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Original Research Article

Diagnostic accuracy of fine needle aspiration cytology for thyroid nodules under the 2023 Bethesda system: a retrospective analysis with future perspectives on artificial intelligence and molecular integration

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ABSTRACT

Background: Thyroid swellings are common clinical presentations in ENT and pose diagnostic challenges in differentiating benign from malignant lesions through clinical and ultrasonographic evaluation alone. FNAC offers a minimally invasive, rapid, cost-effective preoperative tool, though it is limited by an inability to differentiate certain indeterminate categories, such as follicular adenoma from follicular carcinoma. Modern adjuncts, including artificial intelligence (AI) and molecular testing, are increasingly explored to bridge these diagnostic gaps.
Methods: This retrospective study was conducted in the Department of ENT, Father Muller Medical College, Mangalore, Karnataka, India between 1st October 2023 and 31st October 2025, involving 40 patients who presented with thyroid swellings and underwent preoperative FNAC followed by total thyroidectomy. Cytological results were classified utilizing the updated 2023 (3rd Edition) Bethesda system for reporting thyroid cytopathology. The FNAC findings were correlated with postoperative histopathological reports to determine sensitivity, specificity, predictive values, and accuracy.
Results: Out of 40 thyroid swellings, Colloid nodular goitre was the most frequent condition on fine needle aspiration cytology (FNAC) (72.5%). Under the updated 2023 Bethesda system, 80% of cases were categorized as benign (Category II), with 5% demonstrating atypia of undetermined significance (AUS) (category III). Histopathology confirmed colloid nodular goitre in 60% and malignancy in 5% of cases. FNAC-histopathology correlation showed high concordance, demonstrating excellent diagnostic accuracy for benign and malignant lesions, with a sensitivity of 100%, specificity of 88.89%, positive predictive value of 96.88%, negative predictive value of 100%, and an overall diagnostic accuracy of 97.5%.
Conclusions: FNAC is an essential first-line diagnostic tool for the preoperative evaluation of thyroid swellings, guiding surgical planning with high sensitivity and specificity. However, isolated false-positive interpretations highlight the inherent limitations of standard cytomorphology, underscoring the future necessity of integrating emerging molecular classifiers and AI-driven digital pathology to optimize the management of indeterminate nodules.

Keywords: Thyroid swelling, Fine needle aspiration cytology, Histopathology, Bethesda system 2023, Artificial intelligence, Molecular diagnostics, Colloid nodular goitre

INTRODUCTION

Situated in the visceral compartment of the anterior neck, the thyroid gland's intimate proximity to critical structures-including the recurrent laryngeal nerves (RLNs), parathyroid glands, and major cervical vasculature-poses inherent surgical challenges.[1,2] While it functions primarily as an endocrine regulator of metabolism and calcium homeostasis, the gland is highly susceptible to a diverse spectrum of nodular pathologies,

ranging from indolent inflammatory conditions to aggressive malignancies. Because total or partial thyroidectomy carries a risk of significant morbidity, distinguishing between benign and malignant lesions preoperatively is paramount to optimizing surgical extent and preserving these adjacent anatomical structures.[3]

A thorough clinical evaluation provides foundational insights, but distinguishing early malignancies from benign conditions on clinical grounds alone is notoriously difficult. High-resolution ultrasonography (USG) serves as the immediate adjunct, adeptly delineating the morphology and internal composition of these nodules. Yet, while USG excels at characterizing structural features and distinguishing solid from cystic lesions, it cannot reliably determine the definitive histological nature or malignant potential of a lesion.[4]

To bridge this diagnostic gap without immediately resorting to the gold standard of invasive diagnostic surgery, FNAC has become the global standard for preoperative triage.[5] FNAC is minimally invasive, rapid, cost-effective, and demonstrates high diagnostic accuracy for clearly benign or malignant cases.[5,6] However, its utility is significantly constrained within the diagnostic "gray zone"-most notably, its inability to distinguish between follicular adenomas and carcinomas due to overlapping cytological features.[7] Furthermore, approximately 10-15% of aspirates may be non-diagnostic or inadequate, and a non-diagnostic result should never be safely equated with a benign lesion.[8]

While FNAC remains the cornerstone of initial evaluation, the management of these indeterminate thyroid nodules (Bethesda categories III and IV) remains a persistent clinical challenge. Recent paradigm shifts, including the 2023 update to the Bethesda System, alongside the rapid integration of molecular testing and AI in digital cytopathology, are actively reshaping preoperative risk stratification and reducing unnecessary surgical interventions.[9-12,18]

Against this evolving landscape, present study was designed to evaluate diagnostic reliability of FNAC against postop histopathology.

