RESEARCH PAPER

Diagnostic Accuracy of Fine-Needle Aspiration Cytology Reported Using the Milan System in Salivary Gland Lesions: Cytohistopathological Correlation Study

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ABSTRACT

Background: Fine-needle aspiration cytology (FNAC) is a rapid, minimally invasive first-line test for evaluating salivary gland lesions, and the Milan System provides a reporting framework to stratify malignancy risk and guide management, warranting validation against histopathology.

Objective: To determine the diagnostic accuracy of FNAC, reported using the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC), in the evaluation of salivary gland lesions by correlating cytological diagnoses with histopathological findings.

Methods: This analytical cross-sectional study included 32 patients with salivary gland swellings who underwent FNAC, and smears were reported using the MSRSGC. Cytology findings were correlated with histopathology as the reference standard, with Milan categories V–VI considered test-positive for malignancy to compute risk of malignancy (ROM) and validity indices.

Results: The mean age was 42.9 ± 15.8 years; malignant cases were significantly older than benign (57.5 ± 12.4 vs 36.2 ± 12.5; $p < 0.001$), with 70% of malignancies in those ≥50 years ($p = 0.004$). The parotid gland was the commonest site (68.8%), and FNAC was mainly palpation-guided (71.9%), although USG-guided FNAC was used more often in malignancy (60% vs 13.6%; $p = 0.013$). Milan IVa was the most frequent cytology category (37.5%), and histopathology showed 68.8% benign and 31.2% malignant lesions; pleomorphic adenoma (34.4%) was the commonest diagnosis. ROM increased across Milan categories (II 0%, V 75%, VI 100%). Using Milan V–VI as test-positive, FNAC showed 70.0% sensitivity, 95.5% specificity, and 87.5% accuracy.

Conclusion: FNAC reported using the Milan System showed high specificity and good overall accuracy against histopathology, supporting its utility for standardized risk stratification and preoperative management of salivary gland lesions.

Keywords: Salivary gland lesions, Fine-needle aspiration cytology, Milan System, Histopathology correlation, Diagnostic accuracy, Risk of malignancy

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Introduction

Salivary gland lesions encompass a broad spectrum of non-neoplastic conditions and neoplasms, and they remain a recognized diagnostic challenge because of their marked morphologic diversity and overlapping histologic patterns, with the World Health Organization classification containing distinct tumour entities.(1) The parotid gland is the most frequently involved major salivary gland, accounting for the majority of salivary gland tumours, while submandibular, sublingual, and minor salivary gland tumours occur less often and show different benign–malignant proportions.(2) Across populations, pleomorphic adenoma and Warthin tumour are consistently reported among the commonest benign tumours, whereas mucoepidermoid carcinoma and adenoid cystic carcinoma are frequently encountered malignant tumours.(3)

Fine-needle aspiration cytology (FNAC) is widely used in the initial diagnostic work-up of salivary gland swellings because it is rapid, minimally invasive, and can help differentiate non-neoplastic lesions from neoplasms and benign from malignant processes.(4) However, conventional cytology reporting ,interobserver variability and inconsistent terminology, and FNAC performance can vary substantially by lesion type (especially cystic/low-grade tumours), sampling adequacy, and operator experience.(5) To address these issues, the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) was introduced to standardize FNAC reporting of salivary gland lesions into six diagnostic categories linked to an “implied” risk of malignancy (ROM) and recommended clinical management.(6, 7) Literature supported the clinical utility of MSRSGC for risk stratification and quality assurance.(8, 9) The second edition of MSRSGC (published in 2023) further refined ROM estimates and incorporated updates relating to imaging correlation and ancillary testing.(10) In this context, studies correlating MSRSGC-based cytology with histopathology remain important to define local diagnostic performance, identify category-specific pitfalls (atypia, SUMP, and cystic lesions), and strengthen multidisciplinary decision-making. Against this background, the objective of the present study was to determine the diagnostic accuracy of FNAC, reported using the Milan System for Reporting Salivary Gland Cytopathology, in the evaluation of salivary gland lesions by correlating cytological diagnoses with histopathological findings.

