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Diagnostic accuracy and utility of The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), 2017 in an Indian Scenario: A tertiary care appraisal

Sujata Raychaudhuri[1], Dipti Sidam[1], Ruchira Wadhwa[1], Kanika Singh[2*], Mukta Pujani[1] and R.K. Chandoke[1]

[1]Department of Pathology, Employees' State Insurance Corporation (ESIC) Medical College, KL Mehta Road, Faridabad-121012, Haryana, India and [2]Department of Pathology, University College of Medical Sciences (University of Delhi) & Guru Teg Bahadur Hospital, Dilshad Garden, Delhi, India

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Abstract: *Objective:* To categorize the cytological pattern according to Bethesda System for Reporting Thyroid Cytopathology and correlate them with the histopathological diagnosis. Calculate the sensitivity, specificity, accuracy of thyroid lesions and also the risk of malignancy in each category. *Material &Methods:* A retrospective re- evaluation of all consecutive thyroid fine-needle aspiration cytology fine-needle aspiration cytology smears was done from 2016 to 2019. *Results:* 429 patients were subjected to fine-needle aspiration cytology while histopathology was available for 109 cases. The sensitivity was 84.6% and specificity was96.4% with accuracy of 93.6%. The risk of malignancy for categories I, II, III, IV, V and VI was 0%, 1.4%, 20%, 20%, 100% and 100% respectively. *Conclusion:* The study shows application of Bethesda System for Reporting Thyroid Cytopathology in a tertiary care centre in a developing country is a successful step to bring about uniformity and standardise thyroid cytology reporting by reducing the ambiguity.

Keywords: Thyroid, Bethesda, Cytology, Risk of malignancy, NIFTP.

Introduction

Thyroid diseases are prevalent worldwide and accounts for approximately 42 million in India [1]. Palpable thyroid nodules are about 4-7% in the middle-aged population and increase with age [2]. FNAC is the first-line of investigation for evaluating it [3]. It is a quick, easy and cost effective test to differentiate benign from malignant thyroid lesions. India is an endemic zone for goitre due to iodine deficiency hence, it becomes essential to differentiate benign thyroid nodules from malignant ones to avoid unnecessary surgeries [4].

Before the introduction of FNAC of thyroid lesions, amongst the surgically resected thyroid nodules only 14% were malignant which rose to 50% with the advent of FNAC [5-6]. It helps to triage the patients and identify the malignant cases which needs surgery. Thyroid malignancies comprise 1% of all malignancies and 90% of all endocrine malignancies [7]. However, the thyroid FNAC suffers from lack of uniformity of reporting pattern among different cytopathologist and various laboratories at regional, national and international levels which leads to misinterpretation of the findings by clinicians resulting in inappropriate management. To overcome this obscurity, The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) was introduced at the National Cancer Institute, (NCI) Bethesda, Maryland in 2007. TBSRTC categorized the FNAC of thyroid into six diagnostic groups with well defined cancer risks and clear indications for further clinical management [8].

The six diagnostic categories are category I, Non-diagnostic/ Unsatisfactory (ND/UNS), category II, Benign, category III, Atypia of Undetermined Significance or Follicular lesion of Undetermined Significance (AUS/ FLUS), category IV, Suspicious of Follicular neoplasm or Follicular neoplasm (SFN/FN),

category V, Suspicious of Malignancy (SOM) and category VI, Malignant. It aims to bring about uniformity and standardise the thyroid FNAC reporting and enhance the clarity of communication to the clinicians for ease of interpretation to choose a common rationalised protocol of management. It necessitates that each thyroid cytology report begins with one of the six general diagnostic categories [8]. In 2017, there was a revision TBSRTC. It maintained all of the six diagnostic categories with their names since their introduction in 2007.In the revised system the malignancy risks have been updated as a function of their risk associations and has evidence-based clinical management recommendations [9].

It introduced the Non-Invasive Follicular Thyroid neoplasm with Papillary like nuclear features (NIFTP) instead of the conventional encapsulated follicular variant of papillary carcinoma. NIFTP is a non-malignant diagnosis rendered on histopathology. Discretionary notes can be added in cytology report to arouse its suspicion and can be useful to guide the surgical management. The histopathological diagnosis of NIFTP for borderline and malignant cases on cytology reduces the risk of malignancies of these categories when calculated in retrospect [9]. Paucity of studies on accuracy and utility of TBSRTC in an Indian scenario in the era of NIFTP after the 2017 revision was instrumental to conduct the present study.

