International Journal of Medical and Pharmaceutical Research

Online ISSN-2958-3683 | Print ISSN-2958-3675
Frequency: Bi-Monthly
Available online on: https://ijmpr.in/

Original Article

Diagnostic Accuracy of Fine-Needle Aspiration Cytology Across Multiple Organ Systems: A Six-Year Cytohistological Correlation Study

Milind Mahadev Kesarkhane[1], Shrinivasanand Madhavrao Patil[2], Pooja Maroti Nagargoje[3]

[1]Associate Professor, Pathology, Government Medical College, Parbhani
[2]Assistant Professor, Pathology, Dr Shankarrao Chavan Government Medical College, Nanded
[3]Assistant Professor, Pathology, Dr Shankarrao Chavan Government Medical College, Nanded

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Corresponding Author:

Dr Milind Mahadev Kesarkhane
Associate Professor, Department of Pathology, Government Medical College, Parbhani – 431401
Email: milindbims@gmail.com

Received: 30-05-2026
Accepted: 12-06-2026
Available online: 06-07-2026

ABSTRACT

Background: Fine-Needle Aspiration Cytology (FNAC) is a rapid, minimally invasive, and cost-effective diagnostic technique widely used as the first-line investigation for palpable and image-guided lesions. In the era of precision medicine, cytological specimens have gained additional importance owing to their increasing utility in ancillary molecular testing. This study evaluated the diagnostic performance of FNAC across multiple organ systems through cytohistological correlation and analyzed the causes of diagnostic discordance.

Methods: A six-year retrospective study was conducted from January 2020 to December 2025. A total of 3,585 FNAC procedures involving thyroid, breast, lymph node, soft tissue, salivary gland, and other lesions were reviewed. Histopathological correlation was available in 3,123 cases. Cytological diagnoses were rendered using standardized reporting systems wherever applicable, and histopathology served as the reference standard. Sensitivity, specificity, and diagnostic accuracy were calculated.

Results: Of the 3,123 cases with cytohistological correlation, 3,094 (99.07%) showed concordant diagnoses, while 29 (0.93%) were discordant. The overall sensitivity, specificity, and diagnostic accuracy of FNAC were 93.79%, 93.04%, and 96.44%, respectively. Organ-wise diagnostic accuracy was 97.45% for thyroid, 98.32% for breast, 95.45% for lymph node, 94.66% for soft tissue, and 96.33% for salivary gland lesions. Most discordant cases resulted from sampling error, tumor heterogeneity, and inherent limitations of cytomorphological assessment.

Conclusion: FNAC is a highly reliable first-line diagnostic modality demonstrating excellent cytohistological concordance and diagnostic accuracy across multiple organ systems. Standardized reporting systems, image-guided aspiration, optimized sampling techniques, and appropriate ancillary investigations can further enhance its diagnostic utility in contemporary pathology practice.

Keywords: Cytodiagnosis, Histocytology Correlation, Precision Medicine.

INTRODUCTION

Fine-Needle Aspiration Cytology (FNAC) is an established first-line diagnostic technique in contemporary pathology owing to its rapidity, safety, minimal invasiveness, cost-effectiveness, and high diagnostic accuracy. Since its introduction by Martin and Ellis in 1930, FNAC has become indispensable for evaluating palpable and image-guided lesions across multiple organ systems, including the thyroid, breast, lymph nodes, salivary glands, and soft tissues, facilitating early diagnosis and appropriate clinical management while reducing unnecessary surgical interventions. [1,2]

Despite its numerous advantages, FNAC has inherent limitations, particularly the inability to assess tissue architecture, stromal invasion, and histological grading. Diagnostic challenges arising from sampling errors, cystic change, necrosis, and overlapping cytomorphological features underscore the importance of histopathological confirmation and cytohistological correlation as the gold standard for quality assurance. [3–7]

The role of FNAC has expanded considerably with advances in precision medicine. Cytological specimens now support a range of ancillary investigations, including immunocytochemistry, molecular diagnostics, fluorescence in situ hybridization, and next-generation sequencing, thereby extending their diagnostic, prognostic, and predictive value. [5,8–14] Furthermore, standardized reporting systems such as the Bethesda, IAC Yokohama, and Milan systems have enhanced diagnostic reproducibility, risk stratification, and communication between pathologists and clinicians. [2–4]

