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**Original Research Article**

# A Cytologic Study of Thyroid Lesions Utilizing the Bethesda System of Reporting with Histopathologic Correlation

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**Abstract:**

**Background:** Thyroid nodules, affecting 5-10% globally, pose a diagnostic challenge in distinguishing between benign and malignant lesions. Fine-needle aspiration cytology [FNAC], a key diagnostic tool, is guided by the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC). Despite widespread adoption, challenges persist in accurately predicting thyroid lesions solely through cytology. Histopathologic examination remains the gold standard, and up to 20-30% of initially indeterminate nodules are later found to be malignant yet FNAC of the thyroid is the first line procedure that is widely used all over the world in the diagnosis of thyroid lesions.

**Aims:** 1. To study the cytomorphology of various thyroid lesions utilising The Bethesda System of reporting thereby elucidating its utility. 2. Cytohistopathologic correlation; thereby analyzing the cause of diagnostic errors with an eventual aim to improve diagnostic accuracy.

**Methods:** This retrospective study, conducted at Vikram Hospital, Mandya, spanned four years from August 2011 to May 2015. The study encompassed 205 patients who underwent fine-needle aspiration (FNA) of thyroid nodules, either sonographic guided or non-guided. Experienced cytopathologists, going through the clinical information, reviewed slides utilising the Bethesda System categories. Histopathologic slides were reviewed too and diagnoses were correlated with cytology. Descriptive statistics characterized Bethesda System categories and histopathologic outcomes. Concordance rates were calculated, and SPSS version 18.0 was used for statistical analyses ($p < 0.001$ considered significant).

**Results:** In this study encompassing 205 patients, in the age group of 14-61 years, 12.6% were males and 87.3% were females, with the majority (72.7%) aged 20-40 years. Cytologic evaluation categorized 87.8% as benign, predominant lesions being nodular goitre (56.1%) and colloid goitre (38.8%). Follicular neoplasms [3.9%] and Papillary carcinoma [3.9%] showed equal prevalence Follow-up histopathology for 143 cases showed high concordance between cytologic and histopathologic diagnoses, with sensitivity (95.00%) and specificity (97.56%) indicating the reliability of cytology in identifying thyroid lesions. The overall accuracy of cytologic evaluation was commendable at 97.20%.

**Conclusion:** In conclusion, our study contributes valuable insights into the diagnostic accuracy of thyroid cytology, emphasizing its role in stratifying thyroid lesions based on the Bethesda System; being a very useful, uniform and standardized system for reporting thyroid cytopathology. The high concordance rates, along with robust sensitivity and specificity values, highlight the reliability of cytologic assessments.

**Keywords:** Thyroid, Benign, Malignant, Fine-needle aspiration, Histopathologic examination.



## Introduction

Thyroid nodules are a prevalent clinical concern affecting approximately 5-10% of the global population, as reported by various epidemiological studies [1,2]. While the majority of these nodules are benign, the challenge lies in accurately distinguishing between benign and malignant lesions to guide appropriate clinical management. Fine-needle aspiration (FNA) cytology has emerged as a frontline diagnostic tool, offering a minimally invasive means of evaluating thyroid nodules [2]. FNAC is used as a widely used diagnostic tool for its simplicity, diagnostic accuracy and cost effectiveness. Variety of tests give anatomical and functional information about the thyroid gland. FNAC by giving direct morphological information has supplanted most other tests for the preoperative evaluation of thyroid lesions. To standardize the reporting of thyroid cytology and facilitate communication between pathologists and clinicians, the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) was introduced [3]. The TBSRTC, established in 2007 and subsequently revised in 2017, categorizes thyroid FNA results into standardized diagnostic groups, providing a framework for con-

sistent communication of findings and guiding subsequent management decisions [3,4]. The system encompasses six main diagnostic categories, ranging from non- diagnostic, benign, atypia of undetermined significance or follicular lesion of undetermined significance [AUS/FLUS], Follicular Neoplasm/Suspicious for Follicular Neoplasm [FN/SFN], suspicious for malignancy and malignancy, each associated with distinct risk stratifications and management recommendations [4].

Despite the widespread adoption of the Bethesda System, challenges persist in accurately predicting the nature of thyroid lesions solely based on cytologic evaluation [6]. The need for further refinement and validation of this diagnostic approach, particularly through correlation with histopathologic outcomes, is evident [7].

