|| ISSN(online): 2589-8698 || ISSN(print): 2589-868X ||

Available online on http://www.ijmbs.info

International Journal of Medical and Biomedical Studies; 2026; 10(1); 67-74

Original Research

Cytology of Salivary Gland Tumours: Application of the Milan System and Diagnostic Challenges – A Retrospective Study

Parnika[1], Kumari Mona[2], Pulak Raj[3], Shobha Mohindroo[4]

[1]Junior Pathologist, Department of Pathology, Nalanda Healthcare and Diagnostics, Patna, Bihar, India
[2]MBBS MD, Consultant, Department of Community medicine, Nalanda Healthcare and Diagnostics, Patna, Bihar, India
[3]MBBS MD, Consultant Pathologist, Department of Pathology, Nalanda Healthcare and Diagnostics Patna, Bihar, India
[4]Professor, Department of Pathology, KNS Memorial Institute of Medical Sciences, Uttar Pradesh, India

Received: 19-11-2025 / Revised: 22-12-2025 / Accepted: 17-01-2026
DOI: https://doi.org/10.32553/ijmbs.v10i1.3207
Corresponding author: Pulak Raj
Conflict of interest: No conflict of interest

Abstract:

Background: Fine needle aspiration cytology (FNAC) is a primary diagnostic tool for salivary gland lesions. The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) provides standardized categorization with defined risk of malignancy (ROM).

Aim: To evaluate salivary gland cytology cases using the Milan system and analyze diagnostic challenges with histopathological correlation.

Methods: A retrospective study was conducted at Nalanda Healthcare and Diagnostics, Patna, from 10 November 2025 to 10 January 2026. Thirty FNAC cases of salivary gland lesions were categorized according to the Milan system. Histopathological follow-up was available in 26 cases (86.7%). Statistical analysis included calculation of ROM, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and chi-square test.

Results: The most common Milan category was Category IVa (Benign neoplasm) (40%), followed by Category II (Non-neoplastic) (20%). Histologically confirmed malignancy rate was 30.8% (8/26). Sensitivity and specificity of FNAC for detecting malignancy were 62.5% and 94.4%, respectively. Overall diagnostic accuracy was 84.6%. The highest ROM was observed in Categories V and VI (100%).

Conclusion: The Milan system provides structured risk stratification and improves communication between cytopathologists and clinicians. While FNAC shows high specificity, indeterminate categories remain a diagnostic challenge requiring histopathological confirmation.

Keywords: Salivary gland cytology; Milan system; FNAC; Risk of malignancy; Diagnostic accuracy.

Introduction

Salivary gland tumours represent a heterogeneous group of lesions with diverse histomorphological patterns and biological behavior [1]. Although they account for less than 5% of head and neck neoplasms, their wide spectrum poses diagnostic challenges [2].

Fine needle aspiration cytology (FNAC) has emerged as a minimally invasive, cost-effective diagnostic modality for salivary gland lesions [3]. It helps differentiate inflammatory from neoplastic lesions and benign from malignant tumours [4]. However, overlapping cytomorphological features and tumor heterogeneity can lead to diagnostic ambiguity [5].

To standardize reporting and improve clinical utility, the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) was introduced in 2018 [6]. The system categorizes lesions into six diagnostic categories, each associated with an implied risk of malignancy (ROM) and management recommendations [7].

The Milan System classifies salivary gland cytology into six diagnostic categories: Category I (Non-diagnostic), Category II (Non-neoplastic), Category III (Atypia of Undetermined Significance – AUS), Category IVa (Benign neoplasm), Category IVb (Salivary gland neoplasm of uncertain malignant potential – SUMP), Category V (Suspicious for malignancy), and Category VI (Malignant). Each category is associated with an implied risk of malignancy (ROM) and corresponding management recommendations, thereby facilitating standardized reporting and clinical decision-making. Several validation studies have demonstrated improved diagnostic consistency, reproducibility, and better risk stratification with the application of the Milan system [8,9]. The ROM varies significantly across categories, with the highest risk consistently observed in Category VI, reinforcing its strong predictive value for malignancy [10].

