

Fine Needle Aspiration Cytology Reporting of Thyroid Lesions Using Bethesda System- with Histopathological and Ultrasonography CorrelationSandhya M.¹, Raghu S. R.², Sowmya T. S.³¹Assistant Professor, Department of Pathology, Hassan Institute of Medical Sciences, Hassan, Karnataka, India²Associate Professor, Department of Radiology, Hassan Institute of Medical Sciences, Hassan, Karnataka, India³Associate Professor, Department of Pathology, Hassan Institute of Medical Sciences, Hassan, Karnataka, India

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Corresponding Author: Dr. Raghu S. R.

Conflict of interest: Nil

Abstract:**Introduction:** Fine needle aspiration and cytology (FNAC) has been proven to be simple, barely intrusive, secure, and economical in the initial diagnosis of thyroid swellings. Along with ultrasound imaging, FNAC is incredibly helpful in differentiating malignant and benign thyroid swellings.**Aims and Objectives:** (1) To analyse the thyroid cytology smears by TBSRTC. (2) To correlate with radiological findings. (3) To correlate with corresponding histopathological findings.**Materials and Methods:** 100 patients with palpable thyroid swellings referred to the Department of Pathology, Hassan Institute of Medical Sciences, Hassan for fine needle aspiration procedure were included in this study. The cytological findings were reported according to TBSRTC. Among these 62 biopsies were available for histopathological comparison. These were also correlated with the radio- imaging reports.**Results:** Among 100 FNACs, Benign category (TBSRTC II) formed the majority 85% of the cases. Unsatisfactory (TBSRTC I), Atypia of undetermined significance (TBSRTC III), Follicular neoplasm/ Suspicious for Follicular Neoplasm (TBSRTC IV), Suspicious for malignancy (TBSRTC V) and Malignancy (TBSRTC VI) constituted 9%, 1%, 2%, 1% and 2% respectively. On FNAC and Histopathological correlation, the p-value of <0.05 was found which is statistically significant and Kappa value of 0.74 was noted which indicated substantial agreement between FNAC and histopathology. The sensitivity and specificity of FNAC was 98.2% and 100% and USG was 96.4% and 71.4% respectively.**Conclusion:** TBSRTC provides proper communication between the pathologists and treating clinicians and surgeons due to valid and reliable diagnostic categories. The efficiency is further increased when it is used with other diagnostic modalities like ultrasound examination.**Keywords:** Thyroid, Bethesda, Cytology, Ultrasound, Histopathology.

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Introduction

A well-known outpatient technique for the initial diagnosis of thyroid swellings is fine needle aspiration and cytology (FNAC). [1] This method has been proven to be simple, barely intrusive, secure, and economical in identifying a significant percentage of thyroid nodules as benign. [2] It also plays a pivotal role in preventing unnecessary surgery for patients with benign disease. [3] Along with ultrasound imaging, FNAC is incredibly helpful in differentiating malignant and benign thyroid swellings. [4] However, a number of critical contributing factors, such as aspirator expertise, skilful interpretation, and logical analysis, are necessary for FNA to be successful. [5]

When interpreting cytopathology reports, referring physicians have been misled by the pathologists' use

of different terminologies and diagnostic criteria, leading to non-definitive clinical management. [6,7,8] The National Cancer Institute (NCI) held "The NCI Thyroid Fine Needle Symposium" to discuss nomenclature and other concerns regarding thyroid FNACs at Bethesda, Maryland. [9,10] The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) was introduced in 2007 to standardize terminology used in reporting thyroid cytology. TBSRTC is a six-category scheme, these categories are nondiagnostic, benign, atypia of undetermined significance (AUS)/follicular lesion of undetermined significance (FLUS), follicular neoplasm/suspicious for follicular neoplasm (SFN), suspicious for malignancy and malignant. [3,11]

Aims and Objectives

1. To analyse the thyroid cytology smears by TBSRTC.
2. To correlate with radiological findings.
3. To correlate with corresponding histopathological findings.

