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Original Research Article

A RETROSPECTIVE ANALYSIS OF THE CYTOMORPHOLOGICAL SPECTRUM OF SALIVARY GLAND LESIONS USING THE MILAN SYSTEM FOR REPORTING SALIVARY GLAND CYTOPATHOLOGY

Poornima Mishra[1], Marisha Srivastava[1], Anmoldeep[2]

[1]Assistant Professor, Department of Pathology, Motilal Nehru Medical College, Prayagraj, India
[2]Junior Resident, Department of Pathology, Motilal Nehru Medical College, Prayagraj, India

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Corresponding Author:
Dr. Poornima Mishra,
Assistant Professor, Department of Pathology, Motilal Nehru Medical College, Prayagraj, India.
Email: histopath6@gmail.com

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ABSTRACT

Background: Salivary gland lesions are a heterogeneous group ranging from inflammatory conditions to benign and malignant neoplasms. Fine-needle aspiration cytology (FNAC) plays a key role in their evaluation. The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) provides a standardized framework for diagnosis and risk stratification. The aim is to evaluate the cytomorphological spectrum of salivary gland lesions using the Milan system and assess its diagnostic utility.

Materials and Methods: This retrospective study included 45 cases of salivary gland lesions evaluated by FNAC. Cases were categorized according to MSRSGC, and histopathological correlation was performed wherever available. Risk of malignancy (ROM) and diagnostic accuracy parameters were calculated.

Results: The majority of cases were benign, with pleomorphic adenoma being the most common lesion (37.8%). Category IV-a was the most frequent Milan category (37.8%). ROM increased progressively to Category VI (100%). FNAC showed sensitivity of 75%, specificity of 90%, and diagnostic accuracy of 87.5%.

Conclusion: The Milan system is a reliable tool for risk stratification of salivary gland lesions. FNAC demonstrates high specificity and accuracy, supporting its role as an effective initial diagnostic modality.

Keywords: FNAC, Salivary gland lesions, Milan system, Cytology, Risk of malignancy.

INTRODUCTION

Salivary gland lesions comprise a heterogeneous cluster of clinical conditions, ranging from non-neoplastic inflammatory and cystic conditions to benign and malignant neoplasms (Eveson and Cawson, 1985). Although these lesions signify less than 3% of all head and neck tumors, their diagnosis is very challenging, due to significant cytomorphological diversity and considerable overlap among different entities (Colella et al., 2010; El-Naggar et al., 2017).[1,2] Major as well as minor salivary glands may be affected, and accurate characterization typically requires a multidisciplinary approach that integrates with clinical, radiological, and cytological findings (El-Naggar et al., 2017).[3] Moreover, fine-needle aspiration cytology (FNAC) is widely used as a first-line diagnostic tool because of its minimally invasive nature, rapid turnaround time, and cost-effectiveness (Colella et al., 2010).[4] It also facilitates the distinction between neoplastic and non-neoplastic lesions, in addition to informing preoperative decision-making (Colella et al., 2010; Pujani et al., 2018).[5,6] However, due to inadequate sampling, cystic degeneration, and cytomorphological overlap, particularly between benign tumors and low-grade malignancies, interpretation may be affected and also diagnostic decision ambiguity (Hughes et al., 2005; Rohilla et al., 2017).[7,8] Although the Milan System for Reporting Salivary Gland Cytopathology