Materials and Methods

This was a single-centre, hospital-based, analytical cross-sectional study conducted in the Department of Pathology, Chettinad Hospital and Research Institute, Chennai, Tamil Nadu, India over a period of four months between October 2025 and January 2026. The study was approved by the Institutional Human Ethics Committee (IHEC) with reference number IHEC-I/021/10/2025 dated 19/11/2025. This study included patients attending the outpatient or inpatient services of various surgical units suspected salivary gland involvement; referred to the Department of Pathology for FNAC were included and inadequate or unsatisfactory aspirates were excluded from analysis. The sample size was estimated using a single-proportion precision method for diagnostic accuracy at a 95% confidence level ($Z = 1.96$). Assuming an anticipated overall accuracy of 0.90 and an absolute precision (half-width of the 95% CI) of 0.10, the minimum required sample size was calculated to be 32.(11) A two-sided α of 0.05 was applied, with histopathological diagnosis considered as the reference standard for verification, and in cytology categorized as positive for malignancy when reported as Milan categories V–VI.

FNAC was performed under strict aseptic precautions; for deep-seated, small, or radiologically detected lesions, aspiration was performed under ultrasound guidance wherever required. Multiple passes were taken as needed to obtain representative material. Smears were prepared immediately; air-dried smears were stained with Leishman and May–Grünwald–Giemsa (MGG), and alcohol-fixed smears were stained with Papanicolaou (PAP). The stained smears were examined under a light microscope, and cytomorphological features—such as cellularity, architectural patterns, background (mucin, necrosis, inflammation), cytoplasmic characteristics, nuclear atypia, and presence of lymphoid or myoepithelial components—were assessed. Each case was then categorized according to the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) into one of six categories: I (Non-diagnostic), II (Non-neoplastic),

III (Atypia of undetermined significance), IVa (Benign neoplasm), IVb (Salivary gland neoplasm of uncertain malignant potential), V (Suspicious for malignancy), or VI (Malignant).(12) Smears deemed inadequate/unsatisfactory on cytology were excluded from final analysis. For histopathological correlation, specimens obtained by biopsy and/or surgical excision were received in 10% neutral buffered formalin, adequately fixed, and processed. Representative sections were taken after gross examination, processed through graded alcohols and xylene, embedded in paraffin, and sectioned at 3–5 µm thickness using a microtome. Sections were stained with hematoxylin and eosin (H&E) and examined to establish the definitive histopathological diagnosis, including tumour type and benign versus malignant nature, along with relevant morphological characteristics (e.g., capsular invasion, perineural invasion, lymphovascular invasion, and margin status when applicable). Cytology and histopathology findings were recorded independently.

Statistical analysis: Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Armonk, NY, USA). Categorical variables were summarized as frequency and percentage, and continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR), as appropriate. Normality of continuous data was assessed using visual inspection and the Shapiro–Wilk test. Comparisons between benign and malignant histopathology groups were performed using the independent samples t-test for normally distributed variables and the Mann–Whitney U test for non-normally distributed variables, while categorical variables were compared using the chi-square test or Fisher's exact test when expected cell counts were <5. FNAC results were reported according to the MSRSGC, and histopathology served as the reference standard. For diagnostic accuracy analysis, Milan categories V–VI were considered positive for malignancy; sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy were calculated from a 2×2 contingency table with 95% confidence intervals (CIs). A two-sided p value <0.05 was considered statistically significant.

Results

Among the 32 patients evaluated, the mean age was 42.9 ± 15.8 years, with those having malignant histopathology being significantly older than those with benign lesions (57.5 ± 12.4 vs 36.2 ± 12.5 years; $p < 0.001$). Age-group distribution also differed significantly ($p = 0.004$): no malignant cases occurred in patients <30 years (0/10), while 70.0% of malignant lesions were in those ≥50 years (7/10). Males constituted 56.2% overall, and although a higher proportion of malignancies occurred in males (80.0%), the gender difference was not statistically significant ($p = 0.124$). The parotid gland was the most commonly involved site (68.8%), with no significant site-wise difference between benign and malignant groups ($p = 0.575$); laterality was predominantly right-sided (56.2%) without significant association ($p = 0.510$). Clinical/radiological impression correlated with malignancy ($p = 0.008$), as 40.0% of malignant cases were labelled "malignant" clinically/radiologically versus 4.5% among benign lesions. Ultrasound-guided FNAC was used more often in malignant lesions (60.0% vs 13.6%; $p = 0.013$).

