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Original Research Article

Classification of Thyroid FNA Smears into Bethesda Categories and Their Correlation with Thyroid Function Tests

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Abstract: Fine needle aspiration cytology (FANC) of thyroid is a gold standard used in the initial evaluation of thyroid nodules. The Bethesda system for reporting thyroid Cytopathology (TBSRTC) classifies smears into 6 categories each having a malignancy risk and management guideline. When correlated with sonography findings and thyroid function tests(TFT) its diagnostic accuracy is improved. The objective of this study was to classify the thyroid cytology smears by TBSRTC and to correlate these categories with the thyroid function tests. Malignancy risk was calculated for each category in surgically resected specimens. This was prospective study of 287 patients who underwent thyroid FNAC along with sonography and thyroid function tests. All cases were classified into nondiagnostic, benign, atypia of undetermined significance, follicular neoplasm or suspicious of follicular neoplasm and malignant categories. Statistical analysis was done using SPSS software and chi square test was applied to study the statistical significance between TFT and Bethesda categories. The distribution of various categories from 287 evaluated thyroid nodules was as follows 0.68% Non-Diagnostic, 88% Benign, 3.4% Atypia of undetermined significance (AUS) / Follicular lesion of undetermined of significance (FLUS), 4.5% Follicularneoplasm, 1.4% Suspicious for malignancy and 2.06% malignant. Sensitivity, specificity, positive predictive value and negative predictive value were 96%,78%,76%, 97% and accuracy was 85%. Thyroid function tests were altered in 42.06% cases in the benign category whereas the malignant category had normal thyroid function test. TBSRTC is an excellent reporting system for thyroid fine needle aspirates. It also provides clear management guidelines to clinicians to go for follow up or surgery.
Keywords: Bethesda, categories, fine needle aspiration, malignancy risk, thyroid cytology, thyroid function tests

INTRODUCTION

For the initial evaluation of patients with thyroid nodules fine needle aspiration cytology (FNAC) is a widely accepted, minimally invasive and cost effective technique [1]. It is estimated that about 42 million people in India suffer from thyroid disorders but the frequency of thyroid cancer is only 1/lakh population for males and 1.8/lakh population for females [2]. FNAC is extremely useful in differentiating between neoplastic and non-neoplastic lesions of the thyroid which then lead to a correct management decision of surgical intervention or otherwise [3].

It is crucial that cytopathologists communicate thyroid FNA interpretation to referring physicians in terms that are clear, unambiguous and clinically helpful. However due to lack of a standardized system of reporting, pathologists were using different terminologies and diagnostic criteria [4]. This led to confusion among clinicians in the correct interpretation of the report and made optimal clinical management difficult.

To address terminology and other issues related to thyroid FNA, in 2007 the National Cancer Institute (NCI) hosted the Thyroid Fine Needle Aspiration State of Science Conference in Bethesda, Maryland. NCI conference participants acknowledged the importance of developing a uniform terminology for reporting thyroid FNA results which would facilitate effective communication among cytopathologists,

endocrinologists, surgeons, radiologists and other physicians [5].

Subsequently NCI published a monograph entitled "The Bethesda system for reporting thyroid Cytopathology (TBSRTC)", which included definition, diagnostic/morphologic criteria, explanatory notes and a brief management plan for each diagnostic category [6, 7]. TBSRTC is a 6 category scheme of thyroid cytopathology reporting These include non-diagnostic /unsatisfactory, benign, atypical follicular lesion of undetermined significance (AFLUS), suspicious for follicular neoplasm(SFN), suspicious for malignancy(SM) and malignant. Each category has an implied cancer risk which ranges from 0% to 3% for the benign category to virtually 100% for the malignant category [8].

The objective of the present study was to use TBSRTC for classifying thyroid cytology smears and convey brief management plans to clinicians. In addition, various categories were correlated with thyroid function tests. A correlation of cytology was done with final histopathology results and risk of malignancy was calculated for various diagnostic categories.

