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Fine Needle Aspiration Cytology of Thyroid Lesions: A Cytohistological Correlation Study on Diagnostic Accuracy and Pitfalls

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Abstract

Background: Given the low malignancy rate in palpable thyroid nodules (~5%), accurate pre-operative differentiation of benign from malignant lesions is critical to avoid unnecessary surgery. However, thyroid cytology has a diagnostic "grey zone" with reduced classification precision, leading to ambiguity. ***Aims:*** This study aimed to: (1) determine the age and sex distribution of thyroid lesions; (2) evaluate the diagnostic performance (sensitivity, specificity) of fine needle aspiration cytology (FNACC); and (3) identify reasons for cytological-histological discrepancies. ***Methods:*** A retrospective analysis of 64 thyroid lesion cases from a five-year cohort involved cytohistological correlation. FNAC diagnoses were categorized based on agreement or disagreement with histology. Cytological smears and histological sections underwent independent re-evaluation. ***Results:*** Cytopathological diagnoses agreed with histopathology in 58/63 cases (92.06% concordance; 7.93% discordance, 5 cases). No false positive cytological diagnoses occurred. False negative diagnoses were attributed to sampling error and challenges in recognizing concurrent distinct pathologies. ***Conclusion:*** FNAC is an efficient and accurate tool for evaluating palpable thyroid nodules and guiding clinical management. The high malignancy detection rate in resected nodules suggests a reduction in overall thyroid surgeries. Integrating stringent FNAC adequacy criteria with clinical and radiological correlation is crucial to minimize false negative and false positive diagnoses.

Keywords: ***Thyroid Nodule, Fine-Needle Aspiration Cytology, Cytodiagnosis, Diagnostic Accuracy.***

Introduction

Epidemiological data indicate that thyroid nodules represent a prevalent clinical observation, with a reported incidence ranging from 4% to 7% within the general population [1]. Studies suggest a statistically significant higher prevalence of thyroid nodules in the female demographic. Established risk factors associated with increased thyroid nodule occurrence include advancing age, a history of radiation exposure, and dietary consumption of goitrogenic compounds [2]. Palpable thyroid nodules are predominantly benign, with malignancy identified in approximately 5% of cases. Therefore, the accurate preoperative differentiation between benign and malignant thyroid lesions is clinically imperative for optimizing patient management, minimizing unnecessary surgical interventions, and ensuring efficient resource utilization.

Current diagnostic modalities for thyroid nodule evaluation include ultrasonography (USG), thyroid scintigraphy, and fine-needle aspiration (FNAC). While the majority of benign thyroid nodules present as hypo functional ("cold") on scintigraphy, the presence of a hypo functional lesion raises suspicion for malignancy. Conversely, hyper functional ("warm") nodules do not exclude malignancy, even following thyroid-stimulating hormone (TSH) suppression. Although USG exhibits high sensitivity in detecting impalpable thyroid nodules, its specificity for differentiating benign from malignant lesions is limited. USG is particularly valuable for assessing complex cystic masses and non-palpable nodules. According to the 2017 American College of Radiology Thyroid Imaging Reporting and Data System (ACR TI-RADS), risk stratification scores of 3-5 correlate with an elevated probability of malignancy. While USG and FNAC cytology (FNACC) demonstrate comparable sensitivity in diagnosing malignant thyroid nodules, FNAC exhibits superior specificity (90%). This minimally invasive technique offers high diagnostic accuracy (85%) [3]. In distinguishing between benign and malignant thyroid lesions,

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establishing FNAC biopsy as the preferred initial diagnostic procedure for discrete thyroid nodules.

Fine-needle aspiration (FNAC) is a recommended diagnostic modality for pre-operative evaluation and longitudinal surveillance of thyroid nodules [4]. Its increasing clinical utility stems from its procedural simplicity, low cost, and minimal associated morbidity, facilitating the early detection of thyroid malignancies and consequently contributing to improved patient outcomes. This study aims to evaluate the diagnostic performance of thyroid FNAC, specifically focusing on the analysis of discordant cytological and histological findings.