Table 1: Baseline demographic and clinical profile of the study population, overall and by histopathology group

		Overall (N=32)	**Benign on HPE (n=22)**	**Malignant on HPE (n=10)**	**p-value**
Age (years), Mean ± SD		42.9 ± 15.8	36.2 ± 12.5	57.5 ± 12.4	<0.001
Age (years)	<30	6 (18.8)	6 (27.3)	0 (0.0)	0.004
	30-49	16 (50.0)	13 (59.1)	3 (30.0)	
	≥50	10 (31.2)	3 (13.6)	7 (70.0)	
Gender	Male	18 (56.2)	10 (45.5)	8 (80.0)	0.124

	Female	14 (43.8)	12 (54.5)	2 (20.0)	
Gland involved	Parotid	22 (68.8)	14 (63.6)	8 (80.0)	0.575
	Submandibular	7 (21.9)	6 (27.3)	1 (10.0)	
	Sublingual	1 (3.1)	1 (4.5)	0 (0.0)	
	Minor salivary	2 (6.2)	1 (4.5)	1 (10.0)	
Laterality	Right	18 (56.2)	11 (50.0)	7 (70.0)	0.510
	Left	13 (40.6)	10 (45.5)	3 (30.0)	
	Bilateral	1 (3.1)	1 (4.5)	0 (0.0)	
Clinical/radiological impression	Benign	18 (56.2)	16 (72.7)	2 (20.0)	0.008
	Suspicious	9 (28.1)	5 (22.7)	4 (40.0)	
	Malignant	5 (15.6)	1 (4.5)	4 (40.0)	
FNAC approach	Palpation-guided	23 (71.9)	19 (86.4)	4 (40.0)	0.013
	USG-guided	9 (28.1)	3 (13.6)	6 (60.0)	
*Statistically significant at p<0.05					

The parotid gland was the most common site (22/32; 68.8%), followed by the submandibular gland (7/32; 21.9%), with sublingual (3.1%) and minor salivary gland lesions (6.2%) being uncommon. FNAC was predominantly palpation-guided (23/32; 71.9%), while USG-guided aspiration was used in 9/32 (28.1%), most frequently for parotid lesions (8/22; 36.4% USG use within parotid). On cytology, the most frequent Milan category was IVa (benign neoplasm) (12/32; 37.5%), followed by Category II (18.8%), with Categories V and VI contributing 12.5% each; no cases were non-diagnostic (Category I, 0%). Histopathology showed benign lesions in 22/32 (68.8%) comprising non-neoplastic lesions (21.9%) and benign neoplasms (46.9%), while malignancies accounted for 10/32 (31.2%). The commonest diagnosis was pleomorphic adenoma (11/32; 34.4%), followed by chronic sialadenitis (12.5%) and Warthin tumour (9.4%); among malignancies, mucoepidermoid carcinoma was most frequent (9.4%), with adenoid cystic carcinoma and acinic cell carcinoma each comprising 6.2%.

Table 2: Distribution of salivary gland lesions by site and FNAC specimen type

Site	Total n (%)	Palpation-guided n (%)	USG-guided n (%)	USG use within site (%)
Parotid	22 (68.8)	14 (43.8)	8 (25.0)	36.4
Submandibular	7 (21.9)	6 (18.8)	1 (3.1)	14.3
Sublingual	1 (3.1)	1 (3.1)	0 (0.0)	0.0
Minor salivary	2 (6.2)	2 (6.2)	0 (0.0)	0.0
Total	32 (100.0)	23 (71.9)	9 (28.1)	—

Table 3: Cytology reporting distribution according to the Milan System (MSRSGC)