Despite its advantages, certain lesions such as low-grade mucoepidermoid carcinoma, pleomorphic adenoma with atypia, and Warthin tumor with metaplasia may create interpretative difficulties [11,12]. Histopathological correlation remains essential for definitive diagnosis [13].

There is limited data regarding application of the Milan system in smaller diagnostic centers in Eastern India. Therefore, this study aimed to evaluate salivary gland FNAC cases using the Milan system and analyze associated diagnostic challenges [14].

Materials and Methods

Study Design

This was a **retrospective observational study** conducted to evaluate the cytomorphological spectrum of salivary gland lesions and to assess the applicability of the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) along with associated diagnostic challenges.

Study Setting

The study was carried out in the Department of Cytopathology at **Nalanda Healthcare and Diagnostics, Patna, Bihar**, a private diagnostic center catering to both urban and semi-urban populations.

Study Duration

The study period extended from **10 November 2025 to 10 January 2026** (two months). All eligible cases during this duration were included in the analysis.

Sample Size

A total of **30 consecutive cases** of salivary gland swellings subjected to fine needle aspiration cytology (FNAC) during the study period were included.

Inclusion Criteria

- All patients presenting with clinically palpable salivary gland swellings.
- Cases in which FNAC was performed during the defined study period.
- Adequate cytological smears available for evaluation.
- Cases with available demographic and clinical details.

Exclusion Criteria

- Unsatisfactory or hemorrhagic smears insufficient for interpretation.

- Cases lacking essential clinical information.
- Recurrent lesions previously diagnosed outside the study period.

Clinical Evaluation

Detailed clinical information including age, gender, site of swelling, duration, and clinical suspicion was retrieved from laboratory records and requisition forms. Radiological findings, where available, were also noted.

FNAC Procedure

FNAC was performed using a **23–24 gauge disposable needle** attached to a 10 mL syringe under aseptic precautions. Multiple passes were made when necessary to obtain adequate cellular material.

The aspirated material was smeared onto clean glass slides. Smears were:

- Air-dried and stained with **May-Grünwald-Giemsa (MGG)** stain.
- Alcohol-fixed and stained with **Hematoxylin and Eosin (H&E)** stain.

Cytological adequacy was assessed based on cellularity and preservation of morphological details.

Cytological Evaluation and Milan Categorization

All cases were reviewed independently by two pathologists. The lesions were categorized according to the **Milan System for Reporting Salivary Gland Cytopathology (MSRSGC)** into the following six categories:

- Category I – Non-diagnostic
- Category II – Non-neoplastic
- Category III – Atypia of Undetermined Significance (AUS)
- Category IVa – Benign neoplasm
- Category IVb – Salivary gland neoplasm of uncertain malignant potential (SUMP)
- Category V – Suspicious for malignancy
- Category VI – Malignant

Each case was assigned a Milan category based on cytomorphological criteria including cellular arrangement, stromal components, nuclear atypia, background elements, and presence of necrosis or mitosis.

Histopathological Correlation

Histopathological follow-up was available in **26 out of 30 cases (86.7%)**. Surgical specimens included excision biopsies and gland resections. Histopathological diagnosis was considered the gold standard.

Cyto-histopathological correlation was performed to determine concordance and identify discordant cases. Diagnostic discrepancies were analyzed to identify potential cytological pitfalls.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed statistically.

The following parameters were calculated for detection of malignancy:

- True Positive (TP): Cytology malignant and histology malignant
- True Negative (TN): Cytology benign and histology benign
- False Positive (FP): Cytology malignant but histology benign
- False Negative (FN): Cytology benign but histology malignant

From these values, the following were computed:

- **Sensitivity** = TP / (TP + FN) × 100
- **Specificity** = TN / (TN + FP) × 100
- **Positive Predictive Value (PPV)** = TP / (TP + FP) × 100
- **Negative Predictive Value (NPV)** = TN / (TN + FN) × 100
- **Overall Diagnostic Accuracy** = (TP + TN) / Total cases with histology × 100

Risk of malignancy (ROM) for each Milan category was calculated as:

ROM = (Number of malignant cases in a category / Total cases with histology in that category) × 100

Confidence intervals (95% CI) were calculated for sensitivity and specificity. Association between Milan categories and histopathological diagnosis was evaluated using the Chi-square test. A p-value < 0.05 was considered statistically significant.