(MSRSGC) offers a precise and standardized framework for diagnostic reports of salivary gland cytopathology. However, MSRSGC categorizes salivary gland cytology into six diagnostic categories, each allied with an appraised Risk of malignancy (ROM) and corresponding clinical management recommendations (Rossi et al., 2017; Faquin et al., 2018).[9,10] This system is involved in enhancing consistency, reproducibility, and communication between patients (Faquin et al., 2018).[11] On the other hand, various recent studies defined the modernized MSRSGC-2023 (second edition) to enhance the clinical applicability and also incorporate sophisticated ROM, involving evaluations based on large-scale data. Further, integrate improvements in ancillary diagnostic techniques, thereby improving clinical diagnostic precision across various healthcare settings (Wang et al., 2022; Rossi et al., 2024; Prakash et al., 2024).[12-14] Moreover, numerous prospective multicenter studies have accentuated its effective role in guiding clinical management and also enhancing patient outcomes (Manoharan et al., 2025; Mayer et al., 2025).[15,16] Therefore, the MSRSGC is now widely recognized as a global standard for reporting salivary gland cytopathology, with ongoing refinements further enhancing its diagnostic value (Wang et al., 2023; Onyszczuk et al.,2025).[17,18] Despite widespread adoption, region-specific data remain limited, particularly in resource-limited and developing healthcare settings. This present study retrospectively evaluates the cytomorphological spectrum of salivary gland lesions using the Milan System in a tertiary care centre and assesses the system's diagnostic utility, accuracy, and applicability in this context.[19]

MATERIALS AND METHODS

This retrospective study included 45 cases of salivary gland lesions and was conducted in the Department of Pathology at Motilal Nehru Medical College, Prayagraj, Uttar Pradesh, India, spanning from September 2024 to February 2026. Clinical details and cytology smear reports of salivary gland lesions were retrieved from departmental records.

The cytology smears comprised fine-needle aspiration cytology (FNAC) samples from lesions involving both major and minor salivary glands. All obtainable smears were reviewed two times and reclassified into one of the six diagnostic categories according to the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC), following standard diagnostic criteria. Histopathological reports and clinical follow-up data, wherever accessible, were repossessed and correlated with cytological findings. The risk of malignancy (ROM) for each Milan category was calculated by dividing the number of histologically confirmed malignant cases in each category by the total number of cases assigned to that category on cytology. Further, for statistical analysis, cytological diagnoses were categorized as positive (malignant) and negative (benign). Cases with negative cytology but confirmed malignancy on histopathology were considered false negatives, while cases with positive cytology but benign histopathology were considered false positives. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy of FNAC in detecting malignant lesions were calculated, taking histopathological examination (HPE) as the gold standard.

RESULTS

Cytopathological Spectrum of Salivary Gland Lesions (SGL) According to the Milan System (MS): A Retrospective Study- The present study included 45 cases of salivary gland lesions. Patients ranged in age from 8 to 97 years, and an observed male predominance ratio (M: F = 1.5:1). The submandibular gland was the most commonly involved site, 64.4%, followed by the parotid gland (33.3%). Minor salivary gland involvement was rare (2.2%). Cytological Spectrum of Lesions- [Table 1], presented cytomorphological evaluation revealed a wide spectrum of lesions, including non-neoplastic, benign neoplastic, and malignant entities. Pleomorphic adenoma (37.8%) was the most common lesion, followed by non - neoplastic cystic lesions (26.7%) and chronic sialadenitis (11.1%). A smaller proportion of cases were categorized as suspicious or malignant.

Table 1: Cytological Spectrum of Salivary Gland Lesions (n = 45)

Diagnosis	Number of Cases	Percentage (%)
Pleomorphic adenoma	17	37.8%
Non- neoplastic cystic lesion	12	26.7%
Chronic sialadenitis	5	11.1%
Acute on chronic sialadenitis	3	6.7%
Suspicious for malignancy	4	8.9%
Malignant	2	4.4%
AUS	1	2.2%
SUMP	1	2.2%

Milan System Categorizations-According to the Milan System, the majority belonged to Category IV-a (benign neoplasm) (37.8%), followed by Category I (26.7%) and Category II (17.8%), as shown in [Table 2].

Table 2: Milan System Category-wise Distribution

Milan Category	Description	Number of Cases	Percentage (%)
I	Non-diagnostic	12	26.7%
II	Non-neoplastic	08	17.8%
III	AUS	01	2.2%
IV-a	Benign neoplasm	17	37.8%
IV-b	SUMP	01	2.2%
V	Suspicious for malignancy	4	8.9%
VI	Malignant	2	4.4%

Risk of Malignancy (ROM) - Histopathological follow-up allowed calculation of the risk of malignancy (ROM), which showed a progressive increase from lower to higher Milan categories. Categories IV-b and VI demonstrated the highest ROM (100%), while Category V showed intermediate risk (50%), as detailed in [Table 3].