Although the diagnostic performance of FNAC has been extensively studied in individual organ systems, comprehensive multi-organ evaluations incorporating standardized reporting systems and systematic analysis of cytohistological discordance remain limited. Therefore, this six-year retrospective study aimed to assess the diagnostic accuracy of FNAC across multiple organ systems through cytohistological correlation, identify the causes of diagnostic discordance, and highlight strategies to further optimize the diagnostic utility of FNAC in the era of precision medicine.

MATERIALS AND METHODS

Study Design and Population

A retrospective observational cytohistological correlation study was conducted in the Department of Pathology at a tertiary care teaching hospital over a six-year period (January 2020–December 2025). All patients who underwent FNAC for palpable or image-guided lesions involving the thyroid, breast, lymph nodes, salivary glands, soft tissues, and other accessible sites were reviewed. Only cases with corresponding histopathological diagnoses were included for analysis.

Inclusion and Exclusion Criteria

Patients of all age groups and either sex with palpable or image-guided lesions and available cytohistological correlation were included. Repeat FNACs of previously diagnosed lesions, inadequate/unsatisfactory aspirates, metastatic lesions, and cases lacking histopathological follow-up were excluded.

Cytological and Histopathological Evaluation

Demographic and clinical data were retrieved from departmental records. FNAC was performed using standard aseptic techniques, and smears were processed using routine cytological staining protocols. Thyroid, breast, and salivary gland lesions were reported according to the Bethesda, IAC Yokohama, and Milan reporting systems, respectively, while other lesions were interpreted using established cytomorphological criteria.

Histopathological examination of biopsy or surgical specimens served as the reference standard. Tissue processing and reporting followed routine laboratory protocols and College of American Pathologists (CAP) recommendations wherever applicable. Immunohistochemistry was performed in selected cases when required to establish the definitive diagnosis.

Outcome Measures

Cytological diagnoses were correlated with corresponding histopathological findings and categorized as concordant or discordant. Discordant cases were reviewed to identify the probable causes of discrepancy, including sampling error, lesion heterogeneity, interpretative limitations, and inherent cytomorphological constraints.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using appropriate statistical methods. Categorical variables are presented as frequencies and percentages, and continuous variables as ranges. Histopathology was considered the reference standard. Sensitivity, specificity, and overall diagnostic accuracy of FNAC were calculated for individual organ systems and collectively.

The study was conducted after obtaining approval from the Institutional Ethics Committee (IEC)

RESULTS

During the six-year study period (January 2020 to December 2025), a total of 3,585 patients underwent Fine-Needle Aspiration Cytology (FNAC) for the evaluation of palpable and image-guided lesions involving multiple organ systems. The study population comprised 1,234 (34.42%) males and 2,351 (65.58%) females, with patients ranging in age from 5 to 85 years. Thyroid lesions constituted the largest proportion of FNAC cases (1,244; 34.70%), followed by breast (1,351; 37.69%), lymph node (928; 25.89%), soft tissue (97; 2.71%), salivary gland (23; 0.64%), and pancreatic lesions (3; 0.08%) (Table 1).

Sr. No.	Organ	Male	Female	Total No. of Cases
1	Thyroid	646	598	1244 (34.70%)
2	Breast	52	1299	1351 (37.69%)
3	Lymph node	496	432	928 (25.89%)
4	Soft tissue	25	11	97 (2.71%)

Sr. No.	Organ	Male	Female	Total No. of Cases
5	Salivary gland	13	10	23 (0.64%)
6	Others – Pancreatic lesion	2	1	3 (0.08%)
Total		**1234**	**2351**	**3585 (100%)**

Table 1. Distribution of cases diagnosed by FNAC

Histopathological correlation was available in 3,123 (93.34%) of the 3,585 FNAC cases and these cases constituted the study cohort for cytohistological analysis. Breast lesions accounted for the largest proportion of cases with histopathological follow-up (1,268; 40.60%), followed by thyroid (902; 28.88%), lymph node (845; 27.05%), soft tissue (88; 2.81%), salivary gland (18; 0.58%), and pancreatic lesions (2; 0.06%) (Table 2).