MATERIALS AND METHODS

This was a prospective observational study of all patients referred to the Department of Pathology of the Dr. R.N.Cooper hospital for thyroid FNAC between Jan 2009 till May 2014. The duration of study was three and a half years.Institutional ethics committee approval was obtained.The relevant clinical history was taken for all patients along with clinical examination and ultrasonography. Results of TFT (T3, T4 and TSH) were noted. In all suspected cases of thyroiditis ant microsomal antibody levels were done.

Patients were subjected to FNAC sampling under direct supervision. Under aseptic conditions needle was inserted into the lesion and several short rapid strokes were made in different directions with the needle tip in the nodule [9]. In cases of unsatisfactory smears repeat FNAC was done under ultrasonography guidance. Smears were prepared using conventional methods and stained with Giemsa and Papinicolaou Stains. The cytological features were evaluated and the reporting was done according to the morphological criteria given in the monograph of TBSRTC.The implied risk malignancy and recommended clinical management were communicated to the clinicians along with the report. Histopathological specimens of surgically resected specimenswere processed as per the standard method. Cytohistopathological correlation was done. Sensitivity, specificity; positive and negative predictive values were calculated using histopathology diagnosis as gold standard. Thyroid function tests and its association with neoplastic and non-neoplastic lesions were analyzed. Statistical analysis was done using Chi square test and P value was determined
Criteria used for diagnosis.

Nondiagnostic or unsatisfactory-category 1

A smear was considered inadequate if it contained less than 6 groups of well preserved and well stained follicular cells of 10 cells each. However a smear of colloid nodule with abundant colloid or having malignant cells was considered adequate.Smears which were hemorrhagic or cantained only cyst fluid were also put in this category.

Benign-category 2

Cases of colloid goiter, adenomatoid goiter, lymphocytic thyroiditis and granulomatous thyroiditis were included in this group.[Figure 1(A) and 1(B)]

AUS/AFLUS-category 3

Atypia of undetermined significance or atypical follicular lesion of undetermined significance included those cases which had some features of atypia but could not be definitely categorized into either benign or neoplastic category were placed in this group.[Figure 2(A) and 2(B)]

FN/SFN-category 4

Smears showing moderate to high cellularity, scant or absent colloid with microfollicular or trabecular configuration of follicular cells in repetitive patterns were placed in follicular neoplasm or suspicious for follicular neoplasm category. Aspirates with features of Hurthle cells were also placed in this category.[Figure3(A)]

Suspicious of malignancy-category 5

Aspirates that had cytologic features suggestive of but not definitive of papillary carcinoma, medullary carcinoma metastatic carcinoma and lymphoma were placed in this category. [Figure3(B)]

Malignant-category 6

Aspirates that appeared unequivocally malignant were placed in this category. Cases of papillary carcinoma, medullary carcinoma, anaplastic carcinoma, metastatic carcinoma and lymphoma were placed in this category.[Figure 4(A) and 4(B)]

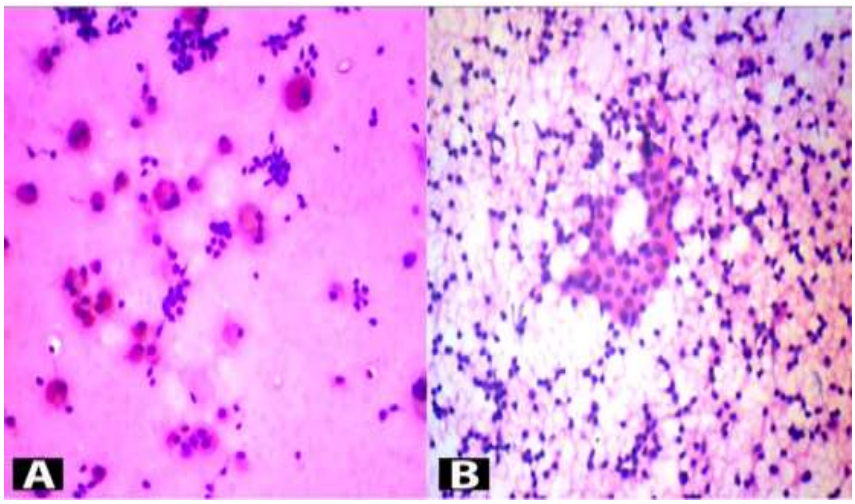

Fig. 1(A): Microphotograph showing benign follicular cells, macrophages against a background of colloid-Colloid goiter. (Benign) Bethesda category 2 (H and E,Low power).1(B): Microphotograph showing presence of hurthle cells against a background of lymphocytes. Lymphocytic Thyroiditis.(Benign)Bethesda category 2(H and E, Low power)

