ORIGINAL ARTICLE

Cytomorphological Findings of Thyroid Lesions Based on the Bethesda System and Their Relation with Thyroid Hormonal Status: A Cross-Sectional Study

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Abstract

Introduction: Swelling of the thyroid gland is a common presentation of thyroid diseases, both benign and malignant, and some are associated with thyroid dysfunction. Fine needle aspiration cytology, along with ultrasound and assessment of thyroid function status, remains the mainstay of evaluation of thyroid swellings. This study aimed to classify thyroid lesions according to the Bethesda system and to assess their relationship with thyroid hormonal status.

Methods: A cross-sectional study was conducted prospectively from April 2023 to April 2024 in a tertiary care center after ethical clearance from the Institutional Review Committee (Reference number: 774). Patients with thyroid lesions were included using convenience sampling, with a sample size of 141. Fine needle aspiration cytology was performed for lesion classification. Data entry and analysis were done using Microsoft Excel and Statistical Package for the Social Sciences version 20.

Results: Among 141 cases, the most common thyroid lesion diagnosed on fine needle aspiration cytology was benign, comprising 87 (61.70%), with benign follicular nodule accounting for 63 (44.70%) among the benign lesions. Among all, 99 (70.23%) patients presented with euthyroid status, and no statistical correlation between thyroid hormone status and benign versus malignant lesions or Bethesda classification was seen in this study. In total, 120 (85.11%) were female. The age group ranged from 18 to 87 years, and the mean age at presentation was 54.8 years.

Conclusions: In this study most thyroid lesions fell into the Bethesda category II. Thus, fine needle aspiration cytology is an important diagnostic tool for thyroid lesions. However, thyroid hormone status and benign versus malignant lesions showed no statistical correlation.

Keywords: *bethesda system; fine needle aspiration cytology; thyroid function test.*

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Introduction

Thyroid gland swelling can occur in both benign and malignant conditions, with some cases presenting with thyroid dysfunction. In adults, the prevalence of thyroid swelling ranges from 4% to 7%, and in children from 0.20% to 1.80%.[1] Lesions of the thyroid can be categorized in hypothyroid (including subclinical hypothyroidism), euthyroid, or hyperthyroid conditions based on the assessment of the level of free triiodothyronine (fT3), free thyroxine (fT4), and Thyroid Stimulating Hormone (TSH).[2]

Fine needle aspiration cytology (FNAC) is a simple, cost-effective, minimally invasive, and quick-to-perform outpatient screening procedure in the diagnosis of diffuse and solitary thyroid nodules.[3] The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) is a uniform, tiered reporting system for thyroid specimens. FNAC, along with ultrasound and assessment of thyroid function status, remains the mainstay of evaluation of thyroid swellings.[4]

This study aimed to classify thyroid lesions according to the Bethesda system and to assess their relationship with thyroid hormonal status.

Methods

This was a hospital based cross-sectional study conducted prospectively in the Department of Pathology, Shree Birendra Hospital, Chhauni, from April 2023 to April 2024, after obtaining ethical clearance (Reg. No: 774) from the Institutional Review Committee. Convenience sampling was done for patients with thyroid lumps presenting to the ENT Outpatient Department (OPD) and referred for FNAC/Ultrasound-guided FNAC to the Pathology/ Radiology Departments of SBH. Inclusion criteria was all thyroid FNAC samples aged above 18 years during the data collection period. Exclusion criteria were repetitive thyroid FNAs of the same individual and patients on thyroid medications.

Informed and written consent was taken from all subject participants involved in this study. Relevant clinical data, including age, sex, site, size, duration, and radiological findings of thyroid swelling, along with the drug history of thyroid medication, were recorded in the proforma. All patients' TFT (fT3, fT4, TSH) values were also retrieved from the Health Management Information System (HMIS) of SBH or from the patients' printed reports after blood sampling into a serum separating tube. The iFlash 1800 Chemiluminescence Immunoassay Analyzer (CLIA) was used to perform TFT in SBH. TFT reference range in SBH was TSH- 0.3-5 µIU/ml, Free T3- 2.3-4.2 pg/ml, Free T4- 0.9-1.68 ng/dl.

The latest 3rd edition of TBSRTC, 2023, recommends six diagnostic categories as follows.[5,6]

(I) Nondiagnostic

(II) Benign

(III) Atypia of Undetermined Significance (AUS)

(IV) Follicular Neoplasm (FN)

(V) Suspicious For Malignancy (SFM)

(VI) Malignant

Among the six categories of TBSRTC, category II (Benign) is considered as non-neoplastic lesion and remaining Bethesda category III (AUS),7 IV (FN), V (SFM) and VI (Malignant) are considered as neoplastic lesions while Bethesda category I (Nondiagnostic) is not included in any category as it does not give any diagnosis.