Sr. No.	Organ	Male	Female	Total No. of Cases
1	Thyroid	309	593	902 (28.88%)
2	Breast	52	1216	1268 (40.60%)
3	Lymph node	511	334	845 (27.05%)
4	Soft tissue	56	32	88 (2.81%)
5	Salivary gland	7	11	18 (0.58%)
6	Others – Pancreatic lesion	2	0	2 (0.06%)
Total		**937**	**2186**	**3123**

Table 2. Distribution of cases with histopathological correlation

Overall, 3,094 of 3,123 cases (99.07%) demonstrated concordance between cytological and histopathological diagnoses, whereas 29 cases (0.93%) showed cytohistological discordance. Based on histopathological diagnosis as the reference standard, FNAC demonstrated an overall sensitivity of 93.79%, specificity of 93.04%, and an overall diagnostic accuracy of 96.44% (Table 3).

Organ	Sensitivity (%)	Specificity (%)	Diagnostic Accuracy (%)
Thyroid	95.43	94.29	97.45
Breast	96.18	95.88	98.32
Soft tissue	92.33	90.67	94.66
Lymph node	94.48	92.14	95.45
Salivary gland	90.56	96.23	96.33
Overall	**93.79**	**93.04**	**96.44**

Table 3. Comparison of diagnostic performance of FNAC

Organ-wise analysis revealed consistently high diagnostic performance across all major organ systems. Breast lesions demonstrated the highest diagnostic accuracy (98.32%), followed by thyroid (97.45%), salivary gland (96.33%), lymph node (95.45%), and soft tissue lesions (94.66%). Sensitivity ranged from 90.56% to 96.18%, while specificity ranged from 90.67% to 96.23%, confirming the excellent diagnostic performance of FNAC across a broad spectrum of pathological lesions (Table 3).

Among the 29 discordant cases, thyroid lesions constituted the largest proportion (12 cases; 41.38%), followed by breast (8 cases; 27.59%), soft tissue (6 cases; 20.69%), lymph node (2 cases; 6.90%), and salivary gland (1 case; 3.45%) (Table 4).

Organ	Cytological diagnosis	Histopathological diagnosis
Thyroid (12)	Bethesda Category II – Benign lesion (8)	Papillary carcinoma (3); Follicular variant of papillary carcinoma (2); Follicular carcinoma (3)
	Bethesda Category IV – Follicular neoplasm (4)	Multinodular goitre (3); Follicular hyperplastic nodule (1)

Organ	Cytological diagnosis	Histopathological diagnosis
Breast (8)	Yokohama Category III – Atypical/Proliferative breast disease with atypia (5)	Ductal carcinoma in situ (4); Invasive breast carcinoma (1)
	Yokohama Category II – Benign breast disease (2)	Atypical ductal hyperplasia (2)
	Yokohama Category II – Inflammatory breast lesion (1)	Intracystic papillary carcinoma (1)
Soft tissue (6)	Lipoma (4)	Pleomorphic lipoma (2); Fibrolipoma (1); Myolipoma (1)
	Benign spindle cell tumour (2)	Dermatofibrosarcoma protuberans (2)
Lymph node (2)	Reactive lymphadenitis (2)	Follicular lymphoma (1); Low-grade lymphoproliferative disorder (1)
Salivary gland (1)	Milan Category IVA – Pleomorphic adenoma (1)	Carcinoma ex pleomorphic adenoma (1)

Table 4. Comparative analysis of discordant FNAC and histopathological diagnoses

In thyroid lesions, discordance predominantly resulted from false-negative diagnoses in lesions initially categorized as Bethesda Category II (Benign) that were subsequently diagnosed as papillary thyroid carcinoma, follicular variant of papillary thyroid carcinoma, or follicular carcinoma on histopathological examination. Conversely, a few lesions categorized cytologically as Bethesda Category IV (Follicular Neoplasm) were ultimately diagnosed as multinodular goitre or follicular hyperplastic nodules. These findings reflect the inherent limitations of cytomorphology in distinguishing follicular-patterned lesions, where assessment of capsular and vascular invasion requires histopathological examination.