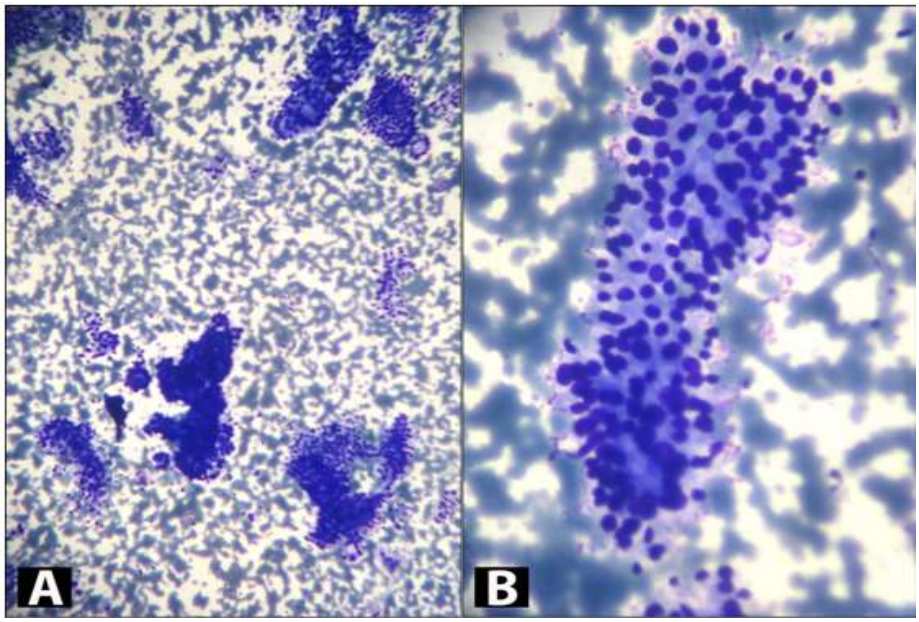

Fig. 2(A): Microphotograph showing presence of clusters of follicular cells with overlapping of nuclei. Atypia of undetermined significance (AUS).Bethesda category 3(Geimsa stain, low power).2(B): Microphotograph showing presence of cluster of follicular cells with anisonucleosis.-Follicular lesion of undetermined significance category. Bethesda category 3(Geimsa stain, high power)