All the thyroid FNACs were performed following standard procedure under aseptic precautions. Palpable thyroid lesions based on size, site, number, and consistency were evaluated. Overlying skin was cleansed using a spirit swab, and the lesion was fixed between two fingers. FNAC was performed with a 23-gauge needle using a non-aspirating technique and attached to a 10 ml syringe when USG-guided. The needle was carefully moved to and fro in all directions of the suspected lesion, and after the needle was withdrawn, the nature of the aspirated material was noted. Content was applied to clean, grease-free glass slides and smeared by applying gentle pressure with a second clean glass slide, moving the upper slide over the lower one to spread the material. At least one air-dried smear for Giemsa stain and one wet smear for Papanicolaou (PAP) stain were prepared. The air-dried smear was then stained with Giemsa, and the wet smear was fixed in 95% ethanol for 15-30 minutes and then stained with PAP. After staining, the slides were mounted with Dibutylphthalate Polystyrene Xylene (DPX) and were examined under the microscope for cytopathological diagnosis. Smears were evaluated by Pathologists and categorized according to TBSRTC.

Cytomorphological details, FNAC diagnosis, and TFT details were entered into Microsoft Excel, and Statistical Package for Social Sciences (SPSS) version 20 was used for statistical analysis. Categorical variables were expressed as percentages and presented in tables wherever possible, and the chi-square test was used to compare qualitative variables. For categories with frequency <5, Fisher's exact test was applied. A p-value of <0.05 was considered significant.

Results

Out of 141 cases, female population was 120 (85.11%)

and male was 21 (14.89%). The female-to-male ratio was 5.7:1. The mean age of the study population was 54.80 years, with an age range spanning from 18 to 87 years. The most affected age group was 51-60 years, 42 (29.80%) followed by 61-70 years. 36 (25.53%), while the least number of cases, 3 (2.13%), were found in individuals aged 18-30 years and those over 80 years each.

Table 1: Distribution of thyroid lesions based on new Bethesda System (n=141).

Bethesda Category		Number of Cases	Percent
I	Nondiagnostic	19	13.47
II	Benign	87	61.70
III	AUS	23	16.32
IV	FN	1	0.70
V	SFM	6	4.26
VI	Malignant	5	3.55
Total		141	100

Based on the diagnosis, most of the lesions on FNA were classified as BFN 63 (44.70%) followed by Lymphocytic thyroiditis (LT) with 14 (9.94%) in the Benign category. The second most common finding was AUS, with AUS-nuclear atypia comprising 14 (9.94%) cases and AUS-O comprising 9 (6.40%) cases. The remaining categories were FN 1 (0.70%), Papillary Thyroid Carcinoma (PTC) 3 (2.13%), and Medullary Thyroid Carcinoma (MTC) 1 (0.70%). (Table 2)

Benign lesions comprised 87 (61.70%), while malignant cases totaled 35 (24.83%), and 19 (13.47%) cases were nondiagnostic. Benign cases outnumbered malignant cases, with a benign-to-malignant ratio of 2.4:1. Based on the new 3rd edition of TBSRTC, most lesions fell into Bethesda category II, 87 (61.70%). (Table 1)

Table 2: Distribution of thyroid lesions based on diagnosis of the lesions (n=141).

Bethesda Category		Diagnosis	Number of Cases	Percentage(%)
I	Nondiagnostic		19	13.47
II	Benign	BFN	63	44.70
		LT	14	9.94
		Colloid Nodule	4	2.84
		LT with Hürthle cell changes	3	2.13
		Adenomatoid Nodule	1	0.70
		Subacute thyroiditis	1	0.70
		Thyroglossal cyst	1	0.70
III	AUS	AUS-nuclear atypia	14	9.94
		AUS-O	9	6.40
IV	FN	Follicular Neoplasm	1	0.70
V	SFM	Suspicious for PTC	5	3.55
		Suspicious for PTC with LT	1	0.70
VI	Malignant	PTC	3	2.13
		MTC	1	0.70
		Metastatic deposit in known case of carcinoma cervix	1	0.70
Total			141	100

Based on TFT, most of the lesions were euthyroid in both groups, comprising benign 61 (43.26%) and malignant 23 (16.32%) cases. No statistically significant correlation between the nature of the lesion (benign versus malignant) and thyroid hormone status indicating that thyroid function test does not necessarily predict the type of thyroid lesion. (Chi-square value: 3.91 df= 4 p=0.422)

The maximum number of lesions fell in the Euthyroid category 99 (70.23%) with most of the lesions being in the Bethesda category II (61 out of 87, 70.11%) which is statistically not significant. (Fisher's exact value: 9.175 p=0.472) (Table 3)

Table 3: Classification of Bethesda categories based on Thyroid hormone status (n=141).

