

Diagnostic Challenges in Indeterminate and Suspicious Thyroid Cytology: A Case Series From A Tertiary Care Center

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Abstract

Background: Thyroid nodules are common, with most being benign; however, indeterminate lesions pose diagnostic challenges due to overlapping cytomorphology.

Aim: To describe the cytomorphological spectrum of thyroid lesions with emphasis on indeterminate categories.

Materials and Methods: A retrospective case series of 31 thyroid FNAC cases (January–December 2025) was analyzed. Smears were stained with May–Grünwald–Giemsa and Hematoxylin and Eosin and categorized using TBSRTC (3rd edition, 2023).

Results: Patients ranged from 16–66 years with female predominance (3.3:1). Most presented with neck swelling; one was an incidental PET-CT finding. Distribution was: Category II (61.2%), III (12.9%), IV (19.3%), V (3.2%), and VI (3.2%). Category III showed nuclear atypia with variable background; Category IV showed microfollicular pattern with scant colloid; Category V/VI showed features of papillary carcinoma. Overlapping features were common.

Conclusion: FNAC is valuable, but indeterminate categories remain challenging. Clinikoradiological correlation and adjunctive methods are essential for accurate diagnosis and management.

Keywords: Thyroid cytopathology; Cytomorphological spectrum; Fine-needle aspiration cytology; Bethesda classification; Indeterminate thyroid lesions; Atypia of undetermined significance; Follicular-patterned lesions; Cytological diagnostic pitfalls; Papillary thyroid carcinoma;

Introduction

BACKGROUND:

Thyroid lesions are commonly encountered in clinical practice, particularly in the adult population. The majority of these lesions are benign (90–92%). While most cases are straightforward, a subset of lesions especially those falling into indeterminate categories pose significant diagnostic challenges. In our institution, we encountered a series of uncommon and diagnostically challenging thyroid lesions across different cytological categories. These cases highlight the complexity and potential pitfalls associated with thyroid cytopathology, particularly in lesions categorized as indeterminate or suspicious for malignancy.

Fine needle aspiration (FNA) remains the first-line diagnostic modality for evaluating thyroid nodules due to its safety, reliability, and high positive predictive value for malignancy. The adoption of standardized reporting systems, particularly The Bethesda System for Reporting Thyroid Cytopathology, has significantly improved diagnostic consistency and clinical management by correlating cytological findings with the risk of malignancy.

The third edition of TBSRTC (2023) further refines the classification of indeterminate lesions into atypia of undetermined significance (AUS), follicular neoplasm (FN), and suspicious for malignancy (SFM). Despite the high sensitivity, specificity, and predictive value of thyroid FNAC, diagnostic dilemmas persist, largely due to overlapping cytomorphological features and variable background findings.

Through this case series, we aim to present rare and diagnostically challenging thyroid lesions encountered in our institution, highlighting the cytomorphological features, potential pitfalls, and the importance of careful interpretation within the framework of TBSRTC. These cases underscore the need for awareness of uncommon presentations to avoid misdiagnosis and ensure appropriate clinical management.

AIM:

To describe the cytomorphological spectrum of thyroid lesions encountered at our institution.

MATERIALS AND METHODS:

Study Design & Settings: This was a retrospective descriptive case series conducted in the Department of Pathology at a Tertiary Care Center. The study includes thyroid fine needle aspiration cytology (FNAC) cases evaluated from January 2025 to December 2025.

Cases: A total of 31 Thyroid FNAC cases were included in the study. Of these, 13 cases categorized under indeterminate and suspicious categories—namely Bethesda categories III (Atypia of Undetermined Significance), IV (Follicular Neoplasm), and V (Suspicious for Malignancy). The remaining cases were Category I: Non diagnostic & Category II: Benign.

Inclusion and Exclusion Criteria: All patients who underwent FNAC for thyroid lesions during the study period were included. Cases with inadequate smears (Bethesda Category I) and Benign cases (Bethesda Category II) were excluded.