Among breast lesions, most discordant cases involved proliferative breast lesions with atypia that were subsequently diagnosed as ductal carcinoma in situ, atypical ductal hyperplasia, or invasive breast carcinoma on histopathology. One inflammatory breast lesion was later confirmed as intracystic papillary carcinoma. These discrepancies were primarily attributable to focal distribution of malignant epithelial proliferation, sampling limitations, and cytomorphological overlap between benign proliferative lesions and low-grade malignancies.

Discordant soft tissue lesions mainly reflected the limited ability of cytomorphology to accurately subtype adipocytic and spindle cell neoplasms. Lesions initially diagnosed as lipoma were subsequently identified as pleomorphic lipoma, fibrolipoma, or myolipoma, whereas benign spindle cell tumors were confirmed as dermatofibrosarcoma protuberans following histopathological examination. The discordant lymph node cases represented follicular lymphoma and low-grade lymphoproliferative disorder initially interpreted as reactive lymphadenitis, while the single discordant salivary gland lesion corresponded to carcinoma ex pleomorphic adenoma that had been reported cytologically as pleomorphic adenoma according to the Milan System (Table 4).

Overall, the findings of the present study demonstrate an excellent cytohistological concordance rate and high diagnostic accuracy of FNAC across multiple organ systems. The small proportion of discordant cases was largely attributable to sampling error, tumour heterogeneity, and the inherent limitations of cytomorphological interpretation, emphasizing the importance of meticulous sampling techniques, clinicoradiological correlation, standardized reporting systems, and appropriate ancillary investigations in diagnostically challenging cases.

A comparison of the diagnostic performance of FNAC observed in the present study with representative contemporary studies is summarized in Table 5. Overall, the sensitivity, specificity, and diagnostic accuracy obtained in the present series are comparable to those reported in previously published literature, further supporting the reliability and reproducibility of FNAC as a first-line diagnostic modality across multiple organ systems. Minor variations among studies may be attributed to differences in study design, sample size, organ-specific case distribution, reporting criteria, observer experience, and the availability of ancillary diagnostic techniques.

Organ	Study	Sensitivity (%)	Specificity (%)	Diagnostic Accuracy (%)
Thyroid	Present study	**95.43**	**94.29**	**97.45**
	Trimboli et al. (2021)	90–98	92–98	95–98
	Baloch & LiVolsi (2021)	91–97	90–97	94–98
Breast	Present study	**96.18**	**95.88**	**98.32**
	Montezuma et al. (2021)	94–98	95–99	96–99

Organ	Study	Sensitivity (%)	Specificity (%)	Diagnostic Accuracy (%)
	Agarwal & Gupta (2022)	93–98	94–99	95–99
Lymph Node	Present study	**94.48**	**92.14**	**95.45**
	Published studies	90–97	90–96	92–97
Soft Tissue	Present study	**92.33**	**90.67**	**94.66**
	Rekhi & Gorad (2021)	89–95	88–95	92–96
	Nambirajan & Jain (2022)	90–95	89–96	92–96
Salivary Gland	Present study	**90.56**	**96.23**	**96.33**
	Milan System studies	88–95	94–98	94–97

Table 5. Comparison of the Diagnostic Performance of FNAC with Published Literature

DISCUSSION

Fine-Needle Aspiration Cytology (FNAC) has evolved into an indispensable first-line diagnostic modality owing to its rapidity, minimal invasiveness, cost-effectiveness, and high diagnostic accuracy. Beyond conventional cytomorphology, cytological specimens now support immunocytochemistry and molecular diagnostics, expanding the role of FNAC in precision medicine.[8–14]