Bethesda Category		Thyroid Hormone Status			Total n(%)
		Euthyroid n(%)	Hyperthyroid n(%)	Hypothyroid n(%)	
I	Nondiagnostic	15(10.64)	3(2.13)	1(0.70)	19(13.47)
II	Benign	61(43.26)	15(10.64)	11(7.80)	87(61.70)
III	AUS	15(10.64)	4(2.84)	4(2.84)	23(16.32)
IV	FN	-	-	1(0.70)	1(0.70)
V	SFM	4(2.84)	-	2(1.40)	6(4.26)
VI	Malignant	4(2.84)	-	1(0.70)	5(3.55)
Total		99(70.23)	22(15.61)	20(14.16)	141(100)

Out of 87 cases in the Bethesda category II, euthyroid cases were 61 (70.11%), hyperthyroid cases were 15 (17.24%), and hypothyroid cases were 11 (12.65%).

Among these 87 Bethesda category II cases, 63 were in the BFN category, of which 47 (74.60%) were euthyroid, 10 (15.87%) were hyperthyroid, and 6 (9.53%) were hypothyroid. (Table 4)

Table 4: Classification of Thyroid lesions based on Thyroid hormone status

	Euthyroid n(%)	Hyperthyroid n(%)	Hypothyroid n(%)	Total n(%)
Nondiagnostic	15(10.64)	3(2.13)	1(0.70)	19(13.47)
Adenomatoid Nodule	-	1(0.70)	-	1(0.70)
BFN	47(33.33)	10(7.11)	6(4.26)	63(44.70)
Colloid Nodule	3(2.13)	1(0.70)	-	4(2.84)
LT	8(5.68)	2(1.42)	4(2.84)	14(9.94)
LT with Hürthle cell changes	2(1.42)	1(0.70)	-	3(2.13)
Subacute thyroiditis	-	-	1(0.70)	1(0.70)
Thyroglossal cyst	1(0.70)	-	-	1(0.70)
AUS- Nuclear	8(5.68)	4(2.84)	2(1.42)	14(9.94)
AUS- O	7(4.98)	-	2(1.42)	9(6.40)
FN	-	-	1(0.70)	1(0.70)
Suspicious for PTC	3(2.13)	-	2(1.42)	5(3.55)
Suspicious for PTC with LT	1(0.70)	-	-	1 (0.70)
MTC	1(0.70)	-	-	1(0.70)
Metastatic Carcinoma Cervix	-	-	1(0.70)	1(0.70)
PTC	3(2.13)	-	-	3(2.13)
Total	99(70.23)	22(15.60)	20(14.17)	141 (100)

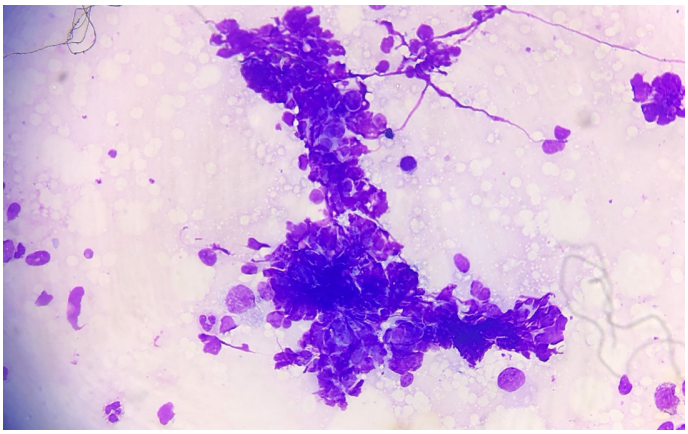

Figure 1: Metastatic deposits in thyroid FNAC (Giemsa stain, 400X)