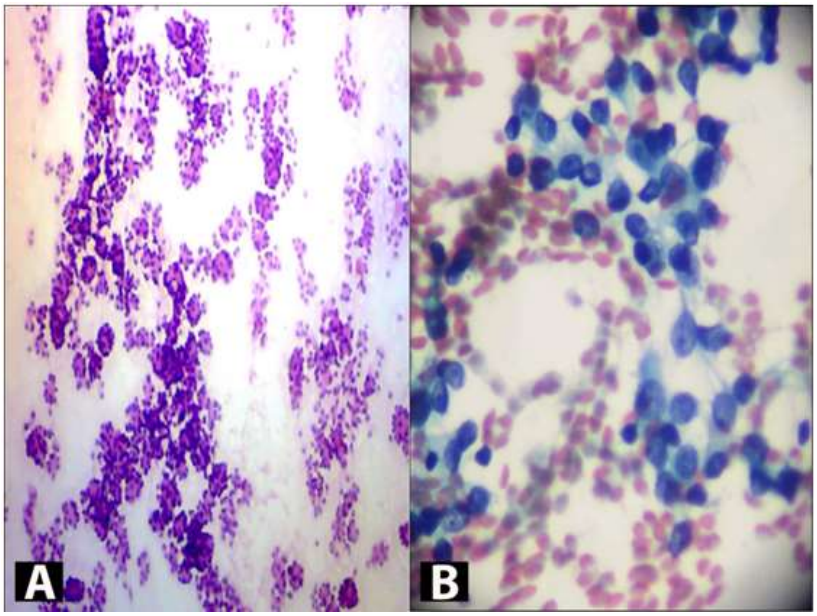

Fig. 3(A): Microphotograph showing cellular aspirate composed of many microfollicles –Follicular neoplasm. Bethesda category 4(H and E, Low power).3(B): Microphotograph showing loose clusters of cells with enlarged pale nuclei and occasitional grooves. Suspicious for malignancy (follicular variant of papillary carcinoma)Bethesda category 5(Pap stain, high power)

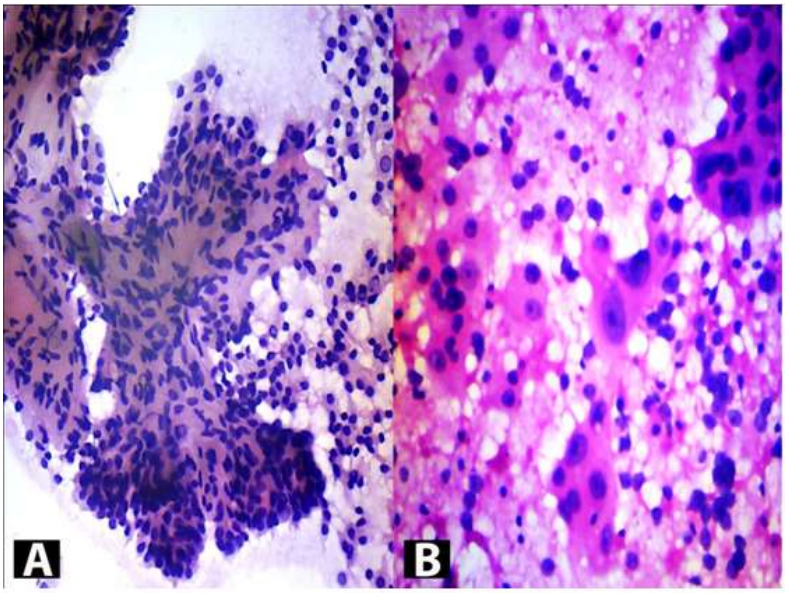

Fig. 4(A): Microphotograph showing cellular aspirate with papillae, enlarged pale nuclei with intranuclear inclusions- Papillary carcinoma. Bethesda category 6(Pap stain, high power).4(B): Microphotograph showing cellular aspirate with pleomorphic cells with enlarged nuclei and prominent nucleoli- Anaplastic carcinoma. Bethesda category 6(H and E, high power)

RESULTS

A total of 287 patients were included in the study on which FNAC was done. The age of the patients ranged from 6 years to 85 years with a mean age of 40.7 years. Benign thyroid lesions were common in 21-49 years age group, while malignant thyroid lesions were common in 40-80 years age group. There were 250 female patients and 27 male patients with a male female ratio of 7:1. The overall distribution of diagnosis was as follows: 2 non diagnostic, 252 benign, 10 AUS, 12 SFN, 5SM and 6 malignant. Table 1 lists the frequency of these diagnoses. Of these cases 63 cases were followed by surgical resection. Table 2 presents the total number of benign and malignant lesions on histopathology of the operated cases. The risk of malignancy for each group was as follows: non diagnostic 0%, benign 3%, AFLUS 20%, SFN25%, SFM 80% and malignant 100%.

Of the thyroid FNA samples, 252 (88%) cases were categorized as benign (201 colloid goiter, 41 lymphocytic thyroiditis, 10 adenomatoid goiter).There were 30 of these cases which underwent surgery. The final diagnosis was colloid goiter in 27 cases, follicular adenoma in 2 cases and follicular variant of papillary carcinoma in 1 case. All 10 cases of AFLUS underwent surgery. The final diagnosis was follicular hyperplasia in a goiter in 5 cases, thyroiditis in 3 cases and follicular variant of papillary carcinoma in 2 cases. In the SFN category the final diagnosis was follicular adenoma in 9 cases, follicular variant of papillary carcinoma in 1 case, follicular carcinoma in 1 case and hurthle cell neoplasm in 1 case. Of the 6 cases reported in the malignant category there were 3 cases of papillary carcinoma, 1 case of medullary carcinoma and 2 cases of anaplastic carcinoma the diagnosis of which were confirmed on histology.