Milan category	n	%
I	0	0.0
II	6	18.8
III	3	9.4
IVa	12	37.5
IVb	3	9.4
V	4	12.5
VI	4	12.5

Cytology–histopathology correlation demonstrated a clear stepwise increase in malignancy rates across Milan categories. All Category II cases were benign on histopathology (6/6), yielding a ROM of 0% with complete concordance. Category III and Category IVb each showed 1 malignant case out of 3 (ROM

33.3%), with one discordant case in each category. Category IVa was predominantly benign (11/12), with a low ROM of 8.3% and one discordant malignant case. In the higher-risk categories, Category V had 3/4 malignant cases (ROM 75.0%) and Category VI was malignant in all 4 cases (ROM 100%). When Milan V–VI were considered test-positive for malignancy, FNAC yielded 7 true positives, 1 false positive, 21 true negatives, and 3 false negatives, giving sensitivity 70.0% (95% CI 39.7–89.2) and specificity 95.5% (78.2–99.2), with PPV and NPV both 87.5% and an overall accuracy of 87.5% (71.9–95.0). Discordant cases included false negatives due to limited or non-representative sampling (e.g., lymphoma reported as Milan III, carcinoma ex pleomorphic adenoma reported as IVa, and cystic mucoepidermoid carcinoma reported as IVb) and a false positive Milan V case where degenerative/metaplastic changes in pleomorphic adenoma mimicked malignancy.

Table 4: Histopathological diagnosis of salivary gland lesions

Non-neoplastic – 7 (21.9%)	**Benign neoplasm – 15 (46.9%)**	**Malignant – 10 (31.2%)**
Chronic sialadenitis – 4 (12.5%)	Pleomorphic adenoma – 11 (34.4%)	Mucoepidermoid carcinoma – 3 (9.4%)
Granulomatous sialadenitis – 1 (3.1%)	Warthin tumor – 3 (9.4%)	Adenoid cystic carcinoma – 2 (6.2%)
Lymphoepithelial sialadenitis – 1 (3.1%)	Basal cell adenoma – 1 (3.1%)	Acinic cell carcinoma – 2 (6.2%)
Benign cyst – 1 (3.1%)		Lymphoma – 1 (3.1%)
		Carcinoma ex pleomorphic adenoma – 1 (3.1%)
		Salivary duct carcinoma – 1 (3.1%)

Table 5: Cytology-histopathology correlation by Milan category and Risk of Malignancy (ROM) for each Milan category

Milan category	**Total**	**Benign on HPE**	**Malignant on HPE**	**Risk of Malignancy**	**Concordant n**	**Discordant n**
II	6	6	0	0.0	6	0
III	3	2	1	33.3	2	1
Iva	12	11	1	8.3	11	1
IVb	3	2	1	33.3	2	1
V	4	1	3	75.0	3	1
VI	4	0	4	100.0	4	0

Table 6: Diagnostic performance of FNAC for malignancy using Milan V-VI as test-positive

	Histopathology		**Total**
	Malignant	**Benign**	
Cytology positive (V-VI)	7	1	8
Cytology negative (I-IVb)	3	21	24
Total	10	22	32
Sensitivity: 70.0 (39.7 to 89.2) Specificity: 95.5 (78.2 to 99.2) PPV: 87.5 (52.9 to 97.8) NPV: 87.5 (69.0 to 95.7) Accuracy: 87.5 (71.9 to 95.0)			

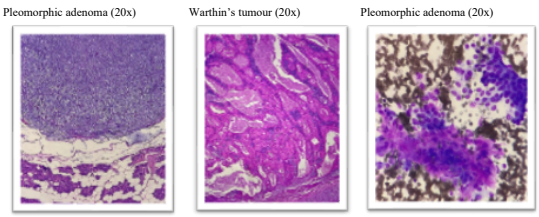

Figure 1: Histopathology and cytology

Discussion

FNAC is widely used as a first-line, minimally invasive test for preoperative evaluation of salivary gland swellings, and the MSRSGC provides a standardized, risk-stratified framework that links cytology categories to an implied ROM and recommended management pathways, as noted by Kala et al. (2019) and Wang & Wang (2023).(12, 13) In the present study, patients with malignant features in histopathology were significantly older than those with benign lesions (57.5 ± 12.4 vs 36.2 ± 12.5 years), and the age distribution showed a clear shift toward malignancy in older patients (70% of