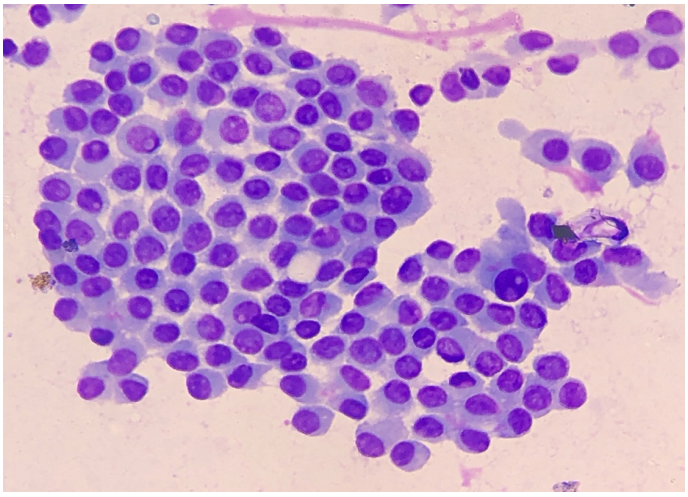

Figure 2: INCIs and nuclear grooving in PTC (Giemsa stain, 1000X)

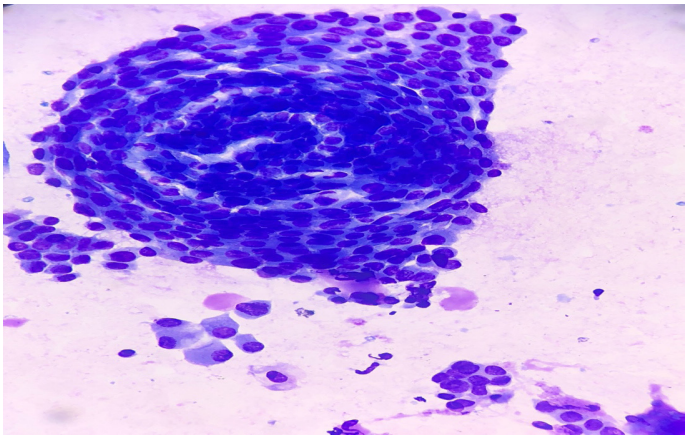

Figure 3: Swirling pattern in PTC (Giemsa stain, 400X)

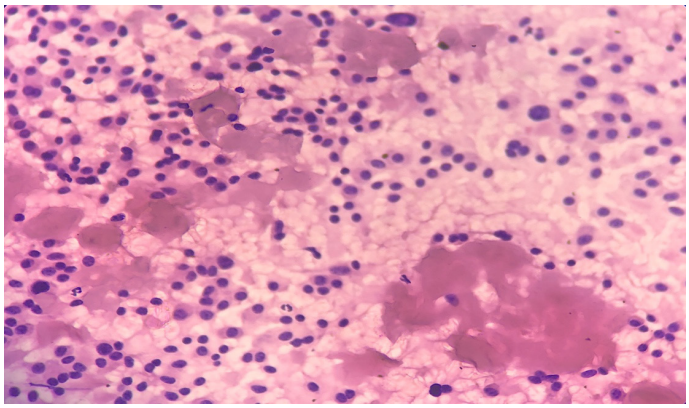

Figure 4: Hyaline deposits in MTC (PAP stain, 400X)

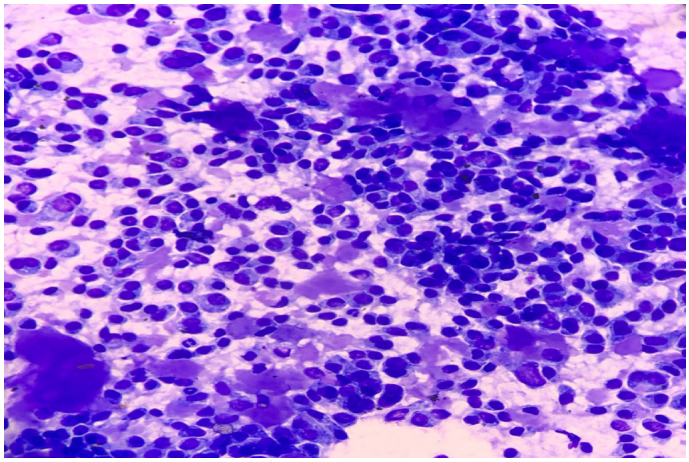

Figure 5: Plasmacytoid and binucleated cells in MTC metastasis to cervical lymph node (Giemsa stain, 1000X)

Discussion

Our result aligns with previously established data showing females with higher prevalence of thyroid disorders. The explanation of more than 2/3rd of the patients being female is likely due to the reason that autoimmune thyroid diseases are less common in males compared to females, as the latter have more hormonal imbalance, particularly estrogen, which can influence the development of thyroid pathology.[7,8] Therefore, female patients who present with thyroid disease frequently have an associated history of weight gain, menstrual irregularities, infertility, and dyspnea.[9] In this study, the age ranged from 18 to 87 years, and the mean age of presentation was 54.80 years, which was similar to studies done by Ha et al. (51.2 years) and Abdelkader et al. (43.7±11.5 years).[10,11] In studies by Jat et al. and Park et al., the mean age at presentation was 35 years and 49.8 years, respectively, which were slightly lower than in our study.[12,13] The present study observed most lesions in the age group 51-60 years, as seen in the study by Chaudhary et al.[14] Studies by Banstola et al., Bhuyar

et al., and Osman et al. showed the most common age group to be 31-40 years.[15–17] This is consistent with the incidence of thyroid nodules increasing with age, with the highest prevalence seen in elderly and middle-aged females.