Material and Methods

In this study, all cytology smears of consecutive Thyroid FNAC performed from 2016 June to 2019 December was revaluated and categorized according to TBSRTC 2017. It was conducted in the department of Pathology in a tertiary care centre. Prior permission of ethical committee was obtained. The study population comprised of cytology reports of all patients attending ESIC hospital, Faridabad who were subject to thyroid FNAC.

The criterion for adequacy of the cytology reports included six clusters of benign thyroid follicular cells of at least ten intact follicular cells in each cluster. Abundant colloid in cases of colloid cyst with scant cellularity and paucicellular smears with atypical cells were considered adequate for diagnosis. All subjects who presented with a palpable thyroid swelling of any lobe (diffuse or nodular) of both sexes and of any age, with and without USG guided FNAC were included. Partial thyroidectomy cases, previous surgeries or known malignancies of thyroid were excluded. FNAC was done under aseptic condition using 23G needle by direct needling or using 10cc syringe.

In most of the cases 2 to 3 passes were taken from different areas of the swelling with the patient in supine position and neck extended. The material obtained was wet fixed and air dried for Pap and Giemsa stain respectively. Histopathology was available for 109 patients and these were correlated with cytology. Sensitivity, specificity, Positive predictive values (PPV) and negative predictive values (NPV) and diagnostic accuracy of the FNAC were evaluated using histopathology as the gold standard. ND, category I was excluded in these calculations.

The cases which were Malignant/SOM (Category VI/Category V) or AUS/FLUS (Category III) on cytology which turned out to be malignant on histopathology were considered true positive (TP). The AUS/FLUS (Category III) and SFN/FN (Category IV) on cytology which turned out to be NIFTP on histology were considered TP. Those which were benign (Category II) or SFN/FN (Category IV) on cytology and turned out to be benign on histopathology were considered true negative(TN).Cases which were malignant on histopathology but were benign(Category II) on cytology were false negative (FN). False positive (FP) were those cases which were AUS/FLUS, SOM or malignant on cytology (Category III, category V, Category VI) but were benign on histopathology. ROM for each of the 6 categories were determined.

Results

Of the total of 429 patients who underwent FNAC, Male to female ratio was 1: 5.8. The 3rd decade was most commonly affected with the average age of 37.99 years. The left sided and nodular lesions were more common. The table 1 shows the total number, distribution and diagnosis of cases in cytology and histopathology in each category.

Histopathology was available for 109 cases. Only one case of ND and 70 benign cases on cytology underwent histopathology. Also, 38 cases out of 109 identified either as AUS/FLUS, FN/SFN, SOM and malignancy on cytology were available for histopathology. The sensitivity and specificity of the study was 84.6% and 96.4 % respectively with accuracy of 93.6 %. The positive and negative predictive rates were 88 % and 95.3%.

Table-1: Total number, distribution and diagnosis of cases in cytology and histopathology in each category

Category According to Bethesda	No of cases in cytology (n=429)	Diagnosis in cytology	Distribution of cases in cytology	No of cases received in histopathology (n= 109)	Histopathological diagnosis	Distribution of cases in histopathology
1, Non diagnostic	8	Non diagnostic	8	1	Colloid goitre	1
2, Benign	373	Colloid goitre	182	70	Colloid goiter	46
		Nodular goitre	29		Lymphocytic thyroiditis	6
		Benign Follicular Nodule	58		Multinodular goitre	12
		Lymphocytic thyroiditis	90		Adenomatoid goitre	5
		Hashimotos thyroiditis	13		Granulomatous goitre	1
3, AUS/ FLUS	10	AUS/FLUS	10	5	Follicular adenoma	3
					NIFTP	1
					Papillary carcinoma	1
4, SFN/FN	17	FN/SFN	14	15	Hurthle cell adenoma	2
		HCN/SHCN	3		Follicular adenoma	9
					Colloid goiter with NIFTP	1
					Medullary carcinoma	1
					Follicular variant of papillary carcinoma	2
5, SOM	12	Susp of malignancy	4	11	Papillary carcinoma	4
		Susp of Papillary ca	3		Follicular variant of papillary carcinoma	6
		Susp of follicular variant of papillary ca	4		Medullary carcinoma	1
		Suspicious of medullary ca	1			
6, Malignant	9	Papillary Carcinoma	9	8	Papillary carcinoma	8

Table-2: Cyto histo correlation and Risk of malignancies in relation to NIFTP.