Table 3: Risk of Malignancy (ROM) According to Milan System

Milan Category	Total Cases	Malignant Cases (HPE)	ROM (%)
I	12	0	0%
II	10	0	0%
III	0	0	0%
IV-a	17	0	0%
IV-b	1	1	100%
V	4	2	50%
VI	1	1	100%

Diagnostic Accuracy of FNAC-Using histopathology as the gold standard, FNAC demonstrated good diagnostic performance. Categories V and VI were considered positive for malignancy. The calculated diagnostic parameters are shown in [Table 4].

Table 4: Diagnostic Performance of FNAC

Parameter	Value (%)
Sensitivity	75.0%
Specificity	90.0%
Positive Predictive Value	60.0%
Negative Predictive Value	94.7%
Diagnostic Accuracy	87.5%

Cytological evaluation -Cytological examination revealed distinct patterns corresponding to specific salivary gland lesions. Pleomorphic adenoma [Figure 1] showed a biphasic population of epithelial and myoepithelial cells arranged in cohesive clusters within a chondromyxoid stromal background. The cells exhibited uniform nuclei and plasmacytoid morphology, with a characteristic fibrillary matrix. In contrast, low-grade mucoepidermoid carcinoma [Figure 2] demonstrated a mixed population of mucous cells with vacuolated cytoplasm, intermediate cells, and epidermoid cells in a mucinous background, seen both singly and in clusters. However, FNAC effectively differentiated benign from malignant lesions.

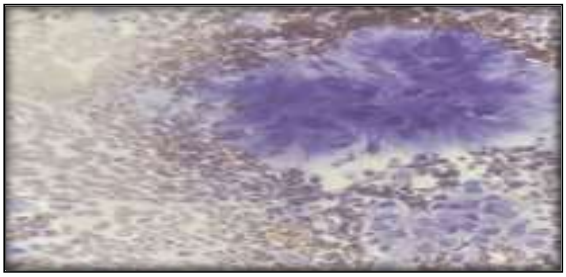

Figure 1: Photomicrograph showing epithelial and myoepithelial cell clusters embedded in a chondromyxoid stromal background, characteristic of pleomorphic adenoma (H&E stain, ×100/×400).

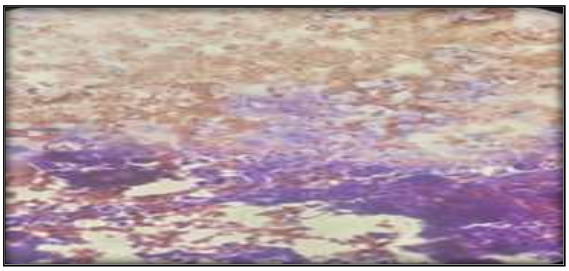

Figure 2: Photomicrograph showing a mixed population of mucous cells with vacuolated cytoplasm, intermediate cells, and epidermoid cells in a mucinous background, consistent with low-grade mucoepidermoid carcinoma (H&E stain, ×100/×400).

Histopathological Findings: Histopathological evaluation revealed distinct morphological features in the examined sections. In [Figure 3], the tumor demonstrates a mixed solid–cystic architecture with prominent nests of epidermoid (squamoid) cells along with intervening cystic spaces. The cystic areas contain mucinous material and scattered mucous cells exhibiting clear cytoplasm. The tumor cells show minimal nuclear pleomorphism, bland chromatin, and low mitotic activity, without evidence of necrosis, consistent with features of low-grade mucoepidermoid carcinoma. In [Figure 4], the section shows a well-organized biphasic pattern composed of epithelial and stromal

components. Multiple duct-like structures and cystic spaces are evident, lined by an inner epithelial layer and an outer myoepithelial layer. These elements are embedded within abundant chondromyxoid (myxoid) stroma. The cells appear cytologically uniform with no significant atypia or mitotic activity, consistent with pleomorphic adenoma.