malignant lesions in ≥50 years, with none occurring in <30 years), which is consistent with Alsanie et al. (2022) demonstrating that malignant salivary gland tumours occur at a higher mean age than benign tumours.(3) Site distribution in this study was dominated by the parotid gland (68.8%), followed by the submandibular gland (21.9%), with sublingual and minor salivary gland lesions being uncommon—an anatomic pattern that aligns with Kalwaniya et al. (2023) and Sudabattula et al. (2024), where the parotid is the most frequently involved major salivary gland and pleomorphic adenoma is typically most common in the parotid.(14, 15) The predominance of pleomorphic adenoma being the most common histopathological diagnosis (34.4%), with Warthin tumour also represented, mirrors Jain et al. (2015) in which pleomorphic adenoma is the most frequent benign salivary neoplasm, and Warthin tumour is strongly occurs with the parotid.(16) Among malignancies, mucoepidermoid carcinoma (MEC) was the most frequent malignant diagnosis, followed by adenoid cystic carcinoma (AdCC) and acinic cell carcinoma, which is consistent with Ullah et al. (2022) describing MEC as the most common malignant tumours of salivary gland tumour.(17)

A notable procedural observation in the present results was the significantly higher use of ultrasound-guided aspiration in malignant lesions. This is plausible because malignant tumours are more likely to be deep-seated, heterogeneous, necrotic/cystic, or associated with ill-defined margins—features that can reduce the representativeness of conventional sampling technique, as noted by Cho et al. (2011), Harb et al. (2020) and Khan et al. (2015).(18-20) Harb et al. (2020) added that ultrasound guidance improves the likelihood of obtaining diagnostic material compared with palpation guidance, particularly for deeper or complex lesions.(20) On cytology, the most common MSRSGC category in this study was Category IVa (benign neoplasm) (37.5%), followed by Category II (non-neoplastic), with Categories V and VI together constituting 25% of cases; importantly, there were no non-diagnostic (Category I) aspirates. The absence of non-diagnostic cases may reflect careful case selection, adequate aspiration technique, and/or selective inclusion of satisfactory smears, since Arashloo et al. (2025) and Kasinathan et al. (2023) report a variable non-diagnostic rate for salivary FNAC depending on operator experience, lesion type (especially cystic lesions), and adequacy thresholds.(21, 22) The MSRSGC framework is designed to communicate this uncertainty explicitly (ND, AUS, SUMP), acknowledging that salivary gland lesions show substantial morphologic overlap and marked histologic diversity, which can limit precise subtyping on cytology alone.(23)

Cyto-histopathology correlation in this cohort demonstrated a clinically meaningful stepwise escalation of malignancy probability across increasing Milan categories. The observed ROMs were 0% in Category II, 33.3% each in Categories III (AUS) and IVb (SUMP), 8.3% in Category IVa, 75% in Category V, and 100% in Category VI. This pattern supports the core conceptual validity of the MSRSGC risk-stratified approach and broadly parallels ROM gradients reported in Farahani & Baloch (2019) and Jaiswal et al. (2018),(9, 24) where lower-risk categories (II, IVa) have low ROM and higher-risk categories (V, VI) have substantially higher ROM. When compared with meta-analytic evidence from Farahani & Baloch (2019), the present ROM estimates for IVa, V and VI fall within the ranges typically seen across studies (recognizing that ROM is sensitive to case mix and verification bias, because only a subset proceed to histopathology in many settings).(9) Using the clinically pragmatic threshold of Milan V–VI as test-positive for malignancy, FNAC in this study achieved high specificity (95.5%) with moderate sensitivity (70.0%), yielding an overall diagnostic accuracy of 87.5%. This performance profile—specificity exceeding sensitivity—is consistent with Gaikwad et al. (2020) and Mezei et al. (2018), where cytology is typically reliable for ruling in malignancy when unequivocal malignant features are present, but can miss malignancy when sampling is non-representative or when tumours show low-grade, cystic, or deceptively bland morphology.(25, 26) Farahani & Baloch (2019) and Wang & Wang (2023) estimates on FNAC accuracy for malignancy detection under MSRSGC reporting also support the notion that pooled sensitivity is often lower than specificity (reflecting false negatives driven by tumour heterogeneity and sampling constraints), while the system improves communication by pairing cytologic interpretation with explicit ROM and management recommendations.(9, 13)