Results

A total of **30 cases** of salivary gland lesions were evaluated by fine needle aspiration cytology (FNAC) during the study period (10/11/2025–10/01/2026). Histopathological follow-up was available in 26 cases (86.7%), which were included for cyto-histological correlation and calculation of diagnostic accuracy parameters.

1. Demographic Profile

The age of patients ranged from **18 to 72 years**, with a mean age of **44.3 ± 13.6 years**. The highest frequency was observed in the **31–50 years** age group (46.7%). There were **17 males (56.7%)** and **13 females (43.3%)**, with a male-to-female ratio of **1.3:1**.

Table 1 shows the age and gender distribution of cases.

Table 1: Age and Gender Distribution of Study Population (n = 30)

Age Group (Years)	Male	Female	Total (%)
≤30	3	2	5 (16.7%)
31–50	8	6	14 (46.7%)
>50	6	5	11 (36.6%)
Total	17	13	30 (100%)

As shown in **Table 1**, the majority of lesions occurred between 31–50 years of age.

2. Site Distribution

The most common site involved was the **parotid gland** (20 cases; 66.7%), followed by the **submandibular gland** (8 cases; 26.7%), and minor salivary glands (2 cases; 6.6%).

The distribution of lesions according to site is illustrated in **Figure 1**.

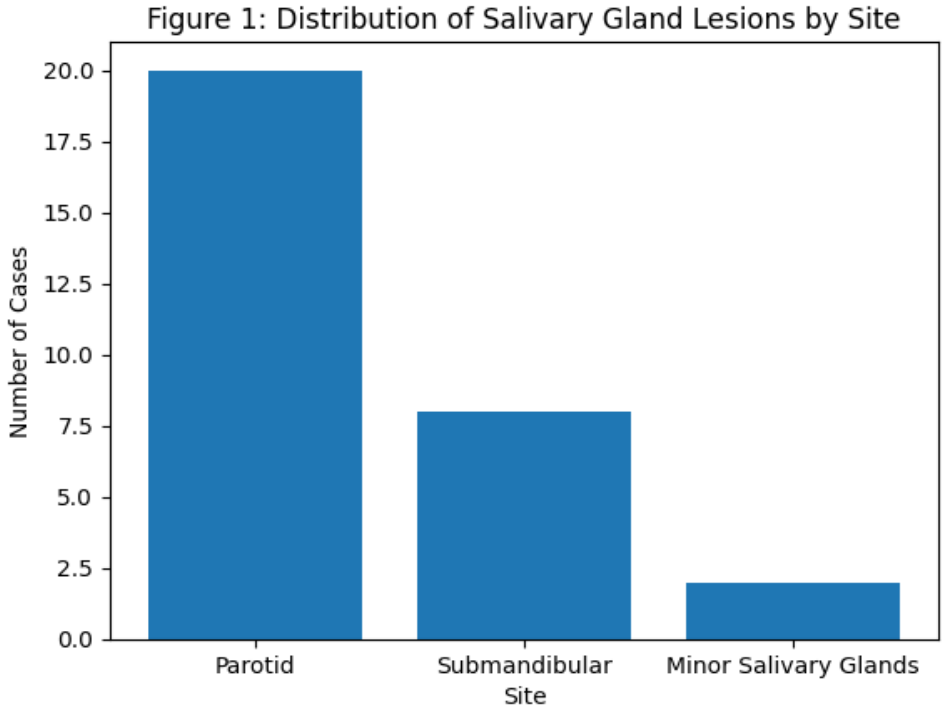

Figure 1: Distribution of Salivary Gland Lesions by Site

3. Distribution According to the Milan System Categories

All 30 cases were categorized according to the **Milan System for Reporting Salivary Gland Cytopathology (MSRSGC)**.

The distribution of cases across Milan categories is shown in **Table 2, Figure 2**.