Materials and Methods

A retrospective analysis was conducted on thyroid lesions diagnosed at Shri Jagannath Medical College and Hospital, Puri, Odisha, India, over a five-year period. The study cohort comprised individuals of all ages and genders presenting with thyroid enlargement who underwent both fine-needle aspiration (FNAC) and subsequent surgical resection. A total of 64 cases with corresponding FNAC cytology and surgical histopathology reports were identified. One case was excluded due to an inadequate FNAC sample, resulting in a final cohort of 63 cases. Cytological and histological records were systematically reviewed. All FNAC procedures were performed by trained pathologists. Smears obtained during FNAC were immediately fixed in a 1:1 solution of 50% ethanol and diethyl ether. Additionally, some smears were air-dried. Standard cytological staining techniques, including Hematoxylin and Eosin (H&E), Papanicolaou (Pap), and May-Grünwald-Giemsa (MGG), were applied for microscopic evaluation.

Formalin-fixed specimens underwent macroscopic evaluation documenting specimen type, dimensions, mass, nodularity, capsular integrity, and secondary alterations including hemorrhage, calcification, and cyst formation. Representative tissue blocks, 2-3 mm in thickness, yielding 5-10 sections, were processed for hematoxylin and eosin (H&E) staining. Special stains were employed as indicated. All cytological smears and corresponding histopathology slides were subjected to blinded, dual pathologist review. Thyroid cytopathology diagnoses adhered to the Bethesda System for Reporting Thyroid Cytopathology (2017): Non-diagnostic/Unsatisfactory (Category I), Benign (Category II), Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance (Category III), Follicular Neoplasm/Suspicious for a Follicular Neoplasm (Category IV), Suspicious for Malignancy (Category V), and Malignant (Category VI) [5]. Fine-needle aspiration (FNAC) and histopathology diagnoses were categorized as concordant or discordant. Discrepant cases underwent a comprehensive review to ascertain the etiology of the diagnostic disagreement, specifically assessing for histological errors, cytodiagnostic inaccuracies (including overlapping criteria or misinterpretation of established criteria), FNAC sampling artifacts, or suboptimal smear quality (characterized by poor cellular preservation, obscuration by blood, hypocellularity, or borderline cellularity).

In this study, a true positive (TP) was defined as a case where a subsequent definitive histopathological examination confirmed a prior cytological diagnosis of malignancy or suspected malignancy. Conversely, a true negative (TN) was characterized by concordant negative cytological and benign histopathological findings. A false negative (FN) diagnostic outcome, when applied to a malignant lesion, denoted a non-neoplastic finding that, if interpreted as such, would typically preclude necessary surgical intervention. Conversely, a false positive (FP) cytological diagnosis occurred when a non-neoplastic lesion was erroneously identified as a neoplasm, potentially leading to unnecessary surgical excision [6]. The diagnostic performance of the index test was evaluated by calculating the positive predictive value (PPV), negative predictive value (NPV), specificity, sensitivity, and the accuracy of fine-needle aspiration (FNAC) in predicting the final histological diagnosis.

Results

The study cohort comprised 63 individuals. The age group of 31-40 years exhibited the highest prevalence of thyroid lesions (37.33%). The demographic distribution of the patient population revealed a female predominance, with a notable concentration of individuals in the fourth and fifth decades of life (Table 1 and Table 2).

Table 1: Distribution of thyroid lesions by age and sex

Age (years)	Male (N)	Female(N)	Total (N)	Male (%)	Female (%)	Total N (%)
11-20	1	3	4	25	75	100
21-30	1	16	17	5.88	94.12	100
31-40	3	18	21	14.28	85.72	100
41-50	1	10	11	9.10	90.90	100
51-60	2	5	7	28.57	71.43	100
61-70	0	3	3	-	100	100
Total(N)	8	55	63	12.70	87.30	100

Table 2: Age and sex-wise incidence of various thyroid lesions including dual pathology

Age (years)	Goiter		Thyroiditis		Benign neoplasms		Malignant neoplasms		Total
	M	F	M	F	M	F	M	F	
11-20	1	2	-	1	-	1	1	-	6
21-30	1	10	-	1	-	2	1	1	16
31-40	2	10	-	3	1	2	-	1	19
41-50	1	7	1	1	-	1	-	1	12
51-60	1	3	-	1	1	1	-	-	7
61-70	-	2	-	1	-	-	-	-	3
Total	6	34	1	8	2	7	2	3	63

M: Male; F: Female.

The prevalence of thyroid disorders in the studied cohort of females aged 31-40 years exhibited the following distribution: goiter at 15.87%, thyroiditis at 4.76%, benign thyroid neoplasms at 3.17%, and malignant thyroid neoplasms at 1.58%. The predominant clinical presentation (75.32%) was isolated thyroid swelling. Hyperthyroidism-related signs and symptoms were observed in 14.7% of the patient population. Isolated occurrences included fever and pain in a single individual, and classic manifestations of Graves' disease in another single case.