When FNAC and histopathology results were compared, FNAC showed sensitivity, specificity and diagnostic accuracy of 96%, 78% and 85%.

Thyroid function tests were performed on all patients. Out of the total number of 287 cases, 64.5% patients were euthyroid, 21.5% patients were found to be hypothyroid and 14% patients were found to be hyperthyroid. Thyroid function tests were altered in 106 out of 252 cases in category 2 leisons (42.06%) while only one cases of category 4 presented with hypothyroidism. All cases of category 3, 5 and 6 were euthyroid. Out of 68 patients of thyroiditis 37 had hypothyroidism (54.4%) and 7 patients were hyperthyroid(10.29%). (Table 3). Antimicrosomal antibodies were positive in 63% cases of thyroiditis

The correlation between thyroid functional status with both benign and neoplastic lesions was analyzed out using chi square test and p value was <0.05.

Table 1: Distribution of cases according to the Bethesda system

Category	Number	Percent
Category 1 (Non diagnostic)	2	0.68%
Category 2 (Benign)	252	88%
Category 3 (Atypia of Undetermined significance)	10	3.4%
Category 4 (follicular neoplasm)	12	4.5%
Category 5 (suspicious for malignancy)	5	1.4%
Category 6 (malignant)	6	2.06%

Table 2: Implied risk of malignancy

Diagnosis of preop FNAC	Actual Diagnosis observed on HPE	Number of malignant cases	Malignancy risk
Nondiagnostic n=2	Colloid goiter n=2	Nil	
Benign n=30	Colloid goiter=27 Follicular adenoma n=2 Follicular variant papillary carcinoma n=1	1	3%
AFUS n=10	Foll hyperplasia goiter n=5 Thyroiditis=3 Follicular variant papillary carcinoma n=2	2	20%
SFN n=12	Follicular adenoma n=9 Follicular variant papillary carcinoma n=1 Follicular carcinoma n=1 Hurthle cell n=1	3	25%
Suspicious for malignancy n=5	Follicular variant papillary carcinoma n=1 Thyroiditis=1 Papillary n=3	4	80%
Malignant n=6	Papillary Ca n=3 Anaplastic n=2 Medullary n=1	6	100%

Table 3: Correlation of Thyroid function tests with Bethesda categories

TFT	Category 2		Category 3	Category 4	Category 5	Category 6
	Colloid	Thyroiditis				
Euthyroid	122	24	10	11	5	6
Hypothyroid	26	37	0	1	0	0
Hyperthyroid	36	7	0	0	0	0

Table 4: Comparison of Distribution of Lesions on FNAC with other studies

Diagnostic Category	Present Study	Jo *et al* [3]	Yassa*et al* [13]	Yang et al [14]	Nayar and Ivanovic [15]	Mondal*et al* [16]
Category 1 (Non diagnostic)	0.68	18.6	7	10.4	5	1.2
Category 2 (Benign)	88	59	66	64.6	64	87.5
Category 3 (Atypia of Undetermined significance)	3.4	3.4	4	3.2	18	1
Category 4 (follicular neoplasm)	4.5	9.7	9	11.6	6	4.2
Category 5 (suspicious for malignancy)	1.4	2.3	9	2.6	2	1.4
Category 6 (malignant)	2.06	7	5	7.6	5	4.7

DISCUSSION

Fine needle aspiration cytology is regarded as the gold standard initial investigation in the diagnosis of thyroid lesions. When coupled with ultrasonography findings and thyroid function tests the diagnostic accuracy of the procedure improves greatly [10].As reported in literature age and gender were associated factors of thyroid lesions. In our study the female male ratio was 7:1.A high female to male ratio has been reported in many earlier studies [11, 12].The mean age at presentation was 40 years which is similar to the studies by Handa *et al* [11] (mean age 38 years) and Pandey *et al* [12] (mean age 39 years).