Category	No. of cases in cytopathology	No. of cases received in histopathology	Benign	Malignant	Risk of Malignancy if NIFTP is not malignant (%)	Risk of Malignancy if NIFTP is malignant (%)
Non diagnostic	8	1	1	0	0 %	0 %
Benign	373	70	69	1	1.42%	1.42%
AUS/FLUS	10	5	4	1	20 %	40 %
SFN/FN	17	15	12	3	20%	26.6 %
Suspicious of malignancy	12	11	0	11	100%	100%
Malignant	9	8	0	8	100%	100%

The ROM for each of the six categories were calculated. (Table 2) These values of ROM were initially obtained considering NIFTP as a non-cancer diagnosis. If the two NIFTP cases on histopathology were considered malignant which were AUS/ FLUS and SFN on Cytology then the ROM would have increased to 40% in the category III and 26.6% in category IV (Table 2).

The discordant cases on cytology and histology which are discussed in Table 3.

Table-3: Dis-concordant cases on Cytology and histopathology

Sl. No	Bethesda category	Cytological diagnosis	Histopathogical diagnosis	Comment
1.	ND/UNS	Non diagnostic	CG with cystic change	Scant cellularity with few cyst macrophages predominance of fluid in the lesion
2.	Benign	Hashimotos thyroiditis	Lymphoma	False negative Focal nature of lesion with selective sampling led to missing the malignant diagnosis on cytology
3.	AUS/FLUS	AUS/FLUS	Follicular adenoma	False positive Low cellularity of lesion prevented the diagnosis of FN/SFN on cytology
4.	AUS/FLUS	AUS/FLUS	Papillary Carcinoma(PC)	False negative Follicular lesion with low cellularity and subtle nuclear changes of PC prevented diagnosis of frank PC
5.	AUS/FLUS	AUS/FLUS	NIFTP	Low cellularity and follicular nature of the lesion lead to diagnosis of FLUS and missing of subtle nuclear changes of PC on cytology
6.	FN/SFN	Suspicious of Hurthle cell neoplasm (HCN)	Medullary carcinoma	False negative Cellular follicular lesion with abundant oncocyticchange lead to the diagnosis of HCN on cytology. Oncocytic morphology mimicked the plasmacytoid nature of MC and overlooking of few spindle cells on cytology led to miss the MC diagnosis.
7.	FN/SFN	Suspicious of Follicular neoplasm	Follicular variant of papillary carcinoma	False negative Follicular pattern was appreciated while Subtle and focal nuclear were missed on cytology
8.	FN/SFN	Suspicious of Follicular neoplasm	NIFTP	Lack of nuclear features of papillary and presence of follicular pattern lead to the diagnosis of FN /SFN

Discussion

The thyroid cytology has evolved as first line diagnostic test over the last 30 years for evaluating thyroid nodules as benign and malignant that aids to streamline the clinical management. It is one of the most commonly done cytology next to gynaecological cytology as the thyroid nodules are common palpable lesions [10]. In the last decade, several classification systems were proposed for thyroid cytology which used 4 to 6 categories by various scientific organisation [11]. These were always not concordant with each other. Bethesda system was introduced to overcome the ambiguity by introducing six categories to standardise the reporting and ensure uniformity of interpretation [8]. The present study used TBSRTC which were compared with histopathology wherever available to calculate the diagnostic accuracy.