In this present study, maximum lesions were found to be benign with a benign to malignant ratio of 2.4:1, similar to Banstola et al. and Park et al.[12,16] Benign to malignant ratios in studies by Bhuyar et al. (7:1), Choudhary et al. (13.4:1), Grandhi et al. (7.6:1), and Vera et al. (13.3:1) showed that the benign lesions significantly outnumbered malignant lesions, especially in middle-aged women.[17–20] Some overlapping features between benign and malignant lesions, such as pseudo-inclusions and nuclear grooves, might have led to misdiagnosis in cytopathology reporting.[16,21] Similar to previous studies by Abdelkader et al., Aydogan et al., Choudhury et al., and Kumari et al., the most common Bethesda classification of lesion in our study was Bethesda II.[10,21–23] In a study by Kumari et al., a higher prevalence of benign findings on TBSRTC could be due to the high incidence of microcarcinoma, which was difficult to diagnose with FNAC.[22] Present study revealed BFN as the most common finding, with 44.70% cases similar to other studies by Bhatta et al. (64.4%), Choudhary et al. (59.4%), Jain et al. (40.6%), Jotva et al. (28%), Karki et al. (45.3%), and Ranabhat et al. (58%), but these were the prevalence of colloid goiter which can be re-classified as BFN according to the new Bethesda classification.[4,19,24–27] This study showed the prevalence of PTC (2.13%), which was similar to studies by Choudhary et al. and Jain et al. (3.6%) and Jotva et al. (2%). Bhatta et al. (10%) and Ranabhat et al. (8%) showed slightly higher prevalence of PTC compared to this study.[4,19,24–26]

The present study showed that most patients were euthyroid (70.23%), consistent with many other studies, including Bhuyar et al. (84%), Devi et al. (70.8%), and Jain et al. (69.1%).[17,26,28] In present study, patients with lymphocytic thyroiditis (8 out of 14, 57.14%) cases were euthyroid followed by hypothyroid (4 out of 14, 28.57%) cases which is similar to the studies by Chaudhary et al. where euthyroid lesions were (7 out of 9, 77.7%) cases and in a study by Devi et al. (4 out of 7, 57.1%) were euthyroid and (2 out of 7, 28.5%) were hypothyroid.[28,29] In this study, PTC was euthyroid (3 out of 3, 100%), which is in concordance with the study by Poudel et al. (1 out of 1, 100%).[30] This shows that there is no significant difference between TFT and various FNA diagnosis indicating that thyroid hormone status alone cannot be a reliable indicator in thyroid lesions.[30] Studies showing correlation between BFN and TFT were not found, this could be a possible area to explore in the future.

Since this is a single hospital-based study, it might have caused selection bias. In this study, of the total 246 thyroid FNACs reported during the data collection period, 141 met the inclusion criteria and 105 were excluded due to repetition of FNA or patients on thyroid medications. Compared to other studies, the variation in findings could be because of sample size as in other studies ranges from 100-1200. In this study, the total AUS cases were 16.32% with an AUS: Malignant ratio of 4.6:1. While this slightly exceeds the ideal recommendations from the 2023 TBSRTC suggesting AUS cases should be <7% and the AUS: Malignant ratio should be 3:1, it is important to note that the study only included 141 cases meeting the inclusion criteria out of a total of 246 thyroid FNACs in the study duration. This selective inclusion may have resulted in a higher AUS count compared to what might have been observed if all thyroid FNAC cases had been included.

Conclusions

In this study, the classification of thyroid FNAC was done according to TBSRTC, which is the universally accepted standardized reporting system for thyroid FNA that correlates with cytomorphological criteria and Risk of Maligancy (ROM). Most thyroid FNAs fall under either “Benign” or “Malignant” entities, but still a significant percentage of thyroid FNAs are placed in the “Indeterminate category”, such as AUS or FN. Like most studies, in this study also thyroid lesions were more prevalent in females and in the age group 51-60 years, with most lesions being benign. However, no significant association was found between thyroid hormone status and TBSRTC and the diagnosis of lesions.

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