Materials and Methods

This cross-sectional study was conducted over a period of 6 months. A total of 100 patients with palpable thyroid swellings referred to the Department of Pathology, Hassan Institute of Medical Sciences, Hassan for fine needle aspiration procedure were examined. Informed consent was taken after explaining the procedure. Using 22 to 25 gauge needle the swelling was aspirated and 4-5 smears were made depending on the swelling. These smears were fixed in ethanol and stained by Haematoxylin and Eosin (H&E) and Papanicolaou (PAP) stains and also air dried and stained by Giemsa stain. The stained smears were analysed under a microscope, classified according to TBSRTC and correlated with the radio- imaging reports.

Inclusion Criteria

All patients with palpable thyroid swellings

Exclusion Criteria

Uncooperative patient.

Subsequently the corresponding 62 biopsies were sent to the Histopathology section, Department of Pathology, Hassan Institute of Medical Sciences, Hassan for examination. These specimens were fixed in 10% formalin for 24 hours. The lesions were grossly examined and all the necessary measurements were recorded. The multiple tissue bits from representative areas were processed and embedded in paraffin wax. Sections of 4-5micron thickness were taken and stained by Haematoxylin and Eosin (H&E) for histopathological examination.

Results

A total number of 100 patients who underwent FNAC of thyroid gland were evaluated. The age distribution ranged from 10 years to 70 years with the majority seen in the age group 41-50 years (32.3%). Table 1, Fig 1

Table 1: Distribution of study subjects with respect to age

Age (Years)	Frequency	Percentage (%)
20-30	18	18
31-40	20	20
41-50	32	32
51-60	19	19
61-70	11	11
Total	100	100