Table 1: Cytology of Thyroid Lesions

Case	Age	Sex	Clinical Presentation	Key Cytological Feature	Bethesda Category
1	52	Female	Swelling in the neck for past 10 years.	Moderate cellularity with nuclear crowding & nuclear inclusions in a background of scant colloid & haemorrhage.	3
3	45	Female	Swelling in the neck for past 6 months. History of pain present.	Moderate cellularity with follicular cells surrounded by lymphocytes. Some follicular cells show nuclear inclusions in a background of colloid & haemorrhage	3
4	45	Male	History of swelling in neck for past 6 months.	Moderate cellularity with cells showing nuclear crowding & few cells showing nuclear inclusions with plenty of cyst macrophages seen in a background of hemorrhage and colloid.	3
5	66	Male	History of swelling in neck for past 2 weeks.	Moderate cellularity with cells showing nuclear crowding & few cells showing nuclear inclusions with plenty of cyst macrophages seen in a background of hemorrhage and colloid.	3
2	49	Female	Swelling in the neck for past few months. History of pain present.	High cellularity with numerous, monotonous, repetitive follicular cells arranged in microfollicular pattern in a background contains scant colloid & hemorrhage	4
6	43	Female	History of swelling in neck for past 3 months.	High cellularity with thyroid follicular cells predominantly in microfollicular pattern in a background of scant colloid & haemorrhage	4
7	16	Female	History of swelling in neck for past 3 months.	Moderate cellularity with thyroid follicular cells predominantly in microfollicular pattern in a background of scant colloid & haemorrhage	4
9	22	Female	History of swelling in neck for past 1 year.	Moderate cellularity with thyroid follicular cells predominantly in microfollicular pattern in a background of scant colloid & haemorrhage	4
10	42	Female	History of swelling in neck for past 3 months.	Moderate cellularity with predominantly Hurtle cells (more than 75%) seen in a background of scant colloid & haemorrhage	4
8	52	Female	USG guided FNA for incidental hyper metabolic thyroid nodule on PET CT	High cellularity with thyroid follicular epithelial cells in singles and sheets, showing nuclear inclusions in a background of scant colloid & hemorrhage.	5
11	52	Female	History of swelling in neck for past 4 months.	High cellularity with thyroid follicular epithelial cells in singles and sheets with thyroid follicular cells showing enlarged nuclei with nuclear clearing, intranuclear cytoplasmic inclusions, overlapping and crowding in a background of scant colloid & haemorrhage.	6

Procedure: Fine needle aspiration of thyroid nodules was performed using a 23 gauge needle under palpation or ultrasound guidance, depending on the clinical indication. Multiple passes were made when required to obtain adequate cellular material.

Cytological Processing and Staining: The aspirated material was smeared onto clean glass slides. Air-dried smears were stained with May–Grünwald–Giemsa (MGG), and alcohol-fixed smears were stained with Hematoxylin and Eosin (H&E). All slides were examined under light microscopy.

Cytological Evaluation: All cases were reported according to The Bethesda System for Reporting Thyroid Cytopathology (3rd edition, 2023). Cytomorphological features assessed included cellularity, architectural patterns, nuclear features, and background characteristics.

Data Collection and Analysis: Clinical details, including age, sex, and presenting complaints, were retrieved from medical records. Cytological findings and Bethesda categorization were documented and analyzed descriptively.

CASE DISCUSSION:

Thyroid nodules are highly prevalent in the general population, with a higher incidence among females and increasing detection due to widespread use of imaging modalities. In the present study, we analyzed 13 thyroid fine needle aspiration cytology (FNAC) cases categorized under indeterminate and suspicious groups, namely Bethesda categories III, IV, and V. The majority of patients were female, and most presented with a palpable neck swelling, consistent with established epidemiological trends [1].

The application of The Bethesda System for Reporting Thyroid Cytopathology has significantly improved the standardization of thyroid cytology reporting by correlating cytological findings with the risk of malignancy (ROM) and guiding clinical management. According to the 3rd edition of TBSRTC (2023), the ROM for Category III (Atypia of Undetermined Significance, AUS) is estimated at approximately 10–30%, Category IV (Follicular Neoplasm) at 25–40%, and Category V (Suspicious for Malignancy) at 50–75%, depending on institutional data and application of molecular testing [2,3].