This six-year study, comprising 3,346 FNACs with 3,123 cytohistological correlations, demonstrated excellent overall performance, with a concordance rate of 99.07%, sensitivity of 93.79%, specificity of 93.04%, and diagnostic accuracy of 96.44%. These findings are comparable with contemporary literature and reaffirm FNAC as a reliable first-line investigation for benign, malignant, and inflammatory lesions. [9–14] Improved aspiration techniques, standardized reporting systems, and better clinicoradiological correlation have further enhanced diagnostic reproducibility. [2–4]

Despite its excellent performance, FNAC remains susceptible to sampling errors in heterogeneous, cystic, necrotic, and deep-seated lesions and cannot assess tissue architecture, capsular or vascular invasion. [1,3,5] Only 29 (0.93%) cases showed cytohistological discordance, predominantly due to sampling limitations and tumour heterogeneity rather than interpretative error, highlighting the importance of meticulous sampling and appropriate ancillary investigations.

Thyroid Lesions

Thyroid lesions constituted the largest study group and showed excellent diagnostic performance (sensitivity 95.43%, specificity 94.29%, diagnostic accuracy 97.45%), consistent with studies using The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC). [2,15,16]

Among 12 discordant cases, eight false-negative lesions were subsequently diagnosed as papillary thyroid carcinoma, follicular variant of papillary thyroid carcinoma, or follicular carcinoma, mainly because of sampling error, cystic degeneration, or tumour heterogeneity. [15,16] Four Bethesda Category IV lesions proved benign on histopathology, reflecting the inability of cytology to assess capsular and vascular invasion required for differentiating follicular-patterned lesions. [2,15] Although molecular testing was not performed, cytological specimens remain suitable for ancillary molecular investigations that may improve risk stratification of indeterminate thyroid nodules. [8–14]

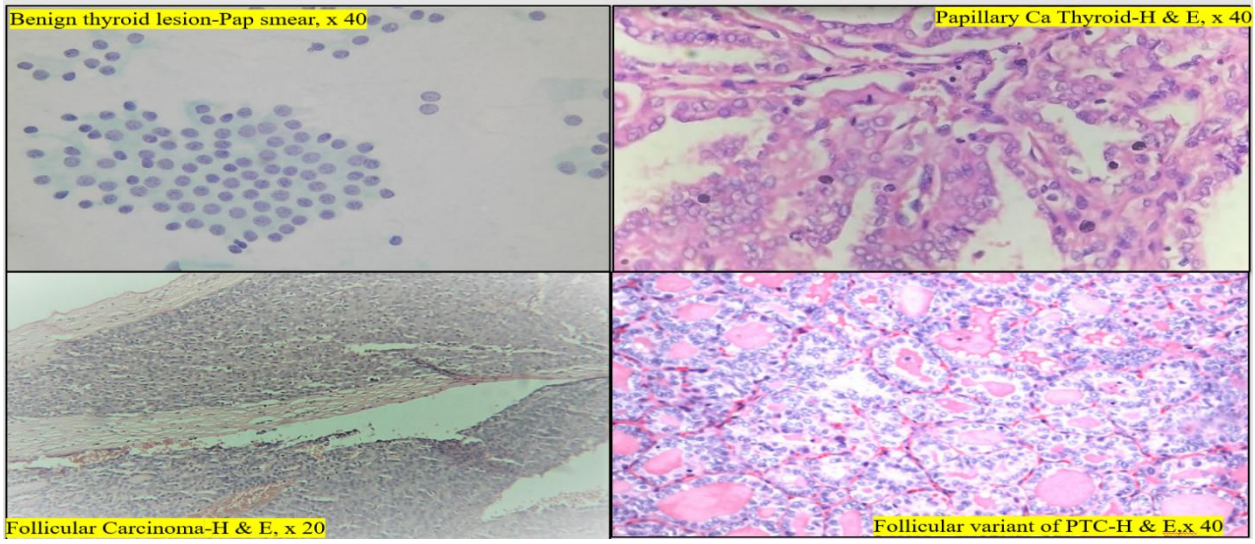

Figure 1- Microphograph of thyroid lesions

Breast Lesions

Breast FNAC demonstrated the highest diagnostic accuracy (98.32%), supporting previous reports and the utility of the International Academy of Cytology (IAC) Yokohama System. [6,7,17–19]

Eight discordant cases primarily involved proliferative lesions with atypia that were subsequently diagnosed as ductal carcinoma in situ, atypical ductal hyperplasia, or invasive carcinoma, reflecting limited sampling and cytomorphological overlap.[17–19] One inflammatory cystic lesion proved to be intracystic papillary carcinoma, emphasizing the importance of image-guided aspiration and clinicoradiological correlation. FNAC remains an important component of the triple assessment approach, complementing rather than replacing core needle biopsy, while also providing material for ancillary testing.