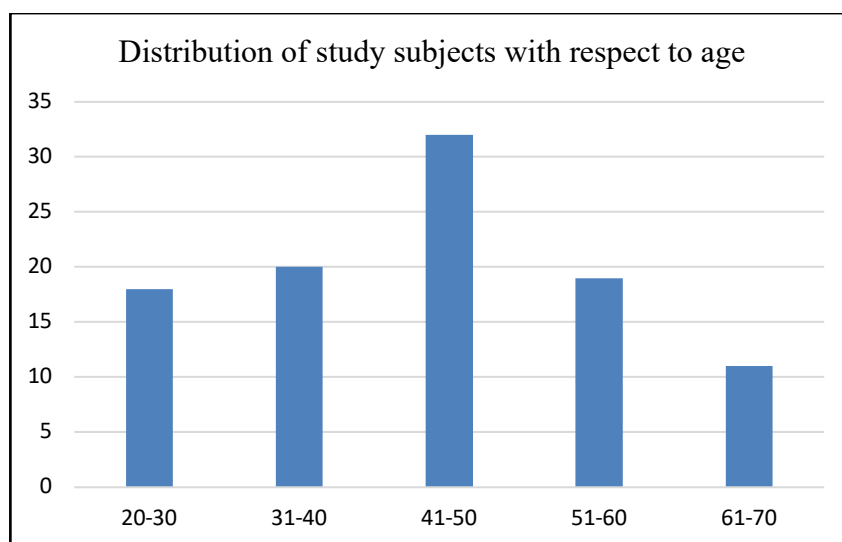


Figure 1: Distribution of study subjects with respect to age

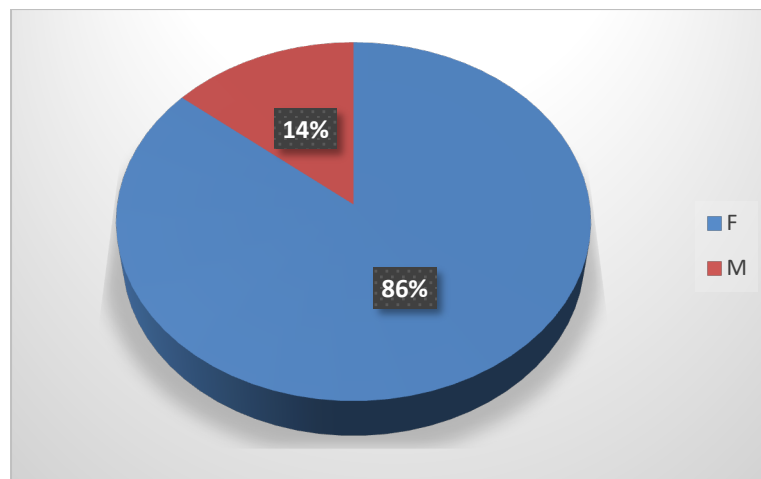


Figure 2: Distribution of study subjects with respect to Sex

Among the 100 cases majority were females 86 (86%). (Fig 2) The distribution of 100 cases according to TBSRTC is shown in Table 2.

Table 2: Distribution of 100 cases according to TBSRTC

TBSRTC Categories	Subcategories	No. of Cases	Total Number of Cases
I- Non-Diagnostic / Unsatisfactory	Cyst fluid only	2	9(9%)
	Acellular	1	
	Obscuring blood	5	
II- Benign	Benign lesion	4	85 (85%)
	Thyroglossal cyst	2	
	Colloid cyst	1	
	Colloid goiter	27	
	Colloid goiter with cystic change	19	
	Hashimoto / Lymphocytic thyroiditis	26	
	Adenomatous goiter	5	
III- Atypia of Undetermined Significance		1	1 (%)
IV- Follicular Neoplasm/ Suspicious for Follicular Neoplasm	Follicular neoplasm	1	2 (%)
	Suspicious for follicular neoplasm	1	
V- suspicious for Malignancy		1	1 (%)
VI- Malignancy	Papillary carcinoma	1	2 (2%)
	Anaplastic carcinoma	1	

Among 100 FNACs, Benign category (TBSRTC II) formed the majority 85% of the cases. This category comprised of benign lesion (5), thyroglossal cyst (2), Colloid cyst (1), Colloid goiter (21), Colloid goiter with cystic change (19), Hashimoto/ Lymphocytic thyroiditis (26) and Adenomatous goiter (2).

Unsatisfactory (TBSRTC I), Atypia of undetermined significance (TBSRTC III), Follicular

neoplasm/ Suspicious for Follicular Neoplasm (TBSRTC IV), Suspicious for malignancy (TBSRTC V) and Malignancy (TBSRTC VI) constituted 9%, 1%, 2%, 1% and 2% respectively. 62 biopsies were available to histopathological examination for comparison after hemithyroidectomy or total thyroidectomy procedures.

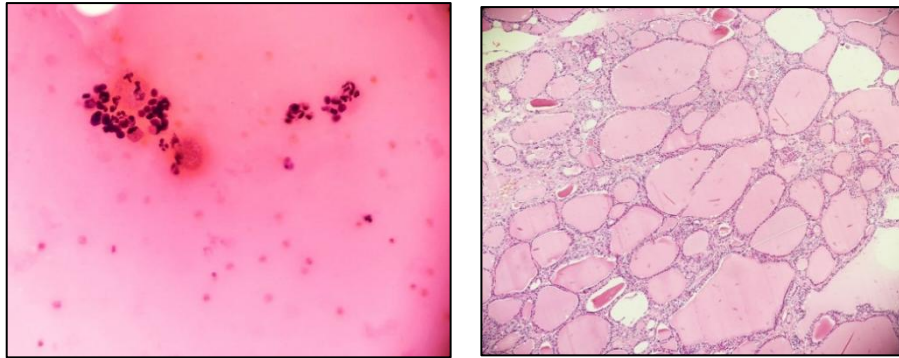


Figure 3: Colloid goiter FNAC (HE, 400X), Tissue section (HE 100X)

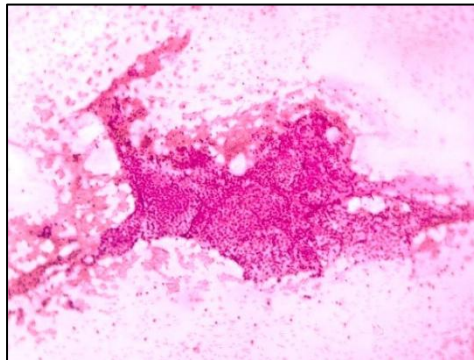


Figure 4: Adenomatoid goiter FNAC (HE, 100X)