In our series, Category III (AUS) constituted a substantial proportion of cases. These cases demonstrated cytological features such as nuclear crowding, occasional nuclear inclusions, and variable colloid background. Such features represent a well-recognized diagnostic gray zone, where reactive, inflammatory, and neoplastic processes may overlap. The presence of cyst macrophages, hemorrhagic background, and lymphocytic infiltration in several cases further complicated interpretation, mimicking papillary carcinoma-like nuclear changes. These overlapping features are a known source of interobserver variability and contribute to the heterogeneity of ROM reported in literature [4].

Category IV lesions in our study were predominantly characterized by high cellularity and a microfollicular architectural pattern in a background of scant colloid. These findings are typical of follicular-patterned lesions, where cytology is inherently limited in differentiating follicular adenoma from follicular carcinoma due to the inability to assess capsular and vascular invasion. The repeated observation of monotonous follicular cells arranged in microfollicles across multiple cases highlights the importance of architectural patterns as a key diagnostic criterion in this category [2,5].

Category V (suspicious for malignancy) cases demonstrated cytological features suggestive of papillary thyroid carcinoma, including nuclear enlargement, chromatin clearing, nuclear overlapping, and intranuclear cytoplasmic inclusions. However, the absence of unequivocal malignant features precluded definitive categorization as malignant (Category VI). This underscores the subjective nature of cytological interpretation and the fine diagnostic line separating Categories III, V, and VI [3,6].

An important highlight of our series is the identification of an incidental hypermetabolic thyroid nodule detected on positron emission tomography–computed tomography (PET-CT) during follow-up for a non-thyroid malignancy. With increasing use of advanced imaging, such incidentalomas are being detected more frequently and often fall into indeterminate cytological categories, posing additional diagnostic and management challenges [7].

A major limitation in thyroid cytology, as reflected in our study, is the significant overlap in cytomorphological features across diagnostic categories. Nuclear atypia, including grooves and inclusions, may be observed in both benign and malignant conditions. Similarly, microfollicular architecture can be seen in hyperplastic nodules as well as follicular neoplasms. Background elements such as hemorrhage, cystic change, and inflammation further obscure interpretation. These factors contribute to potential false-positive and false-negative diagnoses, emphasizing the importance of cautious evaluation [4,8].

Our findings are consistent with existing literature, which highlights the inherent limitations of FNAC in indeterminate thyroid lesions and the need for clinicoradiological correlation. The integration of repeat FNAC, molecular testing, and histopathological follow-up plays a crucial role in improving diagnostic accuracy and patient outcomes [2,6].

In conclusion, this case series underscores the diagnostic challenges associated with Bethesda categories III, IV, and V thyroid lesions. Awareness of cytomorphological overlaps and potential pitfalls, combined with a standardized reporting system and multidisciplinary approach, is essential for accurate diagnosis and optimal patient management.

RESULTS

A total of 31 thyroid fine needle aspiration cytology (FNAC) cases were performed & prominent cases are categorized under Bethesda categories III, IV, V and VI were included in the study. The age of patients ranged from 16 to 66 years, with a mean age in the fifth decade. The majority of patients were female with a female-to-male ratio of approximately 3.3:1. Most patients presented with a history of neck swelling of varying duration. One case was detected incidentally as a hypermetabolic thyroid nodule on positron emission tomography-computed tomography (PET-CT) during follow-up for a prior malignancy.