The acceptable concordant cases in this study are also well-aligned with recognized diagnostic pitfalls in salivary cytology. False negatives attributed to lymphoma (reported as Milan III) are a known

limitation because reactive/inflammatory backgrounds shows less atypical lymphoid populations, and cell block immunocytochemistry/flow cytometry may be required when lymphoma is suspected. Similarly, carcinoma ex pleomorphic adenoma can be missed when FNAC preferentially samples the benign pleomorphic adenoma component and fails to capture focal malignant transformation, a pitfall emphasized in salivary gland cytopathology discussions.(27) The false-negative cystic MEC categorized as IVb in the present study reflects another classic issue: cystic change and low cellularity can lead to aspiration of non-diagnostic cyst contents, and low-grade MEC can cytologically mimic benign cystic lesions unless mucin, intermediate cells, and a representative solid component are sampled—hence the recommendation for multiple passes and image guidance in cystic salivary lesions. Conversely, the false-positive Milan V case (pleomorphic adenoma on histology) is consistent with the fact that degenerative, metaplastic, or atypical stromal/myoepithelial changes in pleomorphic adenoma may mimic malignancy, particularly when the smear is highly cellular, shows squamoid metaplasia, necrotic-like debris, or marked nuclear atypia in a limited sample.(9, 13, 27)

Overall, these findings reinforce the practical value of pairing FNAC with MSRSGC reporting: lower-risk categories (II/IVa) demonstrated predominantly benign outcomes in this cohort, whereas higher-risk categories (V/VI) carried substantial malignancy rates and strong concordance with histopathology, supporting their role in preoperative risk stratification and surgical planning. At the same time, the observed false negatives highlight that cytology performance is heavily influenced by lesion heterogeneity and sampling adequacy, underlining the importance of clinico-radiologic correlation and consideration of repeat sampling or tissue biopsy (including image-guided targeted sampling) in cases with discordance between imaging/clinical suspicion and cytologic category.

The present study had certain limitations. First, the small sample size (N = 32) from a single centre limits the precision of risk estimates (including ROM and diagnostic accuracy) and may reduce generalisability to other settings with different case mix and operator expertise. Second, histopathological verification was available only for cases that underwent biopsy/excision, introducing potential verification (work-up) bias, as lesions managed conservatively after benign FNAC may have been underrepresented. Third, the exclusion of inadequate/unsatisfactory smears may have inflated diagnostic performance compared with real-world practice where non-diagnostic aspirates are encountered. Fourth, salivary gland pathology is heterogeneous, and some entities—particularly cystic lesions, low-grade malignancies, lymphoma, and carcinoma ex pleomorphic adenoma—are prone to sampling and interpretative pitfalls; limited use of cell block, immunocytochemistry, flow cytometry in cytology can further contribute to discordance.

Conclusion

In this study, FNAC reported using the Milan System for Reporting Salivary Gland Cytopathology demonstrated good overall diagnostic performance for detecting malignancy when correlated with histopathology, with high specificity and satisfactory accuracy using Milan categories V–VI as test-positive. The stepwise increase in risk of malignancy across Milan categories supported the system's utility for standardized reporting and clinically meaningful risk stratification. Most discordant results were attributable to recognized sampling and interpretative pitfalls in cystic and heterogeneous lesions, reinforcing the need for careful clinico-radiologic correlation and, where indicated, repeat or image-guided sampling and ancillary testing. Overall, MSRSGC-based FNAC provides a reliable, practical preoperative tool to guide patient counselling and management planning in salivary gland lesions.

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