By analyzing institutional data through modernized lens of 2023 Bethesda criteria, this study aims to assess sensitivity, specificity and accuracy of standard FNAC, while identifying specific diagnostic pitfalls that emerging technological adjuncts could address in future clinical practice.

METHODS

This is a retrospective study where records of patients who presented to the Department of ENT, Father Muller Medical College, Mangalore, Karnataka, India between 1st October 2023 and 31st October 2025 with thyroid swellings were collected. The study included 40 patients for whom preoperatively USG neck and FNAC of thyroid swelling was done and then underwent Total thyroidectomy. Their histopathological reports were reviewed. Preoperative FNAC results were correlated with post operative histopathological diagnosis. Cytological results were classified according to the Bethesda system for reporting thyroid cytopathology (where applicable). Final histopathological diagnoses were correlated with cytological findings, and statistical parameters including sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy of FNAC were calculated. The study was approved by the institutional ethics committee on 10th December 2025. The research protocol was strictly conducted in accordance with the ethical principles for medical research involving human subjects as outlined in the declaration of Helsinki. Informed consent was not required as it is a retrospective record based study.

Inclusion criteria

Patients aged between 18 and 80 years and patients who got preoperative FNAC of the ultrasonographically confirmed thyroid swellings and subsequently underwent total thyroidectomy with available postoperative histopathology reports were included in study.

Exclusion criteria

Patients with incomplete clinical, cytological, or histopathological data, patients who had previously undergone thyroid surgery or radio-iodine therapy and patients with known metastatic lesions in the thyroid were excluded from study.

RESULTS

The study included 40 patients with thyroid swellings, with the highest incidence in the 51-60 year age group, that is 13 cases (32.5%), followed by the 31-40 year group, that is 12 cases (30%) (Table 1).

Colloid nodular goitre was the most frequent condition on FNAC (21 cases of simple colloid goitre and 8 cases with cystic change, together accounting for 72.5% of all cases). There were 4 cases of follicular neoplasm (FN) (10%), 1 case each of lymphocytic thyroiditis (2.5%), adenomatous nodule (2.5%), benign follicular nodule (2.5%), AUS (2.5%), follicular lesion (2.5%), follicular variant of papillary carcinoma (2.5%), papillary carcinoma (2.5%) (Table 2).

On cytological categorization, the results were classified utilizing the updated 2023 (3rd edition) Bethesda system for reporting thyroid cytopathology. Under this current framework, 32 cases (80%) were categorized as category II (Benign). Two cases (5%), which historically may have included the now-retired "FLUS" terminology, were consolidated into category III (AUS). There were 4 cases (10%) classified as category IV (FN), 1 case (2.5%) as

category V suspicious for malignancy (SFM), and 1 case (2.5%) as category VI (Malignant) (Table 3).

Upon final histopathological examination, the majority of cases were diagnosed as colloid nodular goitre (60%), followed by Hashimoto's thyroiditis (17.5%), follicular adenoma (15%), and the follicular carcinoma (5%) (Table 4).

Correlation of FNAC with histopathology for individual diagnostic categories demonstrated high concordance. Among 32 cases diagnosed cytologically as colloid nodular goitre, 31 were confirmed as benign colloid nodular goitre on histopathology, with only one case reclassified as papillary microcarcinoma thyroid.

Similarly, all cytologically reported FNs (4 cases), follicular lesions (1 case), AUS (1 case) and papillary carcinoma (1 case) were confirmed on histopathology as follicular adenoma or its variants, or papillary carcinoma, indicating a strong diagnostic agreement between the two modalities (Table 5).

When benign and malignant lesions were grouped, FNAC showed excellent diagnostic performance. For benign (colloid nodular goitre), there were 31 true positives, 1 false positive, no false negatives and 8 true negatives, yielding a sensitivity of 100%, specificity of 88.89%, positive predictive value of 96.88%, negative predictive value of 100% and an overall diagnostic accuracy of 97.5%.