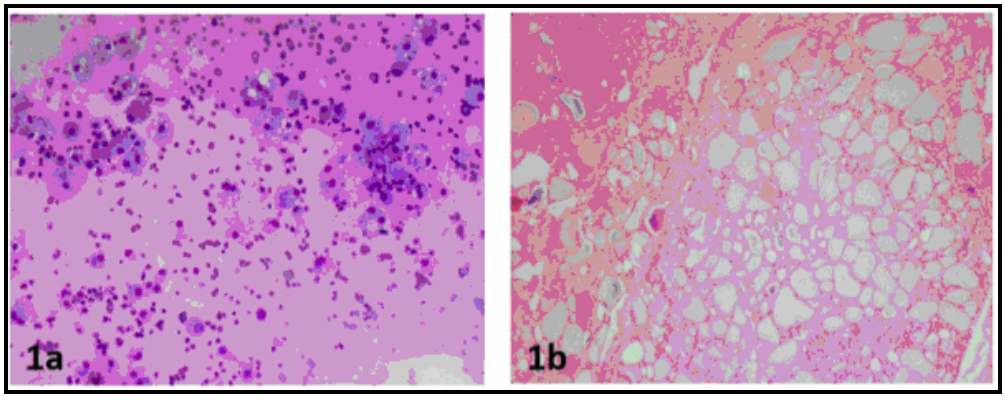
Male: female ratio was 1: 5.8. This finding was concordant with other studies by Handa et al, Murtali et al [4,12-13]. Ratio of males to females was much higher in studies by Silverman et al and Gupta et al [14-15]. The age range was 6 to 83 years, with average age of 37.99 years which correlates with studies by Handa et al, Mandal et al, Bamanikar et al and Nandekar et al.[12,16-18]. Thyroid diseases are most commonly noted in the females of the reproductive age groups who are middle aged [19]. Similar trend was noted in the present study.

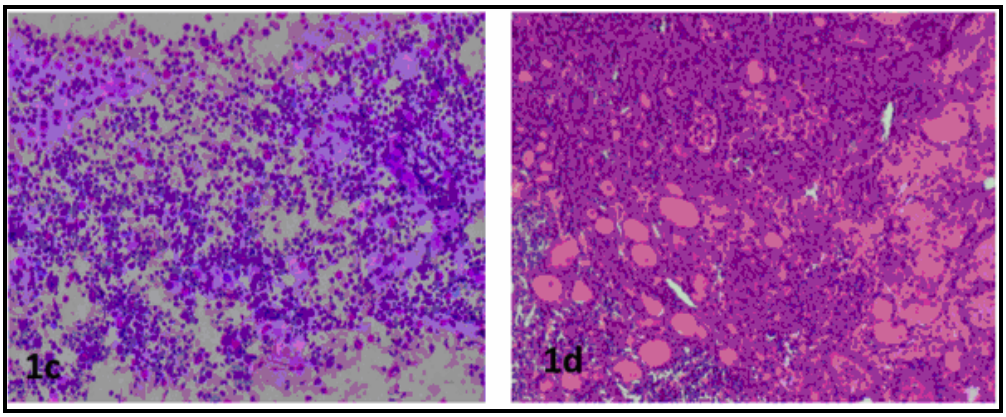
The number of ND/UNS cases in the present study was 1.8 % which is in concordance with studies by Mandal et al [16]. This can be attributed to the fact the trained cyto-pathologist are performing the procedure of FNAC in our institute and also the expertise of the pathologist screening the slides and interpretation of the categories thereby reducing the number of cases in category I.

As per the NCI guidelines of 2007, bloody aspirate, thick smears and paucicellular smears were categorised as ND or unsatisfactory (UNS). Cyst fluid only samples comprising of macrophages only were ND /UNS which needed clinicoradiological follow up. In our study also, these were categorised as benign if no abnormality was detected on ultrasonography. Those with suspicion on ultrasonography (USG) were categorised as ND/UNS and were subjected to repeat Ultrasound guided FNAC. Out of 8 cases, 7 cases turned out to be benign on USG or USG guided FNAC. Only 1 case underwent surgical excision. The facility of USG guided FNAC for small and less palpable nodule also improved the aspirate quality and increased the diagnostic accuracy.