In this cohort, multinodular thyroid enlargement exhibited the highest prevalence (71.2%) as the primary clinical presentation. Cytological analysis revealed nodular goiter as the most frequently identified lesion (40.5%), followed by simple colloid goiter as the second most common cytological finding. For cases with a cytological diagnosis of nodular goiter, subtotal thyroidectomy was the predominant surgical intervention for thyroid lesions. Patients diagnosed with papillary thyroid carcinoma (n=2) underwent total thyroidectomy with radical neck dissection. A cytological diagnosis of suspicious for carcinoma consistently led to total thyroidectomy.

In the evaluation of malignant neoplasms, the diagnostic test correctly identified 3 true positive cases and 60 true negative cases. No false positive results were observed, but 1 false negative result occurred. This yielded a sensitivity of 75%, a specificity of 100%, and an accuracy of 98.43%. For benign neoplasms, the test correctly identified 3 true positive cases and 52 true negative cases, while yielding 2 false positive and 7 false negative results. The calculated sensitivity for benign neoplasms was 30%, the specificity was 96.29%, and the accuracy was 85.93%.

Discussion

A five-year retrospective cytohistological correlation of 63 thyroid lesions revealed a 4% (n=1) diagnostic insufficiency rate after excluding one inadequate sample, lower than previously reported. The study cohort (n=62, age 12-65 years) showed peak prevalence in the 31-40 year age group (33.33%) and a significant female predominance (87.30%, female: male ratio 6.5:1), with most affected females in their fourth decade (90.90%). All patients presented with thyromegaly (100%), with 20.63% exhibiting concurrent symptoms. Hyperthyroidism was present in 14.7% (n=9), including one Graves' disease case. Acute thyroiditis was identified in 1.59% (n=1). Palpation indicated multinodular goiter (71.42%, n=45) as the most common presentation, followed by solitary nodules (15.50%, n=10) and diffuse enlargement (13.08%, n=8). Cytology confirmed toxic goiter in all clinically suspected cases (100%) and detected four (6.34%) previously undiagnosed thyroid malignancies (Table 3).

Table 3: Clinico-cytological correlation of thyroid lesions.

Clinical diagnosis	Colloid goiter	Nodular goiter	Graves's disease	Acute thyroiditis	Chronic thyroiditis	Hashimoto's thyroiditis	Follicular neoplasm	Suspicious of carcinoma	Papillary carcinoma	Total
Thyroid cyst	1	1	-	-	-	-	-	-	-	2
Goiter	21	18		1	1	2	4	2	1	50
Toxic goiter	-	1	1	-	-	-	1	-	-	3
Solitary Thyroid nodule	-	6	-	-	-	-	1	-	1	8
Total	22	26	1	1	1	2	6	2	2	63

Following suspicious (Bethesda V) cytology, total thyroidectomy was performed. Papillary thyroid carcinoma (Bethesda VI) cytology necessitated total thyroidectomy with radical neck dissection. Benign cytology resulted in subtotal thyroidectomy in 62.5% (75/120) of cases, with the remainder undergoing alternative surgical management. Cytological analysis identified goiter in 92.55% (87/94) of clinically diagnosed goiters but failed to detect concurrent adenomatous hyperplasia in 7 cases and chronic thyroiditis in 9 cases (Table 4).

Table 4: Cyto-histo correlation of thyroid lesions

FNAC diagnosis	Number of correlated cases	% of correlated cases	Number of discrepant cases	% of discrepant cases	Total number of cases
Bethesda II (Goiter)	45	93.75	3	6.25	48
Bethesda II (thyroiditis)	4	80	1	20	5
Bethesda IV (benignneoplasm)	6	85.71	1	14.28	7
Bethesda VI (malignant neoplasm)	3	100	0	-	3
Total number of cases	58	92.06	5	7.93%	63

FNAC: Fine needleaspiration cytology

Cytological differentiation between adenomatous nodules and follicular adenomas is challenging due to overlapping features, as exemplified by a misclassified follicular adenoma (Bethesda IV) initially diagnosed as benign goiter (Bethesda II) due to concurrent chronic thyroiditis masking the characteristic microfollicular pattern with flat follicular sheets and sparse nuclear crowding in moderately cellular smears. Histological confirmation highlighted this cytomorphological overlap.