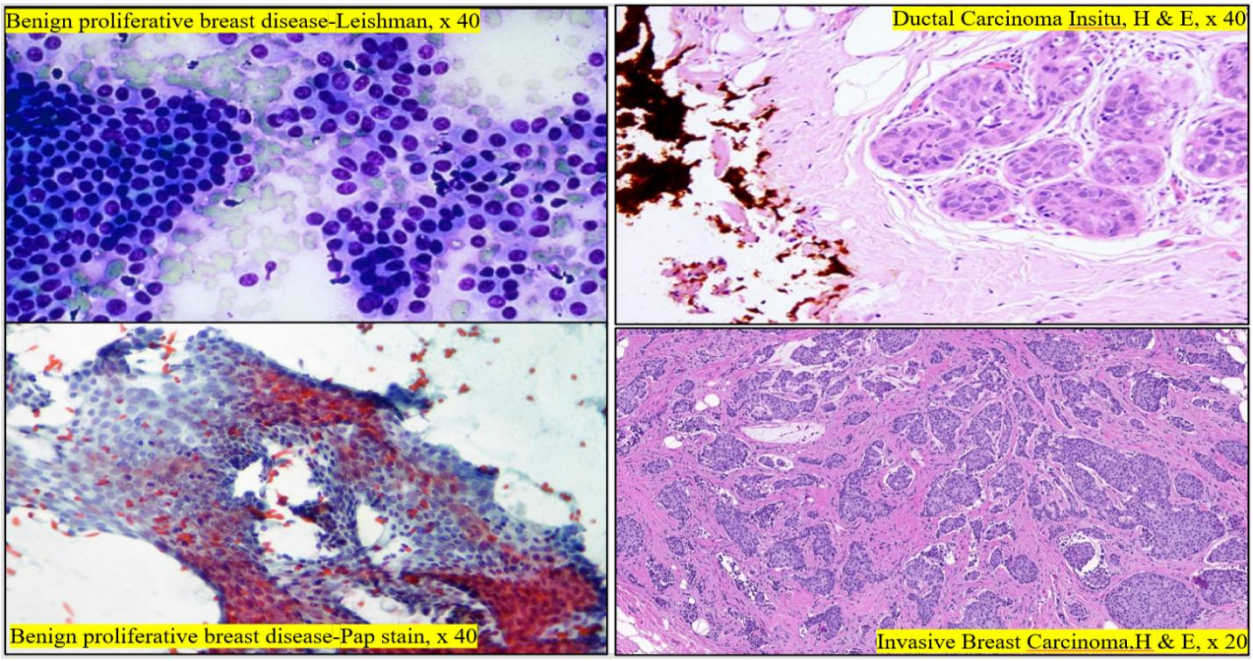

Figure 2: Microphotograph of breast lesions.

Soft Tissue Lesions

Soft tissue FNAC showed excellent diagnostic performance (sensitivity 92.33%, specificity 90.67%, diagnostic accuracy 94.66%). However, accurate subtyping remains challenging because of overlapping cytomorphological features and lack of architectural assessment. [20–22]

Most discordant cases involved adipocytic tumours or spindle cell lesions subsequently diagnosed as dermatofibrosarcoma protuberans, highlighting the need for integration with clinical, radiological, immunocytochemical, and molecular findings for accurate classification. [20–22]