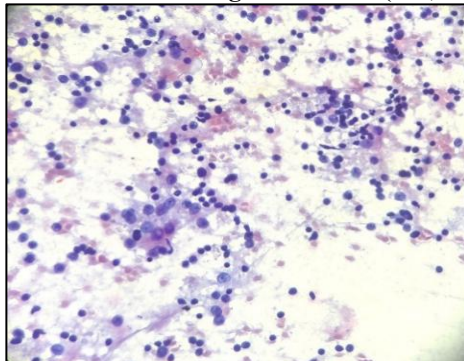


Figure 5: Hashimoto thyroiditis FNAC (HE, 400X)

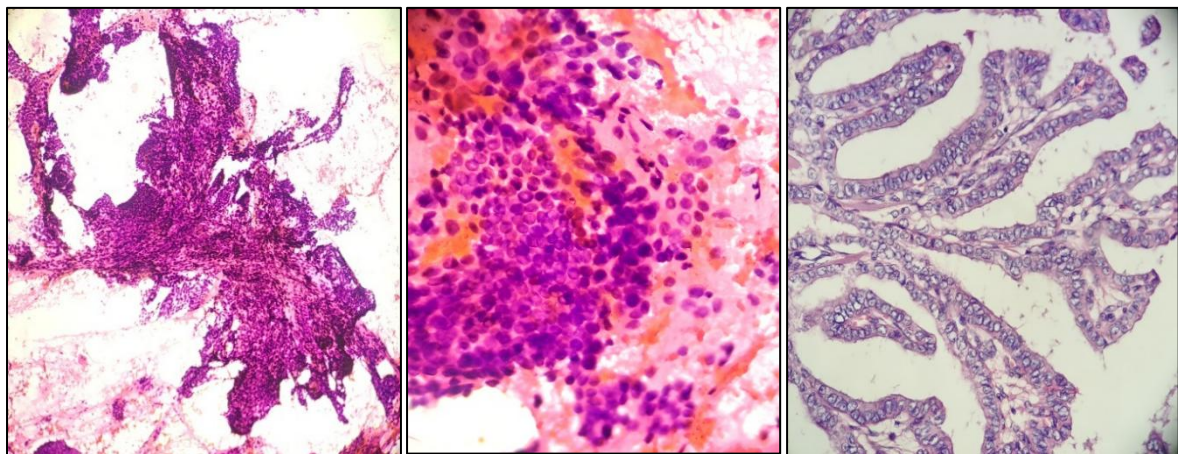


Figure 6: Papillary carcinoma FNAC (HE,40X, 100X), Tissue section (HE, 100X)

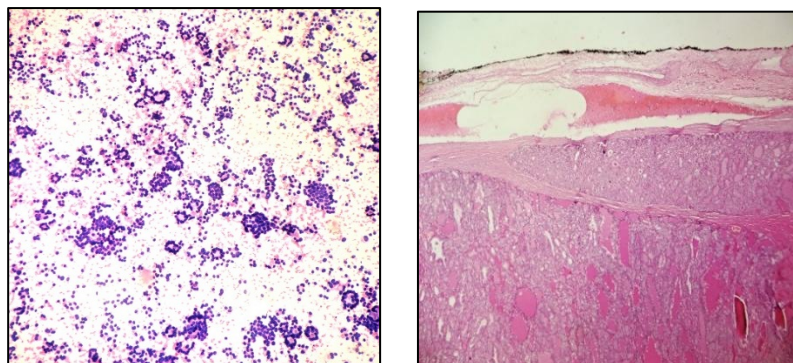


Figure 7: Follicular neoplasm FNAC (HE, 40X), Follicular adenoma, Tissue section (HE, 40X)