Bussenier and Oertel [11] noted that syncytial fragments and microfollicular architecture have a positive predictive value for follicular neoplasm, though these are not entirely specific, occurring in a proportion of nodular goiters. Inverse correlation exists between background colloid and follicular/adenomatoid/hyperplastic nodules (least colloid in follicular neoplasms). Conversely, cellularity and microfollicles directly correlate, with follicular neoplasms exhibiting the highest cellularity and most prominent microfollicular pattern.

The cohort included five thyroiditis cases, one being Graves' disease, presenting with typical hyperthyroidism. Cytology showed moderately cellular smears with follicular clusters/follicles in a

hemorrhagic background, alongside nuclear pleomorphism and marginal vacuoles. Clinical and cytological features favored Graves' disease over toxic goiter in this case.

Cytological-clinical correlation precisely diagnosed one acute thyroiditis case, presenting with fever, neck pain/tenderness, and neutrophilic infiltrate within necrotic tissue on cytology. Cytology identified three chronic thyroiditis cases by follicular cells with moderate lymphoplasmacytic infiltrate and reduced colloid; nodular goiter was absent in two. Histology confirmed fibrosing thyroiditis in one. Literature (Das *et al.*) highlights increased inflammatory cell types in inflammatory goiters versus neoplasms, noting potential diagnostic overlap between papillary carcinoma and thyroiditis due to lymphocytic infiltration [12]. Cytology showed 100% accuracy for three confirmed Hashimoto's thyroiditis cases, consistent with prior sensitivity reports (Nguyen, 92%) [13], characterized by Hürthle cells, lymphocytes, scant colloid, and occasional giant cells. Conversely, one follicular (Hürthle cell) adenoma (histology) was initially misclassified as Hashimoto's thyroiditis (cytology) due to cellular smears with limited lymphocytes and loosely clustered Hürthle cells with focal follicular arrangement. Histological analysis of benign neoplasms (n=9) revealed one Hürthle cell adenoma and eight follicular adenomas.

Cytological analysis of six follicular thyroid neoplasms revealed a 33.33% false-positive rate (n=2), misclassifying a multinodular goiter with adenomatous hyperplasia and a case of chronic thyroiditis. Conversely, histological examination of nine follicular adenomas showed a 55.55% false-negative rate (n=5), with cytology misdiagnosing one as Hashimoto's thyroiditis and four as nodular goiter. A single follicular carcinoma was correctly identified as a follicular neoplasm cytologically. These findings, consistent with Löwhagen and Sprenger [14], underscore the cytomorphological overlap between follicular adenomas and carcinomas, limiting cytology's definitive diagnostic capability. As supported by Deshpande *et al.* [15], histological evaluation for vascular or capsular invasion remains essential for definitive differentiation.

A cohort of nine follicular adenomas showed female predominance (77.8%), with peak incidence in the fourth decade. Single cases of follicular adenoma co-occurred with chronic thyroiditis and multinodular goiter; initial cytology of these yielded false-negative Bethesda II diagnoses for nodular goiter, but this did not alter patient management.

Three malignant thyroid neoplasms (2 papillary, 1 follicular carcinoma) were identified, with cytological diagnostic accuracy of 66.7%. The follicular carcinoma, diagnosed in a 30-year-old male with a solitary nodule, was initially cytologically classified as a follicular neoplasm due to overlapping features, later confirmed as malignant by histology revealing vascular invasion. Cytology showed discohesive clusters, syncytial sheets, and pleomorphic follicular cells in a hemorrhagic, colloid-poor background. Post-cytology, the patient underwent subtotal thyroidectomy. Literature reports a 15-22% malignancy rate in cytologically classified follicular neoplasms [16], consistent with a 16% carcinoma rate in 219 suspected cases [17] and Gharib's ~14% finding [18].