[Figure 4]: Photomicrograph demonstrating tubules and cystic spaces lined by inner ductal epithelial cells and an outer myoepithelial cell layer, embedded in abundant myxoid stroma, characteristic of pleomorphic adenoma (H&E stain, magnification ×100/×200).

Histopathological Correlation: Most **Category** IVa cases were confirmed as pleomorphic adenoma. The Category IVb case corresponded to low-grade mucoepidermoid carcinoma, while Category VI confirmed malignancy. Category V cases showed variable outcomes, requiring histopathological confirmation [Table 5].

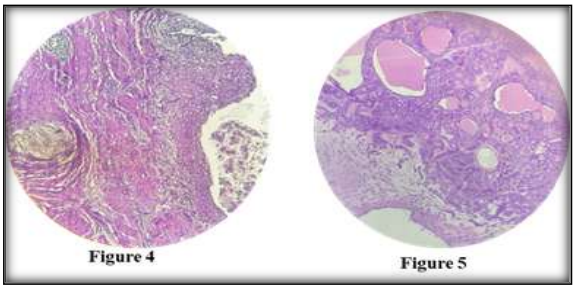

Figure 3: Photomicrograph of salivary gland lesion showing a solid-cystic architecture composed of nests of epidermoid cells with focal clear cell change and minimal nuclear pleomorphism, consistent with low-grade mucoepidermoid carcinoma (H&E stain, magnification ×100/×200).

Table 5: Correlation with FNAC (Milan System for Reporting Salivary Gland Cytopathology)

Lesion	FNAC Diagnosis (Milan Category)	Histopathological Diagnosis	Concordance
Pleomorphic Adenoma	Category IVa – Benign Neoplasm	Pleomorphic Adenoma	Concordant
Mucoepidermoid Carcinoma	Category V – Suspicious for Malignancy / Category VI – Malignant	Low-grade Mucoepidermoid Carcinoma	Concordant

However, the findings of the present study demonstrate that fine-needle aspiration cytology (FNAC), when interpreted using the Milan System for Reporting Salivary Gland Cytopathology, provides reliable diagnostic categorization and effective risk stratification of salivary gland lesions. The observed progressive increase in the risk of malignancy (ROM) across Milan categories (Table 3), along with high diagnostic accuracy [Table 4], supports its utility as a primary diagnostic modality. Therefore, histopathological confirmation remains essential, particularly in cases categorized as suspicious or malignant, to ensure accurate diagnosis and appropriate clinical management.

DISCUSSION

Salivary gland cytopathology remains inherently challenging due to pronounced morphological heterogeneity and substantial overlap between benign and low-grade malignant entities. Within this context, fine-needle aspiration cytology (FNAC), when interpreted using the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC), provides a standardised, risk-based framework that enhances diagnostic precision and clinical decision-making (Rossi et al., 2024; Onyszczuk et al., 2025).

The demographic profile in the present cohort—characterised by a wide age distribution and male predominance—is consistent with contemporary series (Jha et al., 2021; Wang et al., 2022). Notably, the predominance of submandibular gland involvement contrasts with recent multicentric analyses, where the parotid gland remains the most frequently involved site (Rossi et al., 2024; Behaeghe et al., 2023). This discrepancy likely reflects regional epidemiological variations and institutional referral patterns, emphasizing the importance of validating MSRSGC across diverse healthcare settings.

The cytological spectrum in this study was dominated by benign lesions, with pleomorphic adenoma emerging as the most common neoplasm. This finding is consistent with both classical and recent literature, including large cohort analyses (Behaeghe et al., 2023; Prakash et al., 2024). Additionally, the high proportion of non-neoplastic lesions, particularly cystic and inflammatory conditions, highlights the continued relevance of FNAC in differentiating reactive processes from neoplastic pathology, thereby minimizing unnecessary surgical interventions.