Histopathologic examination remains the gold standard for definitive diagnosis, with studies indicating that up to 20-30% of nodules initially classified as indeterminate by cytology are ultimately found to be malignant on histopathologic analysis [8]. Moreover, the Bethesda System's evolving nature, as seen in its revisions, underscores the dynamic landscape of thyroid cytopathology [9].

Ongoing efforts to improve its applicability and diagnostic accuracy highlight the necessity of continued research and validation in diverse clinical settings [9]. In this context, the study aimed to evaluate thyroid lesions through cytologic analysis using the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) and to correlate these findings with subsequent histopathologic examinations.

The integration of both cytologic and histopathologic perspectives holds promise for advancing our understanding of thyroid lesions, guiding therapeutic decisions, and optimizing patient outcomes in the realm of thyroid disorders.

## Materials and Methods

**Study Design:** This retrospective study was conducted at Vikram Hospital, Mandya, spanning for a period of 4 years between August 2011 to May 2015.

**Study Population:** The study included 205 patients who underwent ultrasound guided or non-guided fine-needle aspiration (FNA) of thyroid nodules at Vikram Hospital, Mandya, during the specified time frame. Informed consent was obtained from all patients before FNA procedures.

**Cytologic Evaluation:** FNA samples were collected using aseptic techniques to minimize contamination and ensure sample integrity. Patients were positioned comfortably, and the skin overlying the thyroid nodule was cleansed with an antiseptic solution. Local anaesthesia, typically 2%lidocaine, was administered to minimize discomfort during the procedure in a few cases. A fine-needle aspiration (FNA) was then performed using a thin-gauge needle attached to a syringe. The needle was inserted into the thyroid nodule with a rapid, controlled motion to obtain cellular material. Multiple passes were often performed from different angles to ensure representative sampling. After aspiration, the obtained material was expelled onto glass slides, and immediate smears were prepared. Few slides were fixed in 95% ethanol and remaining were air dried and stained with Papanicolaou and Giemsa stains respectively. Experienced cytopathologists, based on the patients clinical and sonographic information, independently reviewed the cytology slides.

The Bethesda System categories, including "non-diagnostic," "benign," "atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS)," "follicular neoplasm/suspicious for follicular neoplasm (FN/SFN)," "suspicious for malignancy (SFM)," and "malignant," were applied according to the TBSRTC guidelines.

**Histopathologic Correlation:** Histopathology slides were retrieved from the pathology archives and reviewed. The histopathologic diagnoses were classified into benign, malignant [sub classified], or other specific entities.

The correlation between cytologic and histopathologic diagnoses was analyzed, and cases with discordant results were further investigated.

**Statistical Analysis:** Descriptive statistics, including frequencies and percentages, were employed to characterize the distribution of Bethesda System categories and histopathologic outcomes. Concordance rates between cytologic and histopathologic diagnoses were calculated. Statistical analyses were performed using SPSS version 18.0, with p-values less than 0.001 considered statistically significant.

**Ethical Considerations:** The study protocol was approved by the Institutional Review Board (IRB). Patient confidentiality was strictly maintained, and the study adhered to the principles outlined in the Declaration of Helsinki.