A papillary thyroid carcinoma (PTC) case was identified in a male in his second decade. The other three PTC cases occurred in females in their third (n=1), fourth (n=1), and fifth (n=1) decades. These cases, initially presenting as solitary, predominantly euthyroid thyroid nodules (two hypothyroid), showed highly cellular cytological smears with loosely cohesive clusters, papillary fragments, syncytial sheets, and dissociated cells in a hemorrhagic background, exhibiting mild nuclear pleomorphism and grooves. Histological analysis of two cases confirmed PTC with papillary fronds and characteristic nuclear features. A diagnostic score (>7) based on Prabhu and Umashankar criteria was applied [19]. One case was the follicular variant of PTC (FVPC) [20], which, unlike typical PTC in this study, showed less frequent papillary structures and nuclear grooves, but more follicle-like features, dusty chromatin, and intranuclear inclusions. Nuclear grooves were observed on H&E smears. Differentiation from noninvasive follicular thyroid neoplasms with papillary-like nuclear features (NIFTPs) [21-23], characterized by subtle PTC nuclear features and follicular architecture without papillae, psammoma bodies, or necrosis, is critical.

Cytological analysis of two papillary carcinomas, characterized by papillary fragments and definitive nuclear features (fine chromatin, grooves, and intranuclear inclusions, each 100%), yielded accurate diagnoses. Psammoma bodies were not observed. One papillary carcinoma (Bethesda VI) was initially a false negative (nodular goiter, Bethesda II) due to sampling error. The high (100%) positive predictive value of cytology for papillary carcinoma, as reported by Ko *et al.* [24], is consistent with these findings. However, distinguishing nodular goiter, follicular adenoma, and papillary carcinoma cytologically can be challenging due to overlapping nuclear and chromatin characteristics [25].

This five-year study, limited by a small sample size and exclusion of rare thyroid carcinoma subtypes (Hürthle cell, medullary, anaplastic), demonstrated a cytological-histopathological correlation of 92.06% (discrepancy rate: 7.93%). This correlation rate surpasses previous reports. Malignant neoplasm correlation was 100%, and goiter correlation was 93.75%. Fine-needle aspiration (FNAC) accuracy for malignancy diagnosis was 98.43% (sensitivity: 75%, specificity: 100%). Benign neoplasm sensitivity was 75%. Overall for neoplasms, FNAC yielded a sensitivity of 87.5%, specificity of 98.07%, and accuracy of 96.66%, consistent with prior studies [26-30].

The investigated diagnostic method demonstrates significant inter-study heterogeneity in accuracy (specificity: 74%; sensitivity: 61.9-100%). Lower specificity and PPV in one study] likely resulted from binary outcome classification [31]. Variability in sensitivity and specificity is primarily driven by inter-observer disagreement among cytopathologists regarding the "suspicious" category and the definition of false positives (FPs) and false negatives (FNs). While no FPs occurred in this cohort, literature reports a 18% FP rate [6,24,29], often due to misclassification of follicular neoplasms [24]; accepting some FPs may minimize missed malignancies. Three FNs were observed, consistent with the broad literature range (1-16%) [6,27,29], though some studies report extremes [29,32]. FNs in this study likely arose from sampling errors, cytological overlap between follicular adenomas and carcinomas, and missed concurrent pathologies.

Conclusions

Thyroid nodule prevalence peaked in females in their fifth decade. This study underscores fine-needle aspiration (FNAC)'s high accuracy and efficacy in diagnosing palpable thyroid nodules, demonstrating minimal false positive and false negative rates. FNAC exhibits high specificity for nodular goiter, thyroiditis, and particularly papillary carcinoma. However, its utility in differentiating follicular lesions is limited due to overlapping cytological features between benign (e.g., follicular adenomatous goiter) and malignant (e.g., follicular carcinoma, follicular variant papillary carcinoma - FVPC) entities. Despite these limitations leading to indeterminate FNAC results and potential diagnostic errors, especially in hyperplastic nodules, follicular neoplasms, and

FVPC, FNAC remains an effective screening tool. Integrating strict specimen adequacy criteria with clinical and imaging findings is expected to reduce false negative and false positive diagnoses. Close clinical surveillance of cytologically benign nodules is crucial due to rare false negative results, necessitating surgical excision of clinically suspicious lesions regardless of benign cytology.

Declarations

Ethical Approval and Consent to participate

Not applicable as retrospective nature of study. Consent for publication: Not applicable as retrospective nature of study.

Availability of supporting data

Upon request to the corresponding author.

Competing interests

Nil

Funding Statement

Nil

Authors contributions

All authors made substantial contributions to the reported work, including in the areas of conception, study design, execution, data collection, analysis, and interpretation. They participated in drafting, revising, and critically reviewing the article, gave final approval for the version to be published, agreed on the journal for submission, and accepted responsibility for all aspects of the work.

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