A relatively higher proportion of non-diagnostic (Category I) cases were observed in the present study. Although this exceeds the recommended threshold (<10%) suggested by the Milan system, similar findings have been reported in recent studies, particularly in cohorts with a high prevalence of cystic lesions (Onyszczuk et al., 2025). These limitations underscore the importance of incorporating ultrasound-guided FNAC, repeat sampling, and rapid on-site evaluation (ROSE) to improve adequacy and diagnostic yield.

The risk of malignancy (ROM) demonstrated a clear and progressive increase across Milan categories, which is in agreement with recent updates and validation studies of the Milan system (Rossi et al., 2024; Wang et al., 2023). Categories with low malignant potential (II and IV-a) showed minimal or no malignancy, supporting their diagnostic reliability. In contrast, higher categories (IV-b, V, and VI) exhibited significantly elevated ROM, consistent with findings from recent multicentric

and meta-analytical studies (Behaeghe et al., 2023; Prakash et al., 2024). Minor deviations from expected ROM values in intermediate categories are likely attributable to limited case numbers and uneven distribution rather than inherent limitations of the system.

From a diagnostic performance perspective, FNAC in the present study demonstrated high specificity and overall accuracy, consistent with recent systematic reviews and meta-analyses (Wang et al., 2022; Wang et al., 2023). The relatively lower sensitivity observed is a recognized limitation of cytology, particularly in low-grade malignancies and lesions with overlapping morphological features. However, the high negative predictive value reinforces the role of FNAC as a reliable screening and triaging tool in clinical practice.

The variability observed in Category V (suspicious for malignancy) continues to represent a diagnostic gray zone, as emphasized in both earlier and recent studies (Jalaly et al., 2020; Onyszczuk et al., 2025). This highlights the necessity of histopathological confirmation in such cases. Conversely, the high cytohistological concordance observed in Category IV-a supports the robustness of FNAC in diagnosing benign neoplasms, particularly pleomorphic adenoma, a finding consistently reported across recent studies (Behaeghe et al., 2023).

Importantly, recent advancements incorporated in the second edition of the Milan system (2023/2024) have further refined ROM estimates and emphasized the role of ancillary techniques, including immunocytochemistry and molecular diagnostics (Rossi et al., 2024). These developments are expected to improve diagnostic accuracy and reduce indeterminate categories in future practice.

Clinically, the integration of FNAC with MSRSGC facilitates a risk-adapted management approach, enabling more precise decision-making regarding observation, repeat aspiration, or surgical intervention. This is particularly valuable in resource-limited settings, where cost-effective and minimally invasive diagnostic strategies are essential.

Hence, the present study reinforces that FNAC, when interpreted within the Milan framework, provides a reliable, reproducible, and clinically actionable diagnostic approach.

CONCLUSION

Fine-needle aspiration cytology (FNAC), when interpreted using the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC), provides a reliable, reproducible, and clinically meaningful approach for the evaluation of salivary gland lesions. The observed cytomorphological spectrum, predominance of benign lesions, and progressive increase in risk of malignancy (ROM) across Milan categories underscore the robustness of this standardised classification system. However, the high specificity and overall diagnostic accuracy observed in this study reaffirm the value of FNAC as an effective first-line diagnostic modality, particularly in distinguishing benign from malignant lesions and guiding preoperative decision-making. Although limitations such as non-diagnostic samples and reduced sensitivity in certain categories persist, these can be mitigated through improved sampling techniques and adjunctive diagnostic methods. The present findings support the continued integration of the Milan system into routine cytopathology practice, facilitating risk-based stratification and optimised patient management. Further large-scale, multicentric studies incorporating ancillary techniques are warranted to enhance diagnostic precision and validate these findings across diverse clinical settings.

Recommendations

- Adoption of the Milan System (MSRSGC) should be encouraged for standardized reporting and improved clinician–pathologist communication.
- Use of ultrasound-guided FNAC and repeat aspiration is recommended to reduce non-diagnostic rates.
- Incorporation of ancillary techniques (immunocytochemistry/molecular tests) may enhance diagnostic accuracy, especially in indeterminate categories.

Limitations

- Relatively small sample size
- Limited histopathological follow-up in some cases
- Higher proportion of non-diagnostic samples, which may affect ROM estimation

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