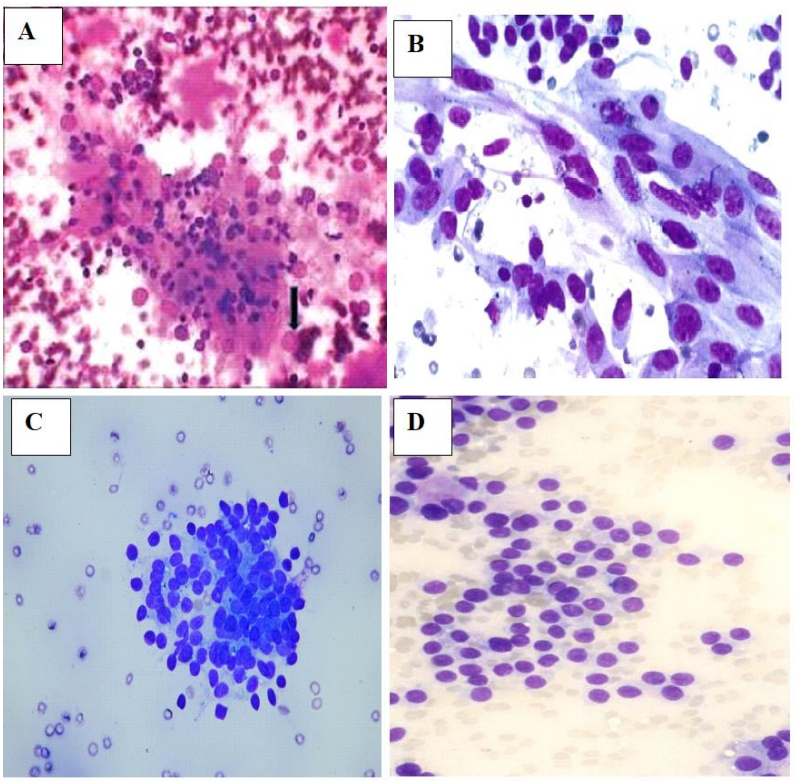


**Figure 1: A. Hashimoto's Thyroiditis (10x) Hurthle cells, few follicular cells & intermingling of lympho-cytes. B. AUS (40 x) Focal collection of cells showing atypia. C. SFN (10 x) Crowded group of follicular cells with mild pleomorphism, nuclear overlapping with a more disordered architecture. D.SFN (10 x) A follicular lesion with mild pleomorphic nuclei & coarser chromatin pattern**

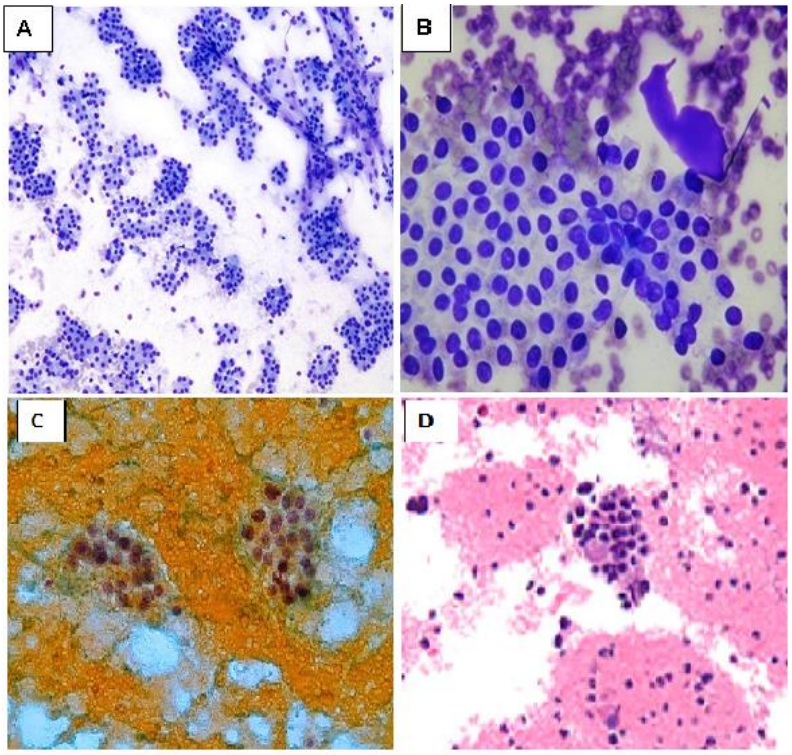


**Figure 2. A. FN (10 x) Hyper cellular smear with cells in micro follicular pattern. B. SF Malignancy (40 x) Sheets of follicular cells which appear uniform, with pseudo inclusion in one cell, flake of dense colloid on the upper right corner. C. Papillary Ca (10 x) Two groups of follicular cells with prominent nucleolus & mild pleomorphism, background of dense haemorrhage. D. Medullary Ca (10 x) Group of plasmacytoid cells, amyloid deposits**

## Results

A total of 205 patients were included in the study, comprising 26 (12.6%) males and 179 (87.4%) females (Table 1). The majority of the study population fell within the age group of 20-40 years, constituting 149 (72.7%) individuals, while 52 (25.4%) participants were aged 41-60 years. A smaller proportion of patients were either below 20 years of age (1.0%, n=2) or over 60 years of age (1.0%, n=2) (Figure3).

The mean age of the study cohort was calculated to be 37.6±17.3 years. Median age for non-neoplastic lesions was 36.1 years and for neoplastic lesions 52.5 years.