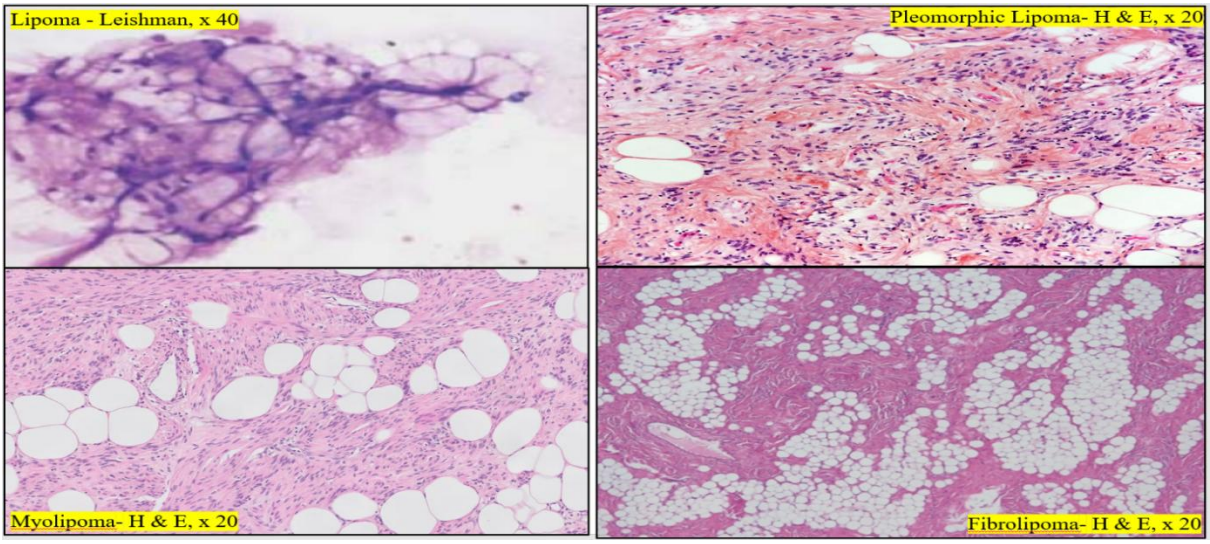

Figure 3 : Microphotograph of soft tissue lesions

Lymph Node Lesions
FNAC demonstrated high diagnostic accuracy for lymphadenopathy (sensitivity 94.48%, specificity 92.14%, diagnostic accuracy 95.45%). The only discordant cases were low-grade lymphoid neoplasms initially interpreted as reactive lymphadenitis, reflecting partial nodal involvement and sampling limitations. Persistent clinical suspicion should prompt repeat aspiration, excisional biopsy, or ancillary investigations such as flow cytometry and immunophenotyping.