The number of benign cases in our study was 86.9%.(373/432) (Table 2)with predominance of colloid goitre (Fig 1a,1b) followed by lymphocytic thyroiditis (1c,1d),benign follicular nodule and hashimotos thyroiditis along with one case of granulomatous thyroiditis. Of the 373 benign cases 70 cases were subject to excision mainly to counter the compression symptoms .Only one case turned out to be lymphoma which was diagnosed as hashimotos thyroiditis on cytology (Fig 2a,2b) and the remaining were benign. The focal nature of the lesion and selective sampling led to missing the diagnosis on cytology. The large number of benign cases may be attributed to the fact that patients come to the hospital directly without being referred. The general population is mainly represented in our study. Similar findings were noted in studies by Mandal et al (87.5 %) and Nandedkar et al (82.67%) in the Indian population [16, 18]. Several non-Indian Studies by Yassa et al, Nayar and Ivanoic et al Jo et al, and have reported 66%,64% and 59% of benign cases respectively which were much lower.[6, 20-21].

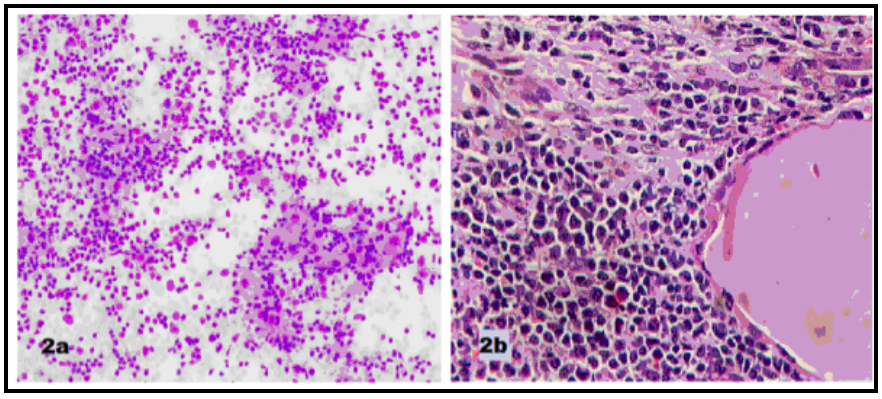
Fig-1a: Thyroid Fna shows singly lying follicular epithelial cells along with cyst macrophages in a background of thin colloid (10x, MGG).
Fig-1b: Photomicrograph shows variable size of follicles lined by flattened epithelium and filled with colloid.
Fig-1c: Thyroid FNA shows scattered follicular epithelial cells along with fair number of lymphocytes in a haemorrhagic background. (10X, MGG).
Fig-1d: Photomicrograph shows numerous follicles lined by flattened epithelial cells and are completely surrounded by lymphoid cells. (100X, H &E).

