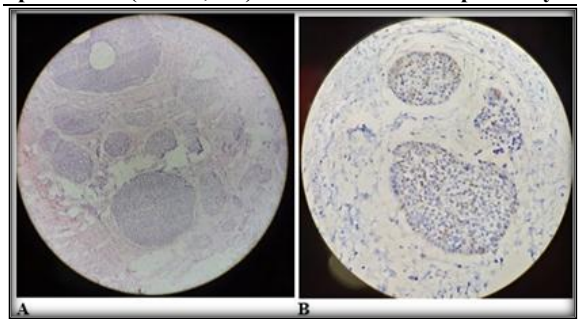

Figure 8: A and B: Histopathology shows malignant tumour cells arranged in nests with a desmoplastic stroma. (H & E,10x). IHC shows patchy focal AR positivity.

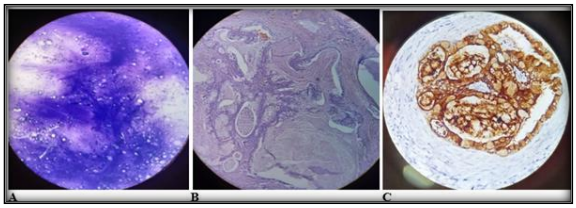

Figure 9: A, B and C: FNA smears shows clusters of squamous cells in a myxoid background diagnosed as pleomorphic adenoma. (MGG, 10x). Histopathology showing predominantly of glandular growth pattern with mucous cells and intermediate cells. (H & E, 10x). IHC shows MUC4 positivity of mucous cells. (40x).

Table 1: Age distribution of cases.

Age group distribution	No. of. Cases	Percentage
0-10	01	1.1%
11-20	09	10%
21-30	10	11%
31-40	18	20%
41-50	22	24.4%
51-60	13	14.4%
61-70	09	10%
71-80	06	6.6%
81-90	02	2.2%

Table 2: Sex wise distribution of salivary gland lesions

Lesion	No of males	No of females
Non-neoplastic	29	25
Benign	19	13
Malignant	03	01
Total	51	39

Table 3: Distribution of cases based on location

Salivary gland involved	Side (Right or left)	No. of. cases	Percentage	Total
Parotid	Right- Left-	33 22	36.6% 24.4%	55
Submandibular	Right- Left-	18 14	20% 15.5%	32

Sublingual	Right- Left-	_____	_____	0
Minor salivary gland		03	3.3%	03

Table 4: FNAC diagnosis of salivary gland lesions

Category	Parotid	Submandibular	Sublingual	MSG	Total	Percentage
Non-neoplastic						
Benign cystic lesion	03				03	3.3%
Sialadenosis	02	03			05	5.5%
Acute sialadenitis	03	05			08	8.8%
Chronic sialadenitis	15	15		01	31	34.4%
Parotitis	04				04	4.4%
Acute suppurative abscess	____	03	_____	_____	03	3.3%
Benign lesion						
Pleomorphic adenoma	17	03	____	01	21	23.3%
Myoepithelioma	01	____	____	___	01	1.1%
Basal cell adenoma		01			01	1.1%
Oncocytoma	01				01	1.1%
Warthin tumour	07	01			08	8.8%
Malignant lesion						
Mucoepidermoid carcinoma	01	01	____	01	03	3.3%
Adenoid cystic carcinoma	01	___	____	___	01	1.1%
Others						

Table 5: Comparison of FNAC and histopathological diagnosis by using MILAN system categorization.

Milan category	FNAC diagnosis	Histopathology diagnosis	No. of .cases
I (Non- diagnostic)	Benign Cystic lesion	Salivary duct cyst Lymphoepithelial cyst	01 02
II (Non -neoplastic)	Sialadenosis Acute sialadenitis Bilateral Parotitis Chronic sialadenitis	---------- ---------- ---------- Chronic sialadenitis	02
III (AUS)	Basal cell adenoma	Basal cell adenoma	01
IV (Neoplasm) A-Benign B-SUMP	Pleomorphic adenoma Warthin's tumour Pleomorphic adenoma	Pleomorphic adenoma Canalicular adenoma Warthin's tumour Oncocytoma Low grade mucoepidermoid carcinoma	07 01 03 01 01
V (SM)	----------	----------	
VI (MN)	Mucoepidermoid carcinoma Adenoid cystic carcinoma	Mucoepidermoid carcinoma Adenoid cystic carcinoma	01 01