**Table 1: Gender distribution of the study subjects (N=205)**

| Gender | Frequency | % |
|---|---|---|
| Male | 26 | 12.6% |
| Female | 179 | 87.4% |

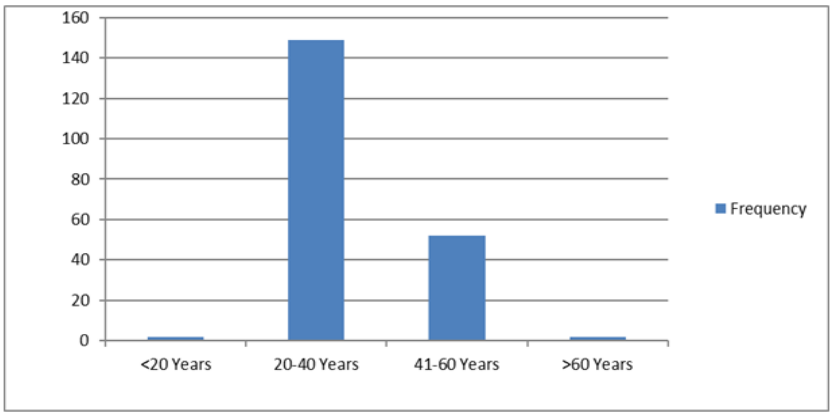


**Figure 3: Age distribution of the study subjects (N=205)**

The cytologic evaluation of thyroid lesions revealed a diverse spectrum of diagnoses within the study cohort.

The majority of cases, constituting 180 (87.8%) diagnoses, were categorized as benign. Nodular goitre was the most prevalent benign entity, identified in 101 (49.3%) cases, followed by colloid goitre in 70 (34.1%) cases. Acute thyroiditis and Hashimoto's thyroiditis were infrequently encountered, representing 1 (0.5%) and 4 (2.0%) cases, respectively. Subacute lymphocytic thyroiditis and cystic nodules were each identified in 2 (1.0%) cases.

Malignant lesions accounted for 10 (5%) diagnoses, with papillary carcinoma being the most prevalent malignancy observed (4.4%) cases. Additionally, a total of 2 (1.0%) cases were categorized as Atypia of Undetermined Significance (AUS), 3 (1.5%) as Suspicious for Follicular Neoplasm (SFN), and 1 (0.5%) as Suspicious for Malignancy. Two (1.0%) cases were non-diagnostic (Table 2).

**Table 2: Distribution of cases according to cytologic diagnosis (N=205)**

| Diagnosis | Frequency | % |
|---|---|---|
| Non diagnostic | 2 | 1.0 |
| Benign | 180 | 87.8 |
| Nodular Goitre | 101 | 49.3 |
| Colloid Goitre | 70 | 34.1 |
| Acute Thyroiditis | 01 | 0.5 |
| Hashimoto's Thyroiditis | 4 | 02 |
| Subacute lymphocytic Thyroiditis | 02 | 1 |
| Cystic Nodule | 2 | 1 |
| AUS/FLUS | 2 | 1 |
| SFN | 3 | 1.5 |
| FN | 7 | 3.4 |
| SFM | 1 | 0.5 |
| Malignancy | | |
| Medullary | 9 | 4.4 |
| Papillary | 1 | 0.5 |

Correlation between cytologic and final HPE Diagnoses (Follow-up histology could be pursued for 143 cases). All cases (n=122) categorized as benign were truly benign on HPE except one (n=1) benign case turned out to be follicular adenoma with Multinodular Goiter on histology. All cases (n=2) of AUS on cytology were truly reparative changes on histology. A case (n=1) of Suspicious for Follicular Neoplasm was diagnosed as Follicular Hyperplasia on histology. Another case (n=1) of suspicious for follicular neoplasm turned out to be Adenomatoid nodule on histologic examination.

Follicular Neoplasms cannot be sub-classified as Follicular Adenoma or Follicular carcinoma on histology since vascular and or capsular invasion to be identified which cannot be done on cytology. All cases (n=7) of Follicular Neoplasms diagnosed on cytology either turned out to be adenoma or carcinoma on histology. A case (n=1) of suspicious for Malignancy on cytology was diagnosed as Follicular variant of Papillary Thyroid carcinoma on histologic examination. All cases diagnosed as Papillary carcinoma (n=7) and Medullary carcinoma (n=1) on cytology were diagnosed the same on histopathologic examination (Figure 3). Sensitivity of cytology is notably high at 99.2%, indicating its strong ability to correctly identify malignancy or suspicious for malignancy (SFN) thyroid lesions, while the specificity is impressively high at 98.4%, minimizing false-negative results for benign cases. The false positive ratio is substantial at 0.02% underlining the strength of a positive cytologic result in predicting malignancy or SFN/FN. Conversely, the false negative ratio at 0.05% suggests that a negative cytologic result significantly reduces the likelihood of malignancy.