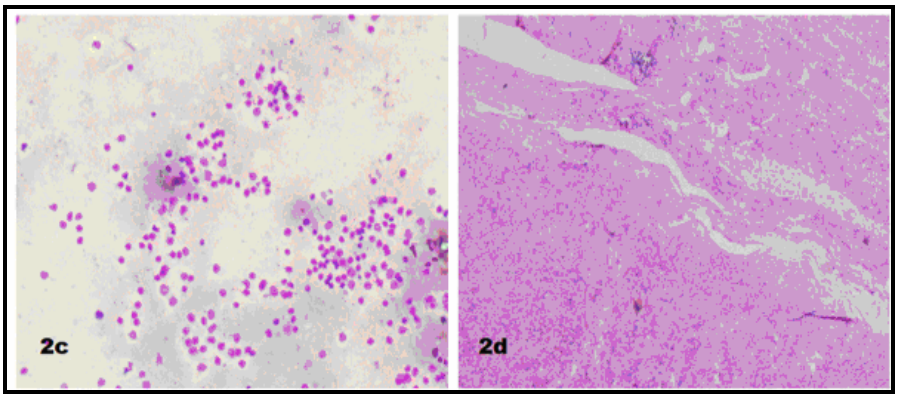




The number of cases in AUS category in the present study is 2.3 % which ideally should be less than 7 percent [6, 22]. Those cases which couldn't be classified as benign, suspicious or malignant were labelled as AUS/FLUS which is a category of last resort and is heterogeneous. This is in concordance with studies by Jo et al (3.4%), Yassa et al (4%) and Nayar and Ivanoic et al (8 %) [6, 20-21]. These lesions were advised for clinical correlation and repeat FNAC after 3 to 6 months with follow up surgery if repeat FNA was AUS or worse. [8, 23]. The lesions were categorised based on atypical nuclear and architectural features into this indeterminate and borderline category. The categorisation is subjective to an extent and overlapping cytological features of follicular lesions of benign and suspicious of malignancy added to it. Out of 10 cases in AUS /FLUS repeat FNAC of 5 cases showed benign lesions. The remaining 5 cases remained AUS on repeat FNAC and when subjected to histopathology showed follicular adenoma (Fig2c,2d) in 3 cases while one case was NIFTP (3a,3b) and one was Papillary carcinoma (PC). In the PC case, scant cellularity with presence of very few cells showing nuclear features of PC restricted its malignant diagnosis, a paucicellular smear with only focal nuclear features of papillary in an otherwise benign appearing follicular lesion prevented the NIFTP case to be diagnosed as Follicular Variant of Papillary thyroid Carcinoma (FVPTC). The three FA cases were moderately cellular with slight predominance of micro follicles but not sufficient to call it as SFN. Mandal et al noted only 1% of lesions in the AUS/FLUS category possibly due to strict adherence of the AUS/FLUS criterion [16].

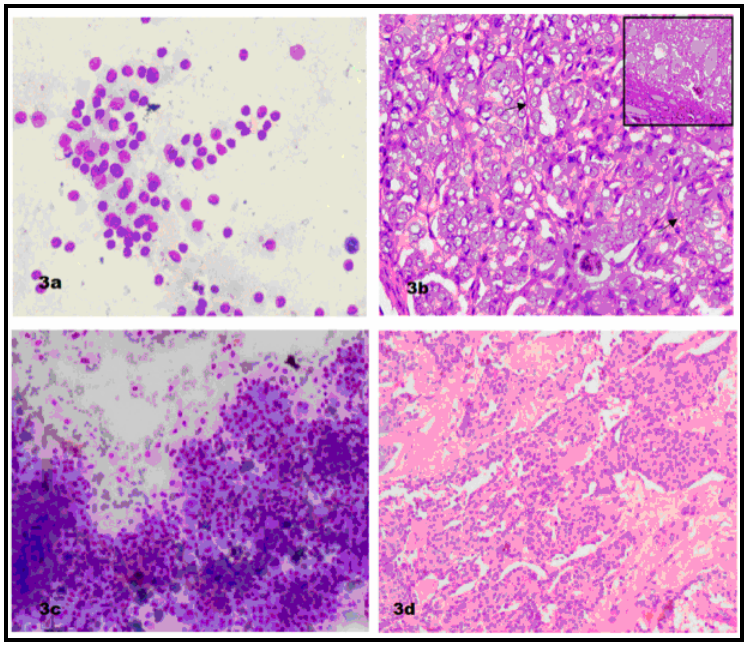
Fig-2a: Thyroid fna shows follicular epithelial cells with few showing hurthle cell change admixed with dense lymphocytic infiltrate (100x, mgg)
Fig-2b: Section shows sheets of medium sized monomorphic population of lymphoid cells surrounding colloid filled follicles (200x, H &E).)
Fig-2c: Smear shows thyroid epithelial cells arranged in follicular pattern with few showing microfollicles. (100x, mgg)
Fig-2d: Photomicrograph shows well encapsulated thyroid tumor composed of uniform sized follicles along with normal thyroid tissue outside the capsule.(40x, H &E).





The SFN/FN category included 4% cases in our study. This in slightly higher in number when compared to a studies by Mandal et al (1.4%), Nandekar et (1.9%) and Mehra and Verma (2.2%). [6,18,24]. The thyroid FNAC is not diagnostic but is a screening test for follicular carcinomas and hurthle cell carcinomas. Most laboratories prefer to use SFN instead of FN as about 35% of lesions are hyperplastic follicular proliferations only and not neoplasms. Most of the lesions are FA or adenomatous nodule in Mutinodular goitre [8, 22].Out of the 17 cases in category IV in cytology, 15 were subject to histopathology and 2 cases were lost to follow up. On histopathology, 9 cases were of FAs and2 were of hurthle cell adenoma (HCA) while 1each was CG with NIFTP and medullary carcinoma (MC) and two were FVPTC. Hence more than 70 percent cases are adenomas and it correlates with the fact that adenomas outnumber the malignancies in this category [8]. The MC and FVPTC were the malignant entities comprising 20% of cases. This is in accordance with the fact that only 15 to 30% cases of FN/SFN are malignant [6, 8, 22, 25].