Based on cytological evaluation using The Bethesda System for Reporting Thyroid Cytopathology:

- Category II (Benign): 19 cases (61.2%)
- Category III (AUS): 4 cases (12.9%)
- Category IV (Follicular Neoplasm): 6 cases (19.3%)
- Category V (Suspicious for Malignancy): 1 case (3.2%)
- Category VI (Malignant): 1 case (3.2%)

Cytomorphological Features

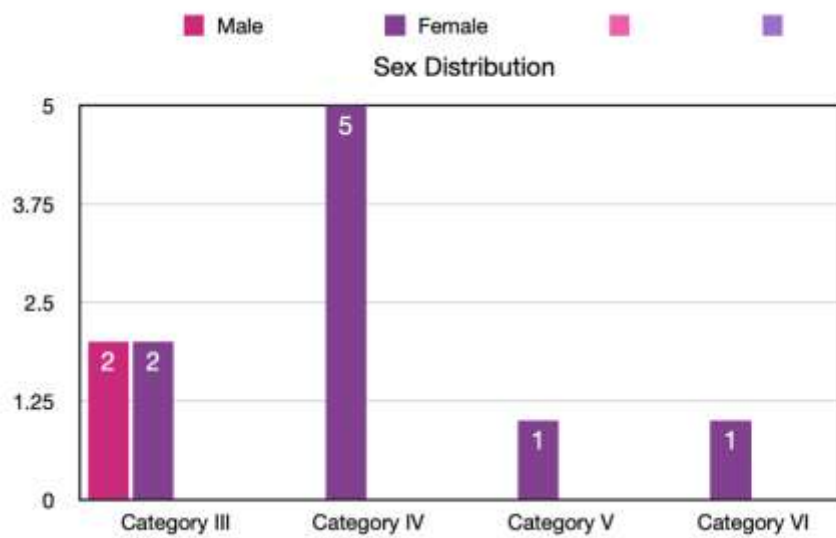
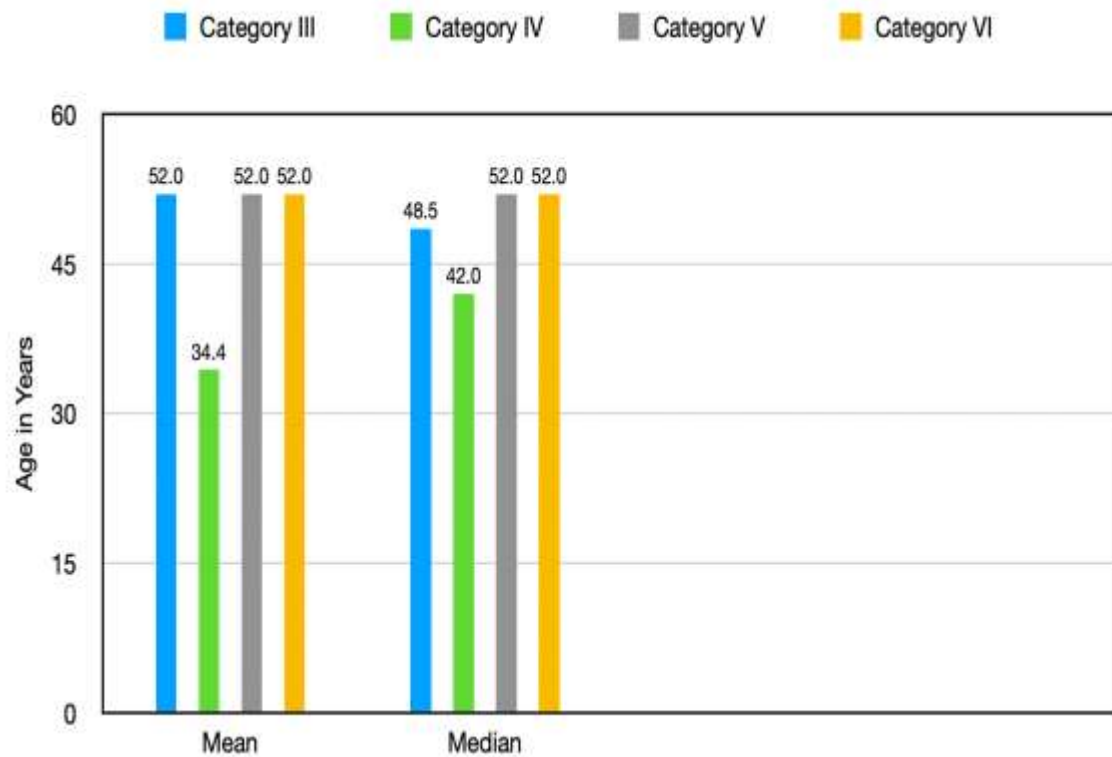
Category III (AUS): Showed moderate cellularity with nuclear crowding, occasional nuclear inclusions, and variable colloid background. Some cases demonstrated cyst macrophages, hemorrhage, and lymphocytic infiltration.