The positive predictive value of 98.3% suggests a substantial increase in the odds of malignancy when cytology indicates so. While the negative predictive value of 99.2% emphasizes the reassuring reduction in the odds of malignancy when cytology is negative, highlighting its strength in clinical decision making. However, it is crucial to acknowledge the challenges encountered particularly in a subset of cases initially classified as SFN on cytologic findings and necessitates exploration into refining the criteria for this category. The overall accuracy of cytologic evaluation is commendable at 97.20%, affirming its effectiveness as a highly reliable diagnostic tool for thyroid lesions (Figure 3).

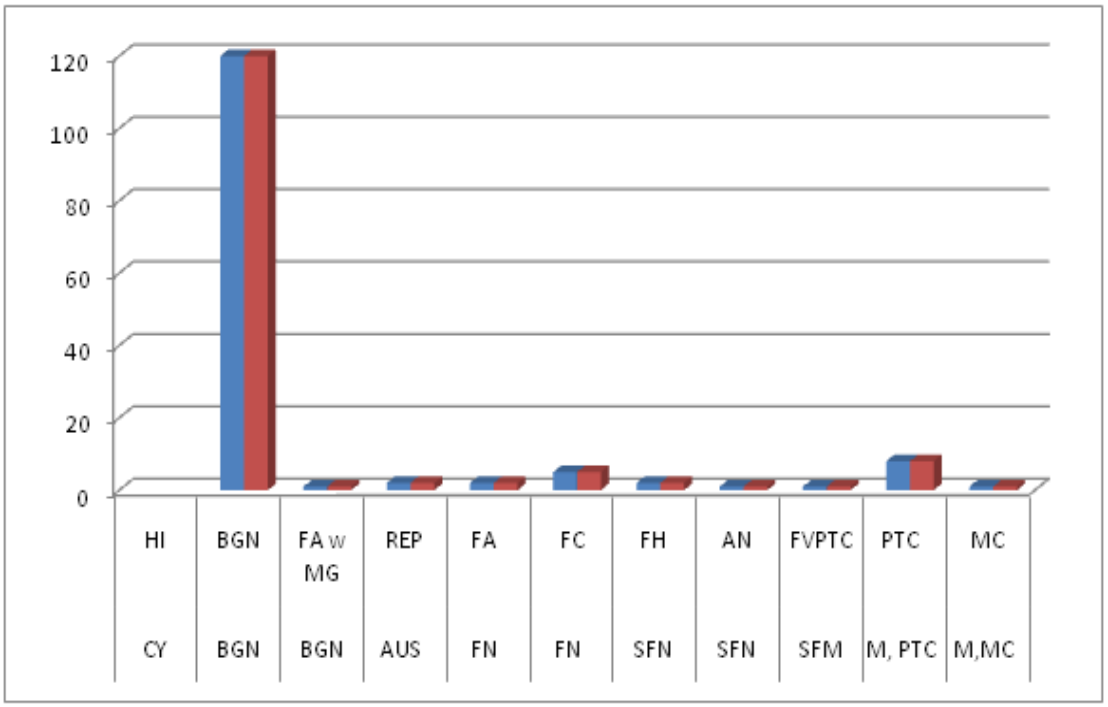


**Figure 4: The distribution of cytopathologic diagnoses alongside their corresponding final histopathologic examination (HPE) outcomes for a subset of cases with available follow-up histology (n=143)**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| TN | FN | TP | TP | TP | FP | FP | TP | TP | TP |

## Discussion

Thyroid lesions present a common clinical challenge, necessitating accurate diagnostic approaches to guide optimal patient management. Our study delves into the comprehensive cytologic evaluation of thyroid nodules using the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), coupled with subsequent histopathologic correlation.

FNA is awell-established outpatient procedure used in the primary diagnosis of thyroid lesions. It should be used as an initial diagnostic test because

of its superior diagnostic reliability and cost effectiveness, before both thyroid scintigraphy and ultrasonography (American Thyroid Association and National Comprehensive Cancer Network) [1]. Thyroid FNA studies was first developed in the 1950s in Sweden in the Radium helmet Hospital of Stockholm[2].

The demographic distribution in our cohort reveals a predominance of females, consistent with the higher prevalence of thyroid disorders in women as shown in the studies by Kapila et al., Doddi et al., and Bagga et al., [10,11,12]. The age distribution highlights a peak incidence in the 20-40 years age group, aligning with previous epidemiological trends.