Fig-3a: Smear shows sheets of follicular epithelial cells forming follicles showing anisonucleosis and nuclear overlapping. (200x, MGG)
Fig-3b: Section tumour composed of follicles with nuclear clearing and grooves but no papilla and intranuclear cytoplasmic inclusion, inset showing colloid goitre (200x, H &E)
Fig-3c: Smears shows sheets of round to ovoid cells having abundant granular cytoplasm with cells arranged in sheets and follicles.
Fig-3d: Photomicrograph shows solid sheets of round to polygonal cells, granular cytoplasm along with amyloid deposition.(200x, H&E)

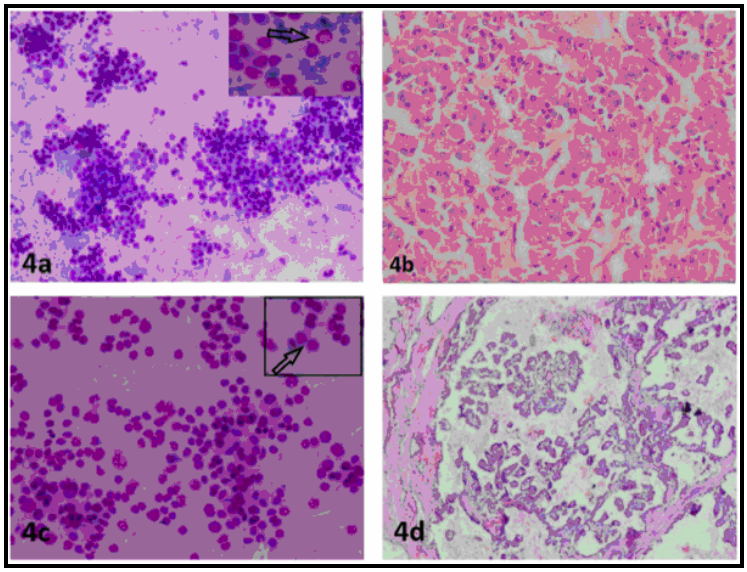


MC was misdiagnosed as hurthle cell neoplasm on cytology.(fig 3c,3d) The predominance of plasmacytoid cells with moderate amount of cytoplasm was mistaken for hurthle cells. Oncocytic variant of MC is a mimicker of hurthle cell tumor [26]. FVPTC comprises the most common malignant diagnosis in category IV. As the nuclear features of FVPTC are mild and focal these are falsely diagnosed as suspicious of follicular neoplasm (SFN). The follicular architecture was seen along with few and focal cells showing the nuclear features of PC like pale powdery chromatin and nuclear grooves but intranuclear cytoplasmic inclusions are rare.

In this study also the NIFTP case which is the encapsulated non- invasive follicular variant of Papillary thyroid carcinoma was falsely diagnosed as SFN/FN. Many cases which are reported as SFN/FN are actually FVPTC [25, 27-28].

The SOM cases in our study is 2.8 % which similar to the studies by Nayar and Ivanoic (2%), Jo et al (2.3%), Mufti et al (2.4%) [20-21, 29]. The nuclear features of FV of PTC are usually mild and localised [8]. The sampling may be partial and only focal nuclear features may be present or aspirate may be paucicellular. This variant of PC poses diagnostic difficulties. In such cases, malignant diagnosis cannot be made with confidence. Such difficulties were noted in the present study as well. Of the 12 cases of SOM, six cases were of FV of PTC of which two showed oncocytic change (fig 4a, 4b) while one was Medullary carcinoma and four others were PTC.(fig 4c,4d).

Fig-4a: Monolayer of sheets of follicular cells which have powdery chromatin having abundant oncocytic cytoplasm and few showing intranuclear inclusion (inset) (100x, MGG).
Fig-4b: Photomicrograph shows oncocytic cells with enlarged pale oval nuclei and occasional cells showing nuclear grooves (100x, H &E).)
Fig-4c: Papillary tissue fragment (left) and sheets of cells which are ovoid with pale nucleus with powdery chromatin and intranuclear groves (inset) (100x, MGG).
Fig-4d: Papillary fronds with fibrovascular core, lined by low columnar cells having optically clear nucleus. (100x, H &E).)