Category IV (Follicular Neoplasm): Characterized by high cellularity with predominantly microfollicular arrangements of follicular cells in a background of scant colloid and hemorrhage.

Category V (Suspicious for Malignancy): Showed high cellularity with follicular epithelial cells exhibiting nuclear enlargement, chromatin clearing, nuclear overlapping, and intranuclear inclusions.

Category VI (Malignant): Revealed classical cytological features of papillary thyroid carcinoma, including nuclear clearing, grooves, intranuclear inclusions, and crowding.

Summary of Findings: Microfollicular architecture was the most common pattern observed in Category IV lesions, while nuclear atypia and inclusions were frequently noted in Category III and V lesions. Overlapping cytological features between categories were noted in multiple cases.



Figures

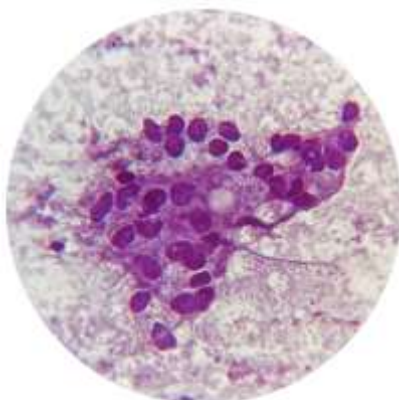


Figure 1: Thyroid follicular cells seen with few nuclei showing nuclear inclusions, TBSRTC Category III. X40 magnification

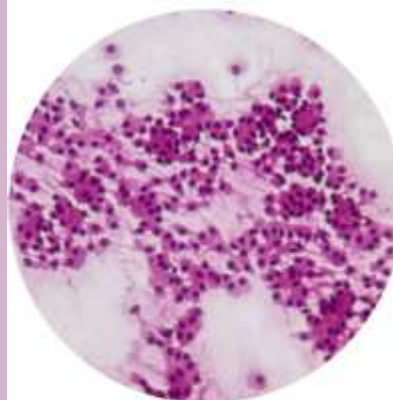


Figure 2: Thyroid Follicular cells seen in repetitive microfollicular pattern. TBSRTC Category IV. X10 magnification

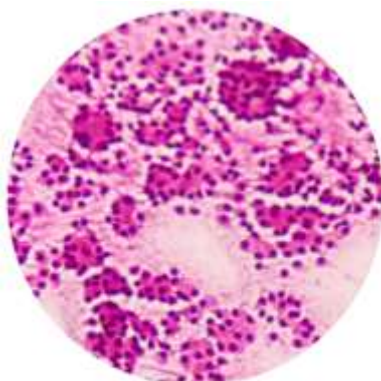


Figure 3: Thyroid Follicular cells seen in repetitive microfollicular pattern. TBSRTC Category IV. X10 magnification

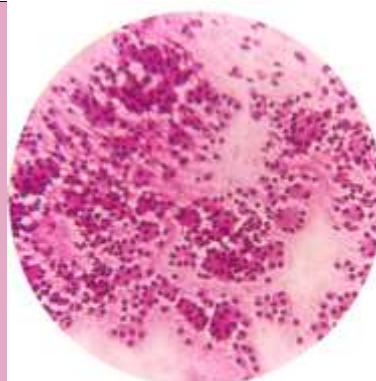


Figure 4: Thyroid Follicular cells seen in repetitive microfollicular pattern. TBSRTC Category IV. X10 magnification

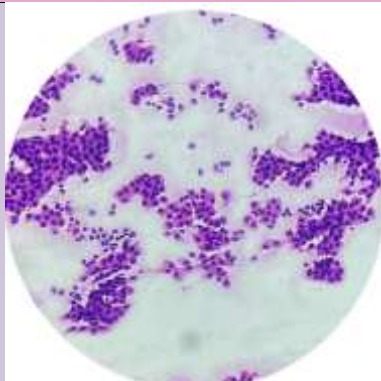


Figure 5: Hurtle cell neoplasm composed of >75% Hurtle cells. TBSRTC Category IV. X10 magnification