The prevalence of benign lesions, particularly nodular and colloid goiters, underscores the importance of distinguishing these from potentially malignant entities, which was similar to the studies by Al Dawish et al., Chan et al., Sekhri et al., Sharma et al., Borgetet al., and Sinna et al., [13-18]. Ultrasound guidance was sought in 12% of the cases reasons being: Inadequacy of samples due to i. Minimal/mild swellingsii. sclerotic or calcified lesions. iii. very few atypical cells iv. mainly blood/haemorrhagic swellings [5,6].

Benign lesions comprised the bulk. Among the malignant cases Papillary Carcinoma was the most common and Medullary Carcinoma the least; similar to other studies [3,4,7,8,9]. FN/SFN formed a gray area giving an array of final diagnoses from Follicular hyperplasia, Adenomatoid nodules of Multinodulargoitre, Follicular Adenoma to Follicular Carcinoma as in other studies [7,8,9].

AUS/FLUS also constituted cases ranging from reparative changes to neoplasm (follicular adenoma) associated with Multinodular Goitre [8,9]. One case which was included under suspicious for malignancy---nuclear features of papillary carcinoma were subtle and focal; final diagnosis was a Follicular Variant of Papillary Thyroid Carcinoma (FVPTC) [2,14].

Hurthle cell neoplasms, Cystic papillary Carcinomas, Lymphomas, Metastatic malignancies were not seen in our study unlike few other studies [2,14,15].

Chan JKC proposed stringent criteria for the diagnosis of FVPTC. Major features: 1.oval rather than round nuclei, 2.Crowding of nuclei with lack of polarity in follicles, 3.Clear or pale nuclear chromatin or prominent nuclear grooves, 4.Presence of psammoma bodies. Minor features: a. presence of abortive papillae, b. elongated or irregularly shaped follicles, c.dark staining colloid, d. presence of nuclear pseudoinclusions [14].

FNAC of thyroid lesions is reported to have a sensitivity range of 65-98%, specificity of 72-100%, accuracy approaching to 95% in the differentiation of benign nodules from malignant ones in few other studies [19,20,21].

This study showed statistical measures which were almost consistent with other studies by Kumar et al., Layfield et al., Leonard etal., [19,22,23]. These likelihood ratios further highlight the diagnostic strength of cytology in clinical decision-making as suggested in the studies by Patel et al., Choden et al., and Erkinuresin et al., [24-26]. Pitfalls in Needle Aspiration Cytology of the thyroid (Shaha et al., [27]). Adequacy of specimens (quantitative and qualitative), accuracy of specimens (non-homogeneity of needle placement), accuracy of cytopathological interpretation, cysts (difficulties with degenerative nodules), follicular lesions (benign versus [vs] malignant). Hurthle cell lesions (benign vs. malignant), and Lymphocytic lesions (Lymphocytic thyroiditis vs. Lymphoma).

**Limitations**

The study has certain limitations that warrant consideration. Firstly, complete clinical information was not uniformly available for all cases, potentially introducing confounding variables and limiting the depth of analysis.

Additionally, the subset of cases with follow-up histology, crucial for correlation, was relatively small, impacting the generalizability of the findings. The study's reliance on a specific timeframe and potential changes in diagnostic criteria over time may also influence the interpretation of results.

Furthermore, the inherent subjectivity in cytologic interpretation and potential interobserver variability, especially in borderline categories, should be acknowledged. External validation in diverse clinical settings and a larger sample size with more extensive follow-up could enhance the robustness and applicability of the study outcomes.

**Conclusion**

FNAC plays an essential role in the evaluation of thyroid lesions. The Bethesda System is very useful, and standardised system of reporting thyroid cytopathology. Facilitates improved communication between cytopathologists and clinicians.

Uniform system for inter-laboratory agreement, leading to more consistent management approaches. Without a uniform system descriptions and diagnoses offered can be vague and the complicated jargon of pathologic terminologies used can be confusing and of no clinical significance to the clinicians. However, a prospective study over a larger population would lend more insight into the merits and demerits of the proposed system.

In addition to the Cytopathologist being aware of potential diagnostic pitfalls, and interpretational

errors that can be reduced further utilizing this system; correlation of cytopathological and histopathological diagnoses is an important quality assurance method. Use of immunocytochemical and molecular markers are to be embraced and facilitated. But the understanding of the molecular pathways of thyroid cancer biology will need to advance substantially before molecular testing can be used with thyroid FNAs. The high concordance rates, along with robust sensitivity and specificity values highlight the reliability of cytologic assessments. This research not only informs current clinical practice but also lays the groundwork for future advancements in the cytologic evaluation of thyroid lesions.

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