Table 2: Distribution of Cases According to Milan System (n = 30)

Milan Category	Number of Cases	Percentage (%)
I – Non-diagnostic	2	6.7%
II – Non-neoplastic	6	20.0%
III – AUS	2	6.7%
IVa – Benign neoplasm	12	40.0%
IVb – SUMP	3	10.0%
V – Suspicious for malignancy	2	6.7%
VI – Malignant	3	10.0%
Total	30	100%

As demonstrated in **Table 2**, the most frequent category was **IVa (Benign neoplasm)** accounting for 40% of cases.

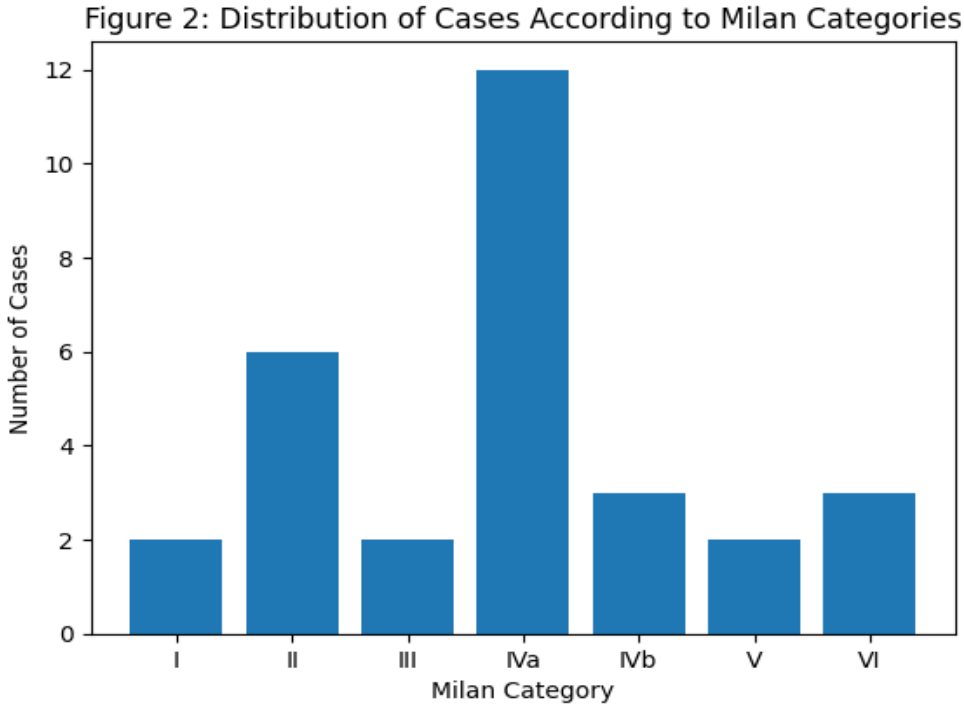

Figure 2: Percentage Distribution of Cases According to Milan Categories

4. Cytological Diagnosis Pattern

Among benign neoplasms, **Pleomorphic adenoma** was the most common lesion (10 cases; 33.3%), followed by **Warthin tumor** (2 cases; 6.7%).

Among malignant lesions, **Mucoepidermoid carcinoma** (2 cases; 6.7%) was the most frequent, followed by **Adenoid cystic carcinoma** (1 case; 3.3%).

The overall cytological diagnosis distribution is summarized in **Table 3**.

Table 3: Cytological Diagnosis of Salivary Gland Lesions (n = 30)

Diagnosis	Number of Cases	Percentage (%)
Chronic sialadenitis	4	13.3%
Acute sialadenitis	2	6.7%
Pleomorphic adenoma	10	33.3%
Warthin tumor	2	6.7%
SUMP	3	10.0%
Mucoepidermoid carcinoma	2	6.7%
Adenoid cystic carcinoma	1	3.3%
Suspicious for malignancy	2	6.7%
Non-diagnostic	2	6.7%
Total	30	100%

5. **Cyto-Histopathological Correlation**

Histopathological follow-up was available in 26 cases (86.7%).