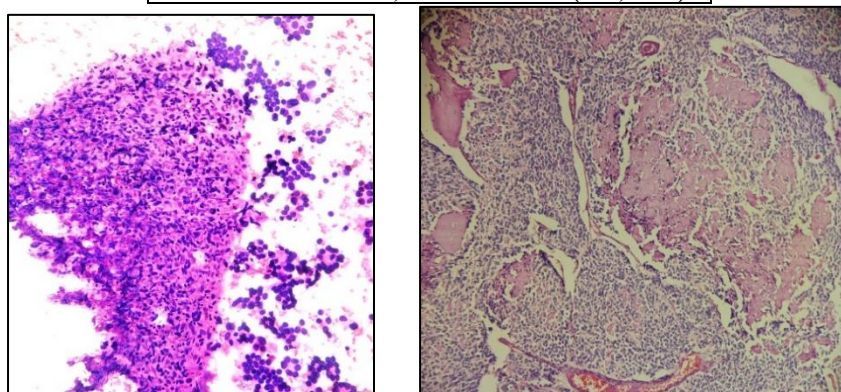


Figure 8: Anaplastic carcinoma FNAC (HE, 40X), Figure 9: Medullary carcinoma, Tissue section (HE, 40X)

Table 3: Cross-Tabulation of FNAC Results with Histopathological Examination (HPE) Outcomes

FNAC	HPE		No. (%)
	Benign No. (%)	Malignant No. (%)	
Benign	55 (98.2)	1(1.8)	56 (100)
Malignant	0	4 (100)	4 (100)
Suspicious	0	2 (100)	2 (100)
Total	55 (88.7)	7 (11.3)	62 (100)

The p-value of <0.05 was found which is significant, we conclude that FNAC shows a significant correlation with HPE results. The diagnostic performance of FNAC in predicting benign and malignant thyroid lesions is not due to random variation, but rather reflects a meaningful association between FNAC and the gold-standard histopathological diagnoses.

The Kappa value of 0.74 was noted, this indicates substantial agreement between FNAC and histopathology.

FNAC correctly identified 55 benign cases, and 1 malignant case was incorrectly classified as benign. The high proportion of correct diagnoses in this group suggests high accuracy of FNAC in diagnosing benign lesions. All 4 cases that FNAC identified as malignant were confirmed by HPE, indicating excellent diagnostic accuracy in detecting malignancies. The indeterminate FNAC cases (n = 2) were ultimately diagnosed as malignant by HPE. This suggests that when FNAC results are indeterminate, there is a moderate risk of malignancy, and further evaluation (e.g., surgical biopsy) is necessary.

Table 4: Distribution of Ultrasonographic findings based on TIRADS

TIRADS	Frequency	Percentage (%)
1	3	4.8
2	41	66.1
3	13	21
4	2	3.2
5	3	4.8

Thyroid Imaging Reporting and Data System (TIRADS) is a reporting system for thyroid nodules on ultrasound which uses a standardised scoring system based on composition, echogenicity, shape, margin and echogenic foci. [12] The cumulative score determines the thyroid lesion belongs to which

category of TIRADS I-V. The risk of malignancy is minimum for TIRADS I (0.3%) and maximum for TIRADS V (35%). [13] In the present study, among 62 cases majority belonged TIRADS II category (66.1%), followed by TIRADS III (21%).