For malignant (papillary carcinoma thyroid), there was 1 true positive, 1 false positive, no false negative and 38 true negatives, corresponding to a sensitivity of 100%, specificity of 97.43%, positive predictive value of 50%, negative predictive value of 100% and overall diagnostic accuracy of 97.5% (Table 6).

Table 1: Incidence of thyroid lesions in age groups.

Age (in years)	Incidence
<20	1 (2.5%)
21-30	2 (5%)
31-40	12 (30%)
41-50	6 (15%)
51-60	13 (32.5%)
61-70	5 (12.5%)
>70	1 (2.5%)

Table 2: Incidence of the types of thyroid lesions according to the fine needle aspiration cytology.

Diagnosis	N
Colloid nodular goitre	21 (52.5%)
Colloid nodular goitre with the cystic change	8 (20%)
Lymphocytic thyroiditis	1 (2.5%)
Adenomatous nodule	1 (2.5%)
Benign follicular nodule	1 (2.5%)
Atypia of undetermined significance	1 (2.5%)
Follicular lesion	1 (2.5%)
FN	4 (10%)
Follicular variant of papillary carcinoma thyroid	1 (2.5%)
Papillary carcinoma thyroid	1 (2.5%)
Total	40

Table 3: The Bethesda system for reporting thyroid cytopathology.

Diagnostic category (Bethesda 2023)	N
Category I: Non-diagnostic or unsatisfactory	0 (0%)
Category II: Benign	32 (80%)
Category III: AUS	2 (5%)
Category IV: FN	4 (10%)
Category V: SFM	1 (2.5%)
Category VI: Malignant	1 (2.5%)
Total	40

Table 4: Incidence of the types of thyroid lesions according to histopathology.

Diagnosis	N
Colloid nodular goitre	24 (60%)
Hashimoto's thyroiditis	7 (17.5%)
Follicular adenoma	6 (15%)
Follicular carcinoma	2 (5%)
Total	40

Table 5: Positive correlation of result of FNAC with the result of histopathology of the different types of thyroid lesions.

Type of thyroid lesion diagnosed by FNAC	Number of the cytological diagnosis	Correlation with the histopathology		Histopathological diagnosis in false cytodiagnosis
		Correct cytodiagnosis	False cytodiagnosis	
Colloid nodular goitre	32	31	1	Papillary microcarcinoma thyroid
Follicular lesion	1		1	Follicular adenoma
FN	4	4		
Atypia of undetermined significance	1		1	Follicular adenoma
Follicular variant of papillary carcinoma thyroid	1		1	Follicular adenoma-Hurthle cell variant
Papillary carcinoma thyroid	1	1		

Table 6: Correlation of cytology versus HPE for benign and malignant thyroid lesions.

FNAC versus histopathology	Observation				Evaluation				
	True positive	False positive	False negative	True negative	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Diagnostic accuracy (%)
Benign (Colloid nodular goitre)	31	1	0	8	100	88.89	96.88	100	97.5
Malignant (Papillary carcinoma thyroid)	1	1	0	38	100	97.43	50	100	97.5

DISCUSSION

Epidemiology and purpose

Thyroid swellings are common clinical entities, predominantly affecting women. Recent epidemiological studies in South India indicate a clinical detection rate of up to 12% in the adult population.[13] Given the gland's superficial location in the anterior neck, it is highly accessible for direct physical examination, ultrasonography, and FNAC. While a wide variety of etiologies manifest as thyroid swellings-most of which are benign-the primary objective of clinical investigation is the accurate exclusion of malignancy.

Management and surgical decision-making

Categorizing thyroid nodules as benign or malignant is critical for determining the optimal management strategy. Although only about 5% of clinically detected nodules harbor malignancy, the burden of thyroid carcinoma in India is notable, accounting for approximately 5-6% of all carcinomas in women and 2% in men.[14] Because not all thyroid swellings require surgical intervention, accurate preoperative screening is essential. When surgery is indicated, these initial investigations play a pivotal role in dictating the extent of the resection, thereby directly influencing patient outcomes and minimizing surgical morbidity.[15]

Utility and pitfalls of FNAC

FNAC has established itself as an indispensable, safe, and cost-effective outpatient procedure, and is now considered a standard prerequisite prior to any thyroid surgery.[16] Its primary utility lies in its ability to differentiate benign from malignant lesions and classify the underlying etiology. While FNAC generally demonstrates high specificity, its sensitivity can be compromised by false-negative results, which carry the severe risk of missing occult malignancies and delaying crucial oncological care.[17] Conversely, false-positive interpretations can lead patients to undergo unnecessary, extensive surgical procedures accompanied by elevated risks of permanent surgical complications.