Table 6: Accuracy of FNAC of various salivary neoplasms

Lesion	No. of cases diagnosed on FNAC	HPE confirmed diagnosis	Accuracy
Pleomorphic adenoma	08	07	87.5%
Warthin tumour	03	03	100%
Basal cell adenoma	01	01	100%
Mucoepidermoid carcinoma	04	02	50%
Adenoid cystic carcinoma	01	01	100%

Table 7: Comparison of diagnostic accuracy with other studies

Study	No of cases histologically confirmed	Diagnostic accuracy
Himani et al.	19	90.45%
GC Fernandez et al.	32	87.50%
Jayram et al.	57	87.70%
Our study	21	95.2%

DISCUSSION

FNAC is a safe, rapid, non-invasive, cost effective and accurate method for diagnosing salivary gland lesions. FNAC reduces the need for surgery by accurate diagnosis of non-neoplastic conditions (category II).

In our study, salivary gland lesions were found to be more common in males as compared to females, with a male: female ratio of 1.3:1. Majority of the lesions are most commonly involved in parotid gland (61.1%) followed by submandibular gland (35.5%) and minor salivary glands (3.3%). Similar findings were observed in studies conducted by Singh et al, with a male: female ratio of 2.6:1 with most commonly involved salivary gland was parotid gland (74.4%) followed by submandibular (21%) and minor salivary glands (4.5%) and Sneha kakoty et al.,9 with a male: female ratio of 1.17:1 1 with most

commonly involved salivary gland was parotid gland (62%) followed by submandibular (32%) and minor salivary glands (6%).

In our present study according to MSRSGC categorization, 3cases (3.3%) were category I, 49 cases (54.4%) were category II, 1(1.1%) case were category III, 33 cases (36.8%) were category IV, 4 cases (4.4 %) were category VI on FNAC. Similar findings observed in study conducted by Sneha kakoty et al.,9 were ND as (6.1%), NN as (38.2%), AUS as (2.7%), NB as (33.4%), SUMP as (2.0%), SM as (2.4%), and Malignant as (15%).

In our study there were one false negative case. The main contributing factor is subjective errors like interpretation of cellularity, obscuring background like haemorrhage and inadequate material. One case of Mucoepidermoid carcinoma was misdiagnosed as Pleomorphic adenoma due to mucinous background resembling myxoid, mildly atypical squamous cells and less cellularity. Similar observations have been made by Sneha kakoty et al,[9] were one case of pleomorphic adenoma turned out to be mucoepidermoid carcinoma.

In our study there were one case of submandibular swelling, on FNAC turns out to be metastatic carcinoma, which had further sent for biopsy and it turns out to be salivary duct carcinoma and had immunohistochemistry correlation.

In the present study, the specificity and the sensitivity were found to be 100 % and 75 % respectively. The positive predictive value of salivary gland cytology was 75 % and diagnostic accuracy was 95.2%. Similar findings were observed in studies conducted by Sneha kakoty et al,[9] sensitivity as 90.91%, specificity as 96.42%, predictive value of salivary gland cytology was 90.91% with diagnostic accuracy of 94.87% and Singh et al, sensitivity, specificity and diagnostic accuracy of 80%, 89.80% and 87.50% respectively.[10-15]

The cytomorphological features suggestive of malignancy are termed as suspicious of malignancy (category V), no cases were observed in our present study. The categories of AUS, SUMP and SM are intermediate category in diagnosis favouring to malignancy in MILAN system categorization.

FNAC is helpful in distinguishing between benign and malignant lesions. Ancillary techniques such as ultrasonography or CT are recommended in patients with small or large cystic or haemorrhagic lesions as many of the neoplasm of salivary gland lesions show cystic change. Proper diagnosis can yield a good treatment plan and better outcomes for the patient.

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Limitations: Limitation of our study includes the smaller number of sample size, very few cases had histopathological follow up, and retrospective nature of the study. Further studies including large number of sample size, multicentric studies are recommended along with proper management plan and histopathological follow-up are required for prospective application of the study.

CONCLUSION

In conclusion, with adequate proper sampling and evaluation by cytopathologists, the majority of salivary gland lesions can be identified through FNAC. It remains the first and most accurate diagnostic tool in majority of the salivary gland lesions. It plays an important role in preoperative evaluation and categorization of various salivary gland lesions for proper management. Even though it has high sensitivity and specificity the ancillary techniques such as biopsy and IHC might be helpful in difficult cases. The FNAC smears may also be used for special stains and immunocytochemistry.

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