FNAC is diagnostic for PTC, MC, undifferentiated carcinoma and lymphoma [10]. The number of malignant cases in our study was 2.1%.Of the9 cases diagnosed on cytology as PTC, 8 were subject to histopathology all of which were confirmed as Papillary carcinoma. PTC can be diagnosed with certainty in thyroid FNA based on characteristic nuclear and cyto architectural features. PC is the commonest malignancy of thyroid and the follicular variant being the commonest type [10]. The findings of our study were in contrast to studies by Yassa (5%), Nayar and Ivanovic (5%) Jo et al (7%), and Mandal et al (4.7%)

[6, 16, 29] who noted higher rates of malignancy on cytology. However, the incidence of malignancy in thyroid nodules in the general population is 0.1% [30].

The experience and the confidence of the cytopathologist are of immense value to quote a lesion as malignant and type it on cytology at the first instance. Many a times the pathologist prefers to use the term SOM and put it under category V. We had a case of MC thyroid which was put in category V and diagnosed as SOM and not typed as MC on cytology. The ROM in the present study is comparable to the other studies by Yassa et al Mandal et al, Jo et al ,Yang et al, Mufti et a land Garg et al.[6, 16, 21-22, 29, 31](Table 4). These studies were prior to the NIFTP era and hence would have considered EFVPTC as a malignant diagnosis. Our values were closest to the study by Mandal et al [16]. Results of various studies have shown the sensitivity of the thyroid FNAC ranges from 50 to 97% while the specificity ranges from 74.9% to 100 % [18]. The sensitivity of our study was 84.6% while the specificity was 96.4 % which are comparable to other studies [13,15-16,22,24,31-32] (Table 5).

Table-4: Comparisons of ROM of different studies

Category	Present study	Mondal et al [16]	Garg et al [31]	Mufti et al [29]	Yang et al [22]	Jo et al [21]	Yassa et al [6]
ND/UNS	0 %	0%	20%	20%	10.7%	8.9%	10%
BN	1.4%	4.5%	0%	3.1%	0.7%	1.1%	0.3%
AUS	20%	20%	25%	50%	19.2%	17%	24%
SFN	20%	30.6%	20%	20%	32.2%	25.4%	28%
SM	100%	75%	66%	80%	64.8%	70%	60%
MGTS	100%	97.8%	100%	100%	98.4%	98.1%	97%

Table-5: Comparisons of sensitivity and specificity with other studies

Studies	Sensitivity	Specificity
Present study	84.6	96.4
Mehra et al [24]	-76.9	88.5
Gupta et al [15]	80	86.6
Mondal et al [16]	86.6	87
Murtali et al [13]	87.1	64.6
Garg et al [31]	88.89	84.31
Yang et al [22]	94	98.5
Bukhari et al [32]	100	82.5

Diagnostic accuracy of thyroid FNAC ranges from 80 to more than 90 percent in competent hands [22]. This is in concordance with accuracy of this study which is 93.6 %. The study showed good concordance of sensitivity, specificity, accuracy and risk of malignancies with other studies establishing the fact that application of TBSRTC is a successful step to bring about uniformity and to standardise thyroid cytology reporting, It aids to provides a simple, clear and common platform of communication of pathologist with the referring clinicians to plan the management protocol universally. The Inclusion of NIFTP as an indolent entity reduced the ROM in category III and IV. This is apilot study andhence larger studies with more no. of cases for histopathological correlation from tertiary care centres in India will improve the diagnostic accuracy. It aims to disseminate TBSRTC at all national labs and facilitate sharing of data at national and international levels.

Conclusion

The study showed good concordance of sensitivity, specificity, accuracy and risk of malignancies with other studies establishing the fact that application of TBSRTC is a successful step to bring about uniformity and to standardise thyroid cytology reporting, It aids to provides a simple, clear and common platform of communication of pathologist with the referring clinicians to plan the management protocol universally. The Inclusion of NIFTP as an indolent entity reduced the ROM in category III and IV.

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*All correspondences to: Dr. Kanika Singh, Assistant Professor, Department of Pathology, University College of Medical Sciences (University of Delhi) & Guru Teg Bahadur Hospital, Dilshad Garden, Delhi-110 095, India. E-mail: kanikasingh263@gmail.com