The diagnostic gray zone and histopathology

Despite its widespread utility, FNAC frequently yields indeterminate or suspicious results.[18,19] A major inherent shortcoming is its inability to cytologically differentiate benign follicular adenomas from malignant follicular carcinomas.[20] Additionally, hyperplastic nodules in Hashimoto's thyroiditis or Hürthle cell adenomas are occasionally misclassified as papillary carcinomas due to overlapping cytomorphological features.[21] Such indeterminate or false-negative diagnoses can result in conservative initial surgeries that subsequently require a high-risk completion thyroidectomy. While intra operative frozen sections are sometimes utilized to guide surgical extent in these scenarios, definitive postoperative histopathology of the excised specimen remains the ultimate gold standard. Histopathological examination not only confirms the diagnosis but uniquely identifies the capsular and vascular invasion features necessary for accurate oncological staging.[18,20]

In this study, FNAC demonstrated high sensitivity and specificity, consistent with earlier reports. Bouvet et al reported a sensitivity of 93.5% and a specificity of 75%, while Handa et al observed even higher values-97% sensitivity and 100% specificity.[15,22] Similarly, Agrawal et al at the Tata Memorial Hospital, Mumbai, documented FNAC accuracy at 90.9%, sensitivity at 76.5%, specificity at 95.9%, with false-positive and false-negative rates of 2% and 4%, respectively.[17] A study by Kessler et al showed sensitivity, specificity and accuracy of FNAC of 79%, 98.5%, and 87% respectively.[23] Another study by Gupta et al showed sensitivity, specificity, and accuracy of FNAC for solitary thyroid nodules as 80%, 86.6%, and 84% respectively.[24] In the FNAC series of a study by Sengupta et al showed sensitivity of 90% while specificity was 100%, accuracy was 98.88%, Predictive value of a positive test and negative test was 100% and 98.75% respectively.[25]

Mandreker et al advocated its routine use, especially for solitary thyroid nodules.[16] Gardner et al highlighted specific cytological features-such as abundant colloid, uniformly spaced large follicles, and macrofollicular patterns-as useful predictors in differentiating lesions with lower malignant potential from those that warrant closer scrutiny.[21] A study by Esmaili and Taghipour showed sensitivity, specificity, and accuracy as 91.6%, 100%, and 97%, respectively.[26]

A study by Kumar et al which was a two year prospective study involving 295 outdoor cases with thyroid lesions at UPRIMS and R, Saifai, Etawah, U.P showed more than half (65.4% cases) the number of thyroid FNACs were diagnosed as colloid goitre. Diagnostic categorization of 295 thyroid FNACs based on Bethesda classification showed that 239 (81.01%) cases were cytologically benign, 2 cases (0.68%) were under AUS while six cases (2.03%) under the neoplasm category- follicular. SFM category included two cases (0.68%)-hyalinizing trabecular adenoma/columnar variant of papillary carcinoma and medullary carcinoma/oncocyticneoplasm. Under the malignant category, there were fourteen cases (4.05%) cytologically diagnosed and in the inadequate/non-diagnostic category there were 32 cases (10.85%) of cases.[27] This was very similar to our study which also concluded that FNAC is a simple, safe and cost effective modality in investigation of thyroid disease with high accuracy and specificity.

Despite these advantages, false-negative and false-positive interpretations do occur. False negatives may result from sampling errors, suboptimal smear preparation, or the presence of occult papillary

microcarcinomas, while false positives can arise from hyperplastic or inflammatory conditions mimicking neoplasia. These limitations highlight the importance of optimal technique, adequate sampling, and continuous quality supervision by experienced cytopathologists. Clinical judgment and imaging correlation remain integral to final management decisions, and FNAC should be interpreted in context rather than in isolation.