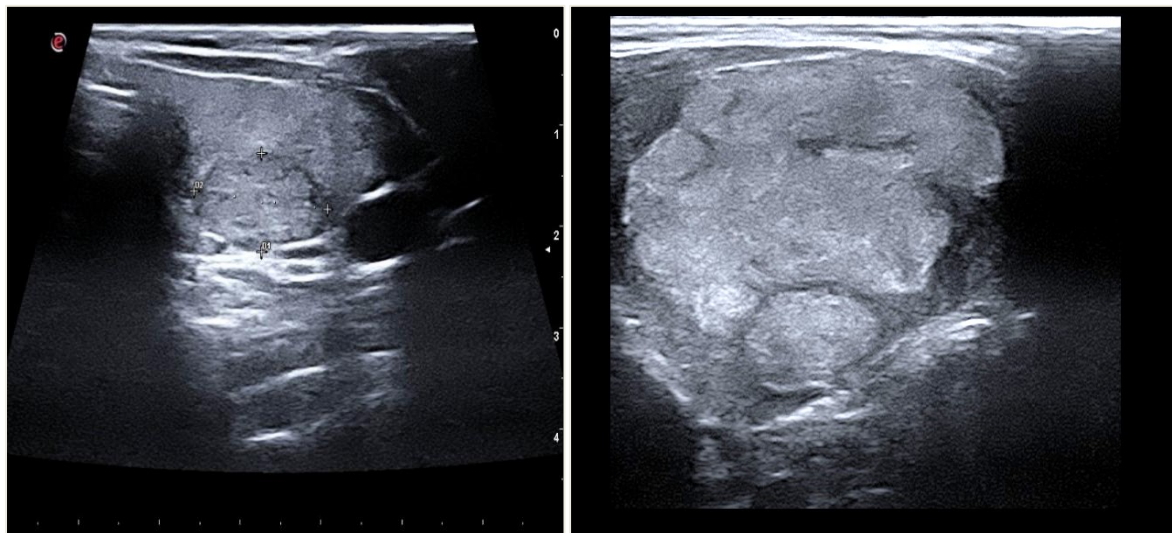


Figure 10: Ultrasonography images of Multinodular goiter

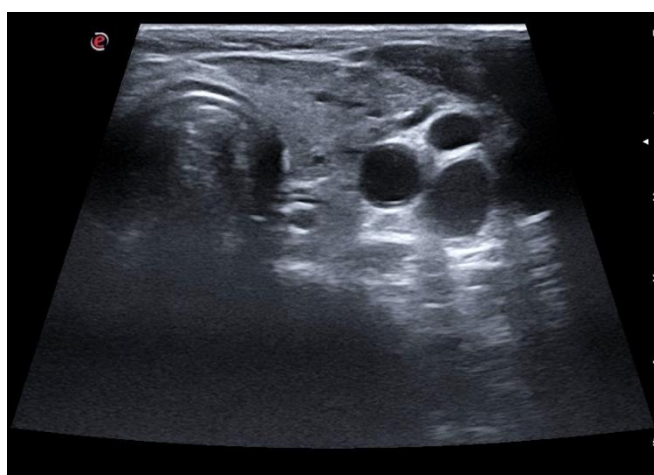


Figure 11: Ultrasonography image of Papillary carcinoma of thyroid

Table 5: Agreement between FNAC Reporting (Bethesda System) and radiological findings

FNAC	Radiological Findings			Total (%)
	Benign	Malignant	Suspicious	
Benign	54(96.4)	1 (1.8)	1 (1.8)	56 (100)
Malignant	1 (25)	3(75)	0	4 (100)
Suspicious	0	1(50)	1 (50)	2 (100)
Total	55 (88.7)	7 (11.3)	2 (3.2)	62 (100)

Here we found the p value <0.05, shows that the agreement is statistically significant and Kappa value of 0.66 which indicates substantial agreement between FNAC and radiological diagnosis.

Table 6: Diagnostic Performance of FNAC, HPE, and Radiology

Diagnostic modality	Sensitivity	Specificity	Positive predictive value (PPV)	Negative predictive value (NPV)
FNAC	98.2%	100%	100%	85.71 %
USG	96.4%	71.4%	96.36%	71.43%
HPE	100%	100%	100%	100%