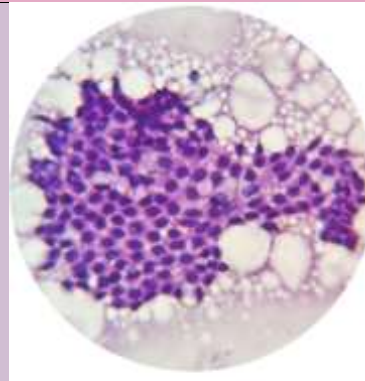
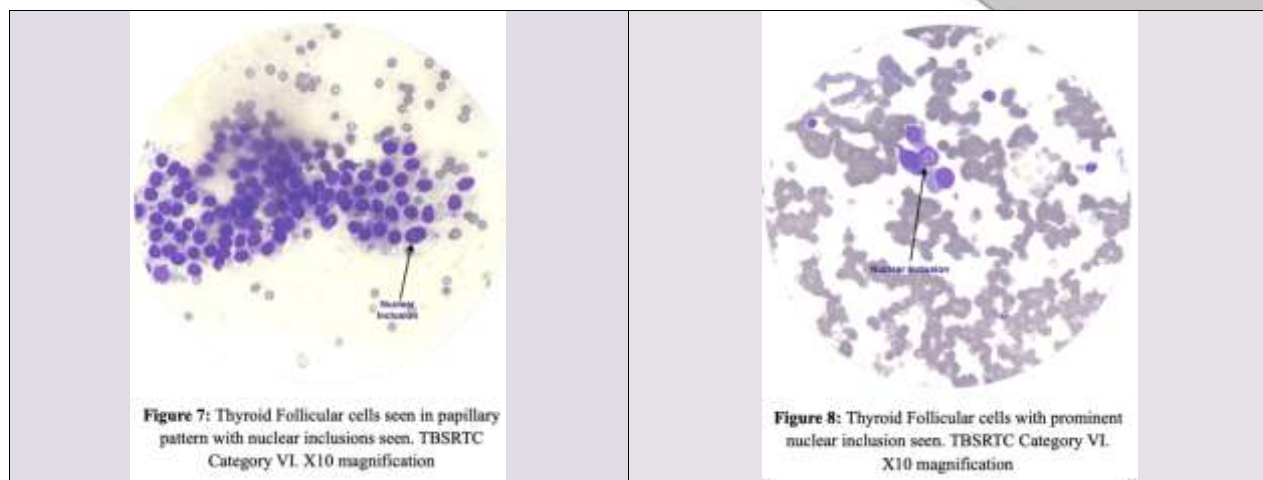


Figure 6: Thyroid Follicular cells seen in papillary pattern with nuclear inclusions seen. TBSRTC Category V. X40 magnification

Figures



CONCLUSION

This case series highlights the diagnostic challenges encountered in thyroid fine needle aspiration cytology, particularly within Bethesda categories III, IV, and V. A significant proportion of cases demonstrated overlapping cytomorphological features, including nuclear atypia, microfollicular architecture, and variable background elements, which contributed to diagnostic ambiguity.

The application of The Bethesda System for Reporting Thyroid Cytopathology provides a standardized framework for risk stratification and clinical management; however, limitations persist, especially in indeterminate categories. Careful cytomorphological assessment, combined with clinical and radiological correlation, remains essential to minimize diagnostic errors.

The presence of incidental thyroid nodules detected on advanced imaging further underscores the evolving diagnostic landscape and the increasing burden of indeterminate lesions. Awareness of potential pitfalls and adherence to a multidisciplinary approach are crucial for improving diagnostic accuracy and guiding appropriate patient management. Further studies with histopathological correlation and adjunctive molecular testing are recommended to enhance diagnostic precision in indeterminate thyroid lesions.

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