Future directions

AI and molecular adjuncts

While FNAC remains the cornerstone of initial thyroid evaluation, the diagnostic gray zone of indeterminate nodules (Bethesda Categories III and IV) continues to pose clinical challenges. In our study, limitations in cytological differentiation were evident, such as a false positive where a benign colloid nodular goiter was misdiagnosed as a papillary microcarcinoma. To address these inherent limitations, the integration of molecular diagnostics and AI is rapidly transforming thyroid cytopathology.

Recent advancements in commercial molecular testing platforms, such as ThyroSeq v3 and Afirma GSC, have proven highly efficacious in the management of indeterminate thyroid nodules.[9,10] These platforms evaluate the genomic landscape of aspirates, offering critical molecular insights that complement traditional cytomorphology. By predicting the benign or malignant nature of Bethesda III and IV nodules at the molecular level, these tests significantly reduce the rate of unnecessary diagnostic hemithyroidectomies. Furthermore, the 2023 update to the Bethesda system, which mandates the subcategorization of AUS into "nuclear atypia" versus "other", aligns directly with the application of these molecular classifiers to stratify malignancy risk more accurately.[19] Clinically, this targeted risk stratification empowers surgeons to confidently recommend active surveillance for molecularly benign nodules, sparing patients the potential burden of lifelong thyroid hormone dependence. Conversely, it ensures nodules with high-risk molecular profiles are triaged for definitive oncological resection upfront, avoiding high-risk, two-stage completion thyroidectomies.

Alongside molecular advancements, AI and digital pathology are emerging as vital diagnostic co-pilots. Deep learning frameworks, particularly transfer learning models, are currently being deployed to objectively quantify nuclear atypia and reduce interobserver variability.[12] Because standard cytomorphology can be challenged by the subjective interpretation of subtle nuclear features, AI serves as an invaluable tool for providing an objective "second read". These AI algorithms demonstrate robust potential in standardizing Bethesda categorization and overcoming preanalytical variability, offering a reliable second opinion in diagnostically challenging cases.[11] As clinical practice evolves, multimodal AI risk-stratification tools that integrate USG, digitized FNAC slides, and molecular data will likely become standard practice, further minimizing false-positive rates and optimizing surgical planning. Ultimately, integrating these AI and molecular advancements transforms subjective interpretations into objective, actionable data-facilitating more transparent patient counseling and significantly alleviating the anxiety traditionally associated with "indeterminate" thyroid swellings.

Clinical translation

Impact on surgical decision-making and patient counseling

For the otolaryngologist, the ultimate clinical utility of these advancements lies in transforming subjective cytological interpretations into objective, actionable data for surgical planning. Historically, an indeterminate FNAC result (Bethesda III or IV) left both the clinician and the patient in a state of diagnostic ambiguity, often leading to a diagnostic hemithyroidectomy by default. However, the integration of AI-based transfer learning models provides an objective "second read." By quantifying subtle nuclear atypia and standardizing the new Bethesda subcategories, these models significantly reduce the interobserver variability that historically plagued indeterminate aspirates.[11,12]

When this AI-enhanced digital cytopathology is combined with molecular testing platforms, preoperative risk stratification shifts from probabilistic to highly precise.[9,10] This multimodal workflow directly alters surgical decision-making: it empowers the surgeon to confidently recommend active surveillance for molecularly benign nodules, sparing patients from the morbidity and lifelong thyroid hormone replacement associated with unnecessary surgery.[10] Conversely, it ensures that high-risk molecular profiles are appropriately triaged for definitive oncological resection upfront, avoiding the need for two-stage completion thyroidectomies. Ultimately, integrating AI and molecular data facilitates more transparent, data-driven patient counseling, alleviating the profound patient anxiety traditionally associated with "indeterminate" thyroid swellings.

CONCLUSION

While FNAC provides highly accurate preoperative triage for the majority of thyroid swellings, the clinical management of indeterminate nodules continues to pose a significant surgical challenge. Our findings reaffirm FNAC as a safe and invaluable cornerstone for initial evaluation, yet they also highlight the inherent limitations of relying exclusively on standard cytomorphology. As clinical practice aligns with the updated 2023 Bethesda criteria, the diagnostic paradigm is shifting. Integrating

digital AI algorithms and molecular testing into the traditional FNAC workflow will be critical for modern surgical practice-ultimately resolving diagnostic ambiguity, reducing the morbidity of unnecessary diagnostic hemithyroidectomies, and optimizing long-term oncological outcomes.

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