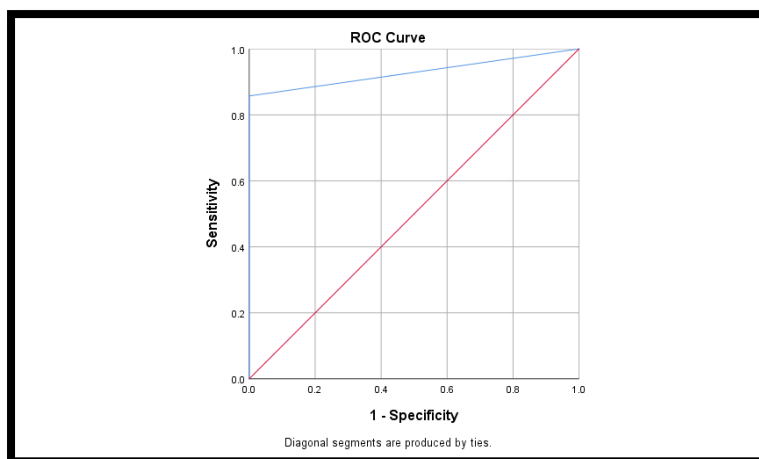


Figure 12: ROC Curve

The ROC curve will plot the true positive rate (sensitivity) against the false positive rate (1-specificity). In our study the Area Under the Curve (AUC) was found to be of 0.929 which is excellent, suggesting that our FNAC/Bethesda system has very strong discriminatory power in differentiating between benign and malignant thyroid lesions compared to the histopathology (HPE) gold standard.

Discussion

Being a tertiary care hospital, we come across thyroid diseases very frequently. Whether it is a multinodular thyroid or diffuse thyroid or solitary nodule thyroid swelling, it welcomes a long list of tests to be done like ultrasound, thyroid hormone profile, FNAC and others. This is basically done to

sort the patients for whom surgery is needed and those who can be managed conservatively. Among this cytology has proved a very significant screening test. For FNAC using Bethesda system by cytopathologists universally as paved a remarkable pathway in thyroid cytology reporting.

In our study in the 100 patients that were included in the study majority were in the age group 41-50 years (32.3%). This was in concordance with Jinadu et al [14] (30.8%). Present study showed predominantly women affected by thyroid pathology with female to male ratio of 6.1:1 which was comparable with Patel et al [15] and Hagezy et al. [16]

Table 7: Comparison of TBSRTC Diagnostic Categories

Studies	Total FNAC Cases (n)	TBSRTC Categories (%)					
		I	II	III	IV	V	VI
Al Dawish MA et al [17]	1433	3.2	75.3	9.1	5	2.2	5.1
Mehra et al [18]	225	7.2	80	4.9	2.2	3.6	2.2
Alshaikh et al [19]	632	10.1	68.8	12.4	2.9	2.6	4.1
Mufti et al [20]	250	11.6	77.6	0.8	4	2.4	3.6
Present study	100	9	85	1	2	1	2

In our study we found 9% cases of Category I which was consistent with study by Mehra et al [18] (7.2%) and Alshaikh et al [19] (10.1%), whereas Al Dawish MA et al [17] study showed lesser frequency (3.2%) and study by Mufti et al [20] showed higher frequency (11.6%). These disparities may be due to differences in the technique and experience of the aspirator. Also using the guidance of ultrasound during the procedure of FNAC can attribute to better quality cellular aspirate.

The highest frequency noted in our study was 85% of Benign- Category II which in concordance with Al Dawish MA et al [17] Mehra et al [18] and Mufti et al. [20] One case of Hurthle cell adenoma was misdiagnosed as colloid goiter, which was diagnosed radiologically as neoplastic lesion. In

thyroid cytology, discrimination between true hurthle cell tumor from other lesions with hurthle cell changes is always difficult. An important parameter to consider for hurthle cell neoplasm would be hurthle cell population of more than 90% of the specimen, a non-macrofollicular cell arrangement, presence of transgressing vessels and prominent macronucleoli. Nevertheless, a false positive case cannot always be avoided. [21]