- True Positives (TP): 5
- True Negatives (TN): 17
- False Positives (FP): 1
- False Negatives (FN): 3

The diagnostic performance of FNAC was calculated as follows:

- **Sensitivity:** 62.5% (95% CI: 24.5%–91.5%)
- **Specificity:** 94.4% (95% CI: 72.7%–99.9%)
- **Positive Predictive Value (PPV):** 83.3%
- **Negative Predictive Value (NPV):** 85.0%
- **Overall Diagnostic Accuracy:** 84.6%

The association between Milan category and histopathological outcome was statistically significant (Chi-square test, p = 0.003).

These findings are summarized in **Table 4**.

Table 4: Diagnostic Performance of FNAC (n = 26)

Parameter	Value (%)
Sensitivity	62.5%
Specificity	94.4%
PPV	83.3%
NPV	85.0%
Accuracy	84.6%

As shown in **Table 4**, FNAC demonstrated high specificity and good overall diagnostic accuracy.

6. **Risk of Malignancy (ROM) According to Milan Categories**

Risk of malignancy was calculated for each category where histopathology was available.

Table 5: Risk of Malignancy (ROM) by Milan Category

Milan Category	Cases with Histology	Malignant Cases	ROM (%)
I	1	0	0%
II	5	0	0%
III	2	1	50%
IVa	10	1	10%
IVb	3	2	66.7%
V	2	2	100%
VI	3	3	100%

As demonstrated in **Table 5**, ROM increased progressively from benign to malignant categories, with **Category V and VI showing 100% ROM**, validating the risk stratification utility of the Milan system.

Summary of Key Statistical Findings

In the present study, benign lesions constituted the majority of cases, with Category IVa being the most frequent Milan category (40%). Histologically confirmed malignancy rate was 30.8%. FNAC demonstrated high specificity (94.4%) and good overall diagnostic accuracy (84.6%), although sensitivity was moderate (62.5%). The highest ROM was observed in Categories V and VI (100%), supporting the risk stratification utility of the Milan system.

Discussion

FNAC remains an indispensable tool in preoperative evaluation of salivary gland lesions [15]. Category IVa (Benign neoplasm) was the most frequent category in our study, similar to findings by Rossi et al. [16].

The proportion of benign neoplasms (40%) is consistent with global literature where pleomorphic adenoma remains most common [17]. The malignancy rate of 30.8% aligns with published validation studies [18].

The ROM observed in Category III (50%) reflects inherent diagnostic uncertainty, as described in earlier Milan validation studies [19]. Category IVb and V showed high ROM, supporting their predictive value [20].

Diagnostic pitfalls include overlapping features between pleomorphic adenoma and low-grade mucoepidermoid carcinoma [21]. Cystic lesions and oncocytic metaplasia also pose interpretative challenges [22].

Sensitivity and specificity values in our study (62.5% and 94.4%) are comparable to previously reported studies, where high specificity but variable sensitivity has been documented [23]. The Milan system improves communication and clinical management decisions [24].

Limitations include small sample size and short duration. Larger multicentric studies are required for robust validation [25].

Conclusion

Application of the Milan system standardizes reporting and provides clinically meaningful risk stratification. Despite high diagnostic accuracy, indeterminate categories require cautious interpretation and histopathological confirmation.

Limitations

The limitations of this study include small sample size, short study duration, and single-center design. Additionally, confidence intervals for sensitivity are wide due to limited malignant cases, reflecting statistical uncertainty. Larger multicentric studies are required for stronger validation of the Milan system in regional settings.

References

1. Speight PM, Barrett AW. Salivary gland tumours. Oral Dis. 2002;8:229–240.
2. El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ, editors. WHO Classification of Head and Neck Tumours. 4th ed. Lyon: IARC Press; 2017.
3. Orell SR, Sterrett GF, Whitaker D. Fine Needle Aspiration Cytology. 5th ed. Philadelphia: Churchill Livingstone; 2012.
4. Stewart CJ, MacKenzie K, McGarry GW, Mowat A. Fine-needle aspiration cytology of salivary gland: a review of 341 cases. Diagn Cytopathol. 2000;22:139–142.
5. Layfield LJ, Gopez EV, Hirschowitz S. Diagnostic accuracy and clinical utility of fine-needle aspiration cytology of salivary gland lesions. Acta Cytol. 2006;50:111–115.
6. Faquin WC, Rossi ED. The Milan System for Reporting Salivary Gland Cytopathology. Cham: Springer; 2018.