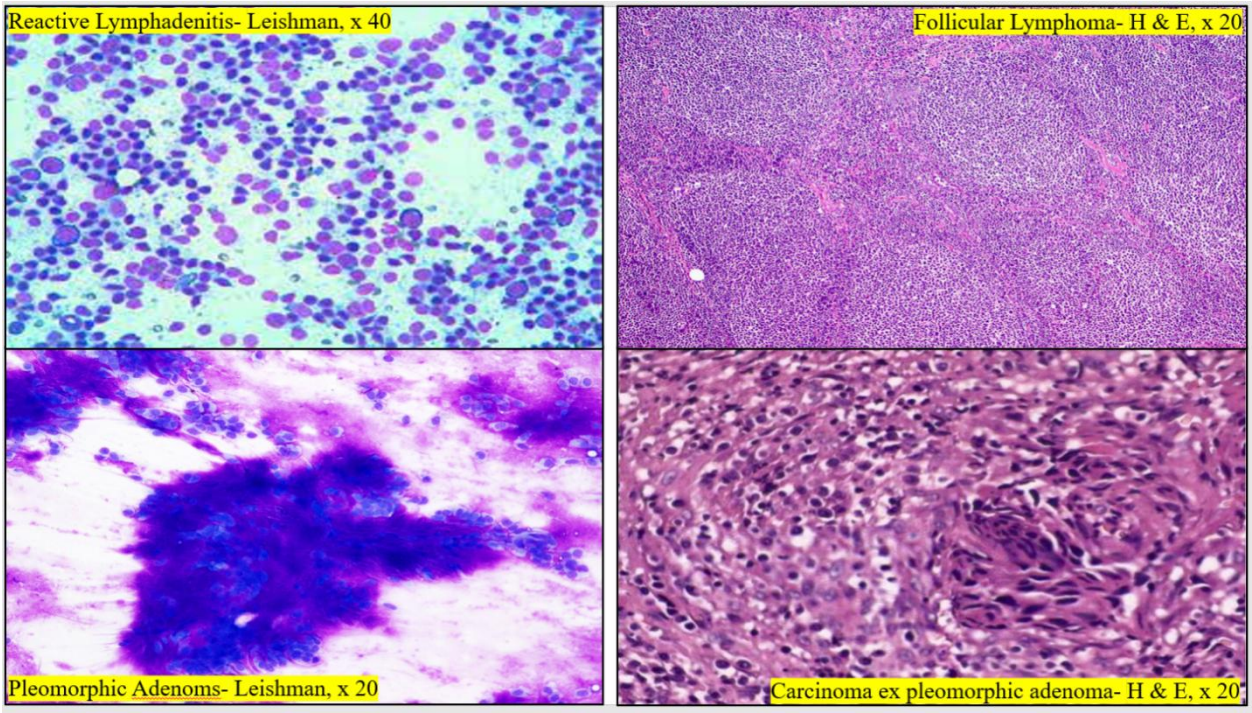

Figure 4: Microphotograph of Lymph node & Salivary gland lesions.

Salivary Gland Lesions
Salivary gland FNAC achieved a sensitivity of 90.56%, specificity of 96.23%, and diagnostic accuracy of 96.33%, supporting the clinical utility of the Milan System for Reporting Salivary Gland Cytopathology. [23,24]

The single discordant case involved carcinoma ex pleomorphic adenoma initially diagnosed as pleomorphic adenoma, most likely because only the benign component was sampled. This underscores the importance of clinicoradiological correlation and repeat image-guided aspiration when malignancy is suspected.

Clinical Implications
Most discordant cases resulted from sampling limitations, tumour heterogeneity, and the inability of cytology to evaluate tissue architecture. Image-guided FNAC, multiple targeted passes, rapid on-site evaluation, and ancillary techniques—including immunocytochemistry, cell block preparation, flow cytometry, fluorescence in situ hybridization, and next-generation sequencing—can further improve diagnostic accuracy and facilitate personalized patient management.[8–14]

Strengths and Limitations
The major strengths of this study include its large sample size, comprehensive multi-organ evaluation, use of internationally accepted reporting systems, and detailed analysis of discordant cases. Limitations include its retrospective single-centre design, potential verification bias due to selective histopathological follow-up, limited use of ancillary techniques, and lack of assessment of interobserver variability.

Future Directions
Prospective multicentre studies incorporating standardized quality assurance, image-guided aspiration, rapid on-site evaluation, digital cytopathology, artificial intelligence, and molecular profiling are warranted to further improve diagnostic performance and expand the role of FNAC in precision diagnostics.

CONCLUSION
This study confirms that FNAC is a highly accurate, minimally invasive, and cost-effective first-line diagnostic modality with excellent cytohistological concordance across multiple organ systems. Although sampling-related limitations account for occasional diagnostic discrepancies, standardized reporting systems, meticulous sampling, clinicoradiological correlation, and ancillary investigations can further optimize its performance. The expanding integration of cytomorphology with molecular diagnostics ensures that FNAC will continue to play a pivotal role in precision medicine.

FNAC remains an indispensable diagnostic modality that continues to evolve from conventional cytomorphology to an integrated platform supporting precision oncology.

What this study adds: This large six-year cytohistological correlation study demonstrates consistently high diagnostic accuracy of FNAC across multiple organ systems while systematically analyzing the causes of diagnostic discordance. The findings provide practical recommendations to minimize diagnostic pitfalls and highlight the evolving role of FNAC as a reliable source of material for ancillary and molecular investigations in contemporary cytopathology.

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