The cytologic diagnosis was recorded as per the criteria laid down in the standard nomenclature of the Bethesda system. It was easy to classify the results in the 6 categories as opposed to earlier reporting system. The risk of malignancy in our study was similar to that given in the Bethesda system except for the AUS category which was higher in our study. We compared the results obtained in our study with the studies of Jo *et al* [3], Yassa *et al* [13], Yang *et al* [14], Nayar and Ivanic [15] and Mondal *et al*[16](Table 4). The present study had 2 (68%) cases in the non-diagnostic category. Other studies had 1.6% to 16.4% in this category [17].The percentage of cases in the non-diagnostic category was lower as compared to other studies. This could be attributed to the fact that a repeat FNAC is done by the pathologist herself under sonography guidance in all initial nondiagnostic aspirates. Both patients were operated and the final diagnosis was colloid goiter. The benign category had 252(88%) cases. Other studies have reported 34% to 87.5% cases in this category [17].The percentage of cases in the benign cases was higher in our study. The reason could be that ours being a peripheral hospital a large number of patients come directly without referral. There were 30 cases in this category which were operated for cosmetic reasons or to relieve pressure symptoms. Out of these cases the final histopathological diagnosis was colloid goiter in 27 cases, follicular adenoma in 2 cases and FVPC in one case.The malignancy risk was 3%. The cytologic criteria of nodular goiter can overlap with follicular adenoma and in certain cases it is difficult to distinguish between them.

We had 10 cases in the AFLUS category which comprise 20% of the cases. A wide variation ranging between 3 to 29% has been reported in this category. The malignancy risk in our study was 20 % which is similar to the rates found by Yassa *et al* [13], (24%) and Yang *et al* [14] (19%).There is a variation in this category because it is somewhat heterogeneous and subjective [18].The Bethesda systems recommend a limited use of this category and not more than 7% cases should be included in this group. A repeat FNAC and follow-up is recommended management for this category. However in our setup all cases were operated which has also been reported in other studies.

The diagnosis of SFM and malignant category warrant immediate surgical intervention in all cases. The malignancy risk of 80% and 100% in these 2 categories is similar to other studies [19].Only one case of thyroiditis was misdiagnosed as SFM because of marked an isonucleosis which is found in these cases. Moreover the presence of malignancy in association with thyroiditis can further compound this problem. A correlation with sonography findings along with abnormal thyroid function tests can be helpful in these cases. There were 2 cases of FVPC in AFLUS group

and 1 case in the benign group. FVPC on cytology shows follicular pattern of cells and nuclear features may be focal and subtle, hence it can be missed [20].

Hence though FNAC can provide an accurate diagnosis in majority of cases, there are problems in the indeterminate categories of AUS/AFLUS and SFN [21].In these cases molecular testing for somatic mutations like BRAF,RAS,RET/PTC and PAX8/PPAR-y can complement the cytology findings, leading to better management decision [22, 23].

The sensitivity specificity diagnostic accuracy positive predictive value and negative predictive value was comparable to studies by Handa *et al* [11] and Bagga *et al* [24].

Thyroid function tests were performed in all cases.They were altered in majority (106) cases of benign lesions while only one case of AFLUS category was hypothyroid. All cases in category 5 and 6 were euthyroid. This is in concordance with the study by Boigen *et al*[25] who reported that thyroid function tests were altered in majority of benign lesions. Nuclear enlargement and atypia can be seen in cases of benign thyroid disorders like thyroiditis and Graves' disease which can be mistaken for malignancy. However correlation with TFT can be helpful in these cases.

CONCLUSION

The classification of thyroid FNA smears into the Bethesda categories is a simple, convenient and standardized method of reporting which also provides management guidelines. However there is a great variation in the reporting and management of AFLUS category. Molecular testing may be helpful in these cases and can complement cytology findings. Alteration in thyroid function tests is associated with benign conditions and can aid in the diagnosis of the benignconditions with atypical features.

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