7. Rossi ED, Faquin WC, Baloch Z, Barkan GA, Foschini MP, Pusztaszeri M, et al. The Milan System for Reporting Salivary Gland Cytopathology: analysis and suggestions of initial survey. Cancer Cytopathol. 2018;126:754–766.
8. Song IH, Song JS, Sung CO, Roh JL, Choi SH, Nam SY, et al. Accuracy of the Milan System for Reporting Salivary Gland Cytopathology: a multi-institutional study. Acta Cytol. 2019;63:146–154.
9. Gargano SM, Sebastiano C, Griffin AC, Cangiarella JF, Simsir A, Baloch ZW, et al. The Milan System for Reporting Salivary Gland Cytopathology: a retrospective analysis of 285 cases. Cancer Cytopathol. 2019;127:613–621.
10. Wei S, Layfield LJ, LiVolsi VA, Montone KT, Baloch ZW. Reporting of salivary gland fine-needle aspiration specimens: comprehensive analysis of diagnostic categories and risk of malignancy. Am J Clin Pathol. 2019;151:613–621.
11. Griffith CC, Pai RK, Schneider F, Duvvuri U, Johnson JT, Ferris RL, et al. Salivary gland tumor fine-needle aspiration cytology: proposal for a risk stratification classification. Am J Clin Pathol. 2015;144:20–27.
12. Baloch ZW, Faquin WC, Layfield LJ, Rossi ED, Pusztaszeri M, Vielh P. Standardized terminology and nomenclature for salivary gland cytology: the Milan proposal. Diagn Cytopathol. 2017;45:109–117.
13. Schmidt RL, Hall BJ, Wilson AR, Layfield LJ. A systematic review and meta-analysis of the diagnostic accuracy of fine-needle aspiration cytology for salivary gland lesions. Head Neck. 2011;33:1396–1403.
14. Kala C, Kala S, Khan L. Application of the Milan System for Reporting Salivary Gland Cytopathology: a retrospective study. J Cytol. 2020;37:152–157.
15. Ali SZ, Cibas ES. The Bethesda System for Reporting Thyroid Cytopathology: definitions, criteria and explanatory notes. New York: Springer; 2010.
16. Pusztaszeri MP, Rossi ED, Baloch ZW, Faquin WC. Salivary gland cytopathology: an update. Adv Anat Pathol. 2019;26:33–45.
17. Eveson JW, Cawson RA. Salivary gland tumors: a review of 2410 cases with particular reference to histological types and site distribution. J Pathol. 1985;146:51–58.
18. Tandon S, Shahab R, Benton JI, Ghosh SK, Sheard J, Jones TM. Fine-needle aspiration cytology in diagnosis of salivary gland lesions. J Laryngol Otol. 2008;122:120–124.
19. Seethala RR, Barnes EL, Hunt JL. Epithelial-myoepithelial carcinoma: a review of clinicopathologic spectrum and immunophenotype. Mod Pathol. 2007;20:52–60.
20. Zhang S, Bao R, Bagby J, Abreo F. Application of the Milan System for Reporting Salivary Gland Cytopathology in Asian population. Diagn Cytopathol. 2020;48:829–835.
21. Nagel H, Laskawi R, Büter JJ, Schröder M, Chilla R. Cytologic diagnosis of mucoepidermoid carcinoma: pitfalls and limitations. Cancer. 1997;81:82–89.
22. Kumar N, Gupta R, Gupta S. Diagnostic pitfalls in salivary gland fine-needle aspiration cytology. Cytojournal. 2017;14:10.
23. Söderström N. Fine-needle aspiration biopsy: a review of 2200 cases. Acta Med Scand Suppl. 1966;451:1–235.
24. Pusztaszeri M, Bongiovanni M, Faquin WC. Update on salivary gland cytopathology. Semin Diagn Pathol. 2020;37:169–177.
25. Griffith CC, Pai RK, et al. Risk of malignancy and diagnostic performance of the Milan System for Reporting Salivary Gland Cytopathology. Cancer Cytopathol. 2019;127:713–723.