The category AUS/FLUS is an area of uncertainty for both pathologists and physicians. According to TBSRTC, the incidence of Category III diagnosis should be less than 7%. [19] In our study it was 1% which was similar to Mufti et al [20] (0.8%). Whereas Al Dawish MA et al [17] and Alshaikh et al [19] showed increased incidence, 9.1% and 12.4%

respectively. For this category lesions, FNA procedure has to be repeated after 3 months, in keeping with TBSRTC guidelines.^[19] In our study one case which was diagnosed as Atypia of undetermined significance cytologically turned out to be Papillary carcinoma. Atypia of undetermined significance is a category in thyroid Bethesda that describes nodules with ambiguous cytological findings. Recent studies show increased risk of malignancy of 38-55% [22]. In such cases ultrasound correlation will be more helpful as radiological features like hypo or anechogenicity, tall than wide shape, irregular borders and microcalcifications show increased risk of nodule being malignant. [22]

In the present study, Category IV showed 2% incidence which was in agreement with Mehra et al [18] (2.2%) and Alshaikh et al [19] (2.9%). Al Dawish MA et al [17] (5%) and Mufti et al [20] (4%) showed slightly high preponderance.

We noted that the frequency of Category V (1%) and VI (2%) in this study was in consonance with the other studies. 1 case of Papillary carcinoma and 1 case of Anaplastic carcinoma was reported in our study.

One case of papillary carcinoma- follicular variant was underdiagnosed as follicular neoplasm. Causes of misinterpretation could be due to the fact that nuclear features of papillary carcinoma may be present focally in the nodule. [23]

In the present study we could diagnose a case of Anaplastic carcinoma transformed from co-existing well differentiated follicular carcinoma. Cytological features showed features of both follicular neoplasm and anaplastic carcinoma. Preoperative diagnosis of such conditions is very helpful for deciding therapeutic approach as anaplastic carcinoma is resistant to radioactive iodine and require multimodality therapy (surgery, external beam radiation and chemotherapy). [24]

We found that in our study the evaluation of thyroid gland by ultrasound had sensitivity, specificity, positive and negative predictive value of 96.4%, 71.4%, 96.36% and 71.43% respectively when compared with FNAC and HPE in determining benign and malignant nodules. A related study Jinadu et al [14] found 100% sensitivity, specificity, positive and negative predictive value. Similarly in a study conducted by Popli et al [25] the sensitivity, specificity, positive and negative predictive value for ultrasound diagnosis of benign and malignant thyroid nodules were 81.8%, 87.2%, 59.0% and 95.5% respectively. Similar findings were obtained from study done by Ram et al. [26] Malik et al [27] in a related study found a sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 70.59%, 96.39%, 80%, 94.12% and 92% respectively.

When comparing FNAC diagnoses with the histopathological diagnoses which is the gold standard we found 98.2%, 100%, 100% and 85.71% of sensitivity, specificity, positive and negative predictive value respectively. This was similar to the findings by the study by Abdullahi et al [28] where the overall sensitivity, specificity, and accuracy rate of cyto-histopathology correlation was 91.1%, 96.6%, and 94.9% respectively. Babu et al [29] found sensitivity in the range of 80-100%, specificity and the positive predictive value was almost 100% and the negative predictive value was between 98-100% for all the lesions detected by FNAC.

We encountered few false negative cases in our study such as 4 cases of follicular adenomas and a case of follicular carcinoma were underdiagnosed as nodular colloid goiter on FNA study. Although FNAC is a reliable method for preoperative evaluation of thyroid nodules, sometimes it is difficult to differentiate nodular hyperplasia from follicular neoplasm. Many studies have shown that alongwith high cellularity and microfollicular pattern, other cytological features like trabecular pattern and cell clusters with cellular overlapping should be examined as they are more specific for follicular neoplasm [30] False negative chances in FNA can be reduced by avoiding suboptimal samples and multiple passes should be performed in various parts of large nodule or from different nodules. An ultrasound examination and ultrasound guided FNAC always increase the accuracy of cytological diagnosis.

Conclusion

The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) provides proper communication between the pathologists and treating clinicians and surgeons. Due to valid and reliable diagnostic categories, implementing TBSRTC in diagnosis and management of thyroid lesions universally has proved advantageous. The efficiency is further increased when it is used with other diagnostic modalities like ultrasound examination and thyroid hormone levels.

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