COMPARISON OF DIAGNOSTIC EFFICACY BETWEEN CONVENTIONAL CYTOPATHOLOGY & LIQUID BASED CYTOLOGY IN THYROID FNA SMEARS USING BETHESDA SYSTEM OF REPORTING

Swagatika Agrawal[1], Pranita Mohanty[2], Raghumani Mohanty[3]

[1]Assistant Professor, Department of Pathology, Hi-Tech Medical College & Hospital, Bhubaneswar.
[2]Associate Professor, Department of Pathology, IMS & SUM Hospital, Bhubaneswar.
[3]Professor, Department of Pathology, Hi-Tech Medical College & Hospital, Bhubaneswar.

ABSTRACT

BACKGROUND
Conventional cytology is a minimal invasive procedure used for all palpable lesions. Liquid Based Cytology (LBC) is a modified technique used in the current study.

The objective of the study is to find out the diagnostic accuracy of Liquid Based Cytology (LBC) as compared to Conventional FNAC (CP) in thyroid cases.

MATERIALS AND METHODS
A hospital-based cross-sectional study was conducted in eastern India. All thyroid swelling cases referred for FNAC were included. Results were captured with general profile of the patients. Data was analysed for diagnostic accuracy using Stata.

RESULTS
Concordance rate between LBC and CP was 70.8% (highest for malignancy (92.8%)). Sensitivity CP and LBC was 82.4(56.6 – 96.2) and 75.0% (47.6 – 92.7) respectively, and specificity was 100.0(93 – 100.0) and 97.1% (74.6 – 97) respectively. PPV and NPV for CP were 100.0% (76.8 – 100.0) and 94.4% (84.6 – 98.8) respectively and for LBC were 92.3% (64.0 – 99.8) and 89.2% (74.6 – 97.0) respectively. LR+ in LBC was found to be high (25.5).

CONCLUSION
LBC can help ruling in malignancy in thyroid but FNAC (CP) remains the procedure of choice.

KEYWORDS
Fine Needle Aspiration Cytology, Thyroid Gland, Bethesda System, Diagnostic Accuracy, Predictive Value, Concordance Rate, Clinical Pathology General.

BACKGROUND
Fine needle aspiration cytology (FNAC) is well established as the first line diagnostic tool for evaluation of palpable head & neck swellings. In the past open surgical biopsy was practiced yielding a definitive histopathological diagnosis. FNAC of thyroid lesion is a merely painless, rapid, direct & safe adjunctive procedure for obtaining material for cytologic analysis. FNAC leave no scars & it also has a very low risk of seeding of tumours along the needle tract. The procedure is cost effective, minimally invasive & free of complications, well tolerated by the patient, can be done on an outpatient basis, repeatable and most important aspect & avoidance of surgery in situation, like non-neoplastic or inflammatory conditions.[1-3]

Currently this technique is practiced worldwide for thyroid lesion. It has got high sensitivity and specificity rates.[4] Diagnosis is usually done by conventional cytopathology (CP).

Liquid-based cytology (LBC) is based on the use of a semi-automated device for collecting and processing both gynaecologic and non-gynaecologic cytologic specimen. Its diagnostic sensitivity is as accurate as conventional preparations, especially due to its excellent cell preservation (especially cells in suspension and smears) and lack of undesired background. It provides cells in monolayer, superior to direct smear.[5,6]

Though LBC cannot replace conventional cytopathological diagnosis, the confusions in diagnostic terminology and clinician perception of its inconsistency was addressed in 2007 by the National Cancer Institute (NCI) who hosted thyroid FNA state of the science conference wherein the terminology & morphologic criteria for repeating thyroid FNA were concluded thus forming the framework for The Bethesda System of Reporting Thyroid Cytopathology (TBSRTC).[7,8] The system improves the clarity of

communication among cytopathologists and other health care providers, predicts the cancer risk & reduces unnecessary surgery for patients of benign nodules & appropriately triages patients with malignant nodules for timely clinical intervention.[9,10]

Objectives

To find out the different pathological diagnoses using liquid based cytology preparation, along with its concordance rate and diagnostic accuracy compared to fine needle aspiration cytology for cases of thyroid swelling at a tertiary care centre of Odisha, India.

MATERIALS AND METHODS

The study was cross-sectional in nature and was conducted in the department of pathology (of the primary author) of a tertiary care center located at Bhubaneswar, Odisha, India from July 2013 to October 2015. All the patients attending different outpatient departments of the hospital with thyroid swellings were referred for FNAC. Universal sampling method was used for the study.

Inclusion Criteria included patients who opted for fine needle aspiration cytology and having thyroid swelling. Age and sex were not deciding factors for excluding the patients. But those patients with inadequate material for interpretation during cytology and those not willing for these invasive procedures for obtaining their samples even after explaining the purpose, utility and consequence of the procedure were excluded from the study.

Data were collected using a pre-designed and pre-tested tool for data collection which included specific components for each case like general profile of the patients (age, gender, chief complains, duration of swelling, etc.) and results of each case after doing FNAC using CP and/or LBC. Results by CP and LBC after FNAC were also compared with histopathology wherever possible. Data were then entered in excel sheet, imported and analysed using licensed version of Stata SE v 12.1 for diagnostic accuracy of the test using sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratios. Concordance rate of LBC with FNAC was also calculated.

Sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, and accuracy were calculated using Stata SE v. 12.1 while accuracy was calculated using the formula below:

$$Accuracy = \frac{TP + TN}{Total\ number\ of\ cases} \times 100$$

RESULTS

A total of 295 consecutive thyroid FNACs were examined during the study period using, Conventional Preparation (CP). Out of these, 120 (40.68%) cases were prepared by LBC and were compared with CP. Histopathological findings were available in 68 (23.05%) cases, those who were operated or gave consent for tissue biopsy. But LBC was

available for comparison with HP in only 50 cases. A detailed clinical history was included for every case before obtaining the sample. General profile along with important clinical features of the participants are given in table 1. Mean age of the study participants was found to be 35.5 ± 0.7 years (Fig. 1).

Sl. No.	Parameter	No. s (%)
1	**Age**	**35.5 ± 0.7 years[#]**
2	**Gender**	
	Female	269 (91.2)
	Male	26 (8.8)
3	**Duration of Symptoms**	**23.95 ± 2.82 months[#]**
4	**Mean Tumor size^**	
	≤ 4 cms	216 (73.7)
	>4 cms	77 (26.3)
5	**Consistency of tumor^**	
	Soft	18 (6.2)
	Firm	163 (55.6)
	Mixed	95 (32.4)
	Cystic	14 (4.8)
	Hard	3 (1.0)
6	**Tenderness^**	
	Present	33 (11.3)
	Absent	260 (88.7)
7	**Distribution of cases^**	
	Solitary	248 (84.6)
	Multi-nodular	45 (15.4)
8	**Type of aspirate**	
	Haemorrhagic aspirate	144 (48.8)
	Blood mixed colloid	117 (39.7)
	Frank colloid	34 (11.5)
9	**Diagnostic rate**	
	Confirmed diagnosis	263 (89.2)
	Non-conclusive	32 (10.9)
	Table 1. General Profile and Clinical Features of the Study Participants (n=295)	

[#]Represents Mean ± SE of mean; ^ n = 293 as thyroid swelling was not palpable in 2 cases.
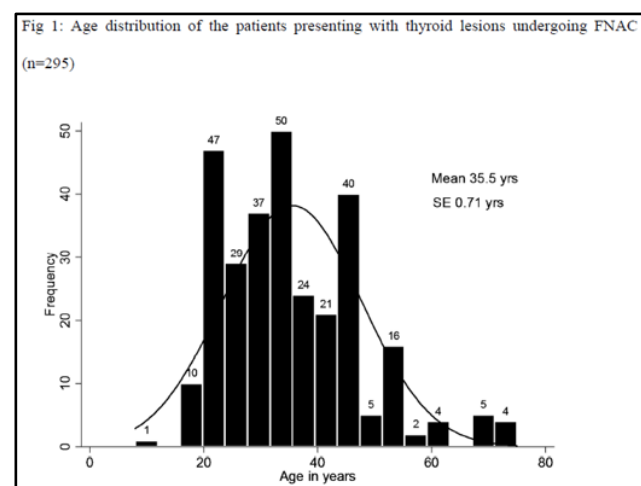

Figure 1

Routine cytological diagnosis done for the study participants yielded colloid goitre (62.0%) as the most

common form of thyroid swelling in routine CP and was also the most common type in LBC though the proportion was remarkably less (33.3%) (Table 2). Atypia of undetermined significance was 5% of LBC but was nil in routine FNAC (CP).

Similar was the scenario for non-diagnostic cases which was seen only in LBC cases (12.5%) (Table 2).

Sl. No.	Diagnosis	Routine FNAC (n=295) No. of Cases (%)
1	Colloid goiter	183 (62.0)
2	Hyperplastic nodule / Adenomatoid goiter	40 (13.6)
3	Granulomatous thyroiditis	5 (1.7)
4	Acute Thyroiditis	0 (0.0)
5	Hashimoto's thyroiditis	32 (10.9)
6	Follicular neoplasm	13 (4.4)
7	Papillary Carcinoma	17 (5.8)
8	Anaplastic Carcinoma	2 (0.7)
9	Medullary Carcinoma	3 (1.0)

Table 2. Diagnosis of the Cases Using Routine Fine Needle Aspiration Cytology and Liquid Based Cytology

Grouping the diagnosis using Bethesda classification showed that benign lesions were the most common form of thyroid cases (75.6% in CP and 56.7% in LBC). Malignant cases were found comparatively more in LBC than routine FNAC (15.0% vs 7.5%) as was suspicious for malignancy and follicular neoplasm cases (Table 3). Non-diagnostic cases also contributed to around one-tenth of the cases in both methods as can be seen from table 3.

Categories	Routine FNAC (n = 295) No. (%)	LBC (n = 120) No. (%)
Group 1: Benign	223 (75.6)	68 (56.7)
Group 2: Atypia with Undetermined Significance (AUS)	3 (1.0)	6 (5.0)
Group 3: Suspicious for Follicular Neoplasm (SFN)	13 (4.4)	10 (8.3)
Group 4: Suspicious for malignancy	2 (0.7)	3 (2.5)
Group 5: Malignant	22 (7.5)	18 (15.0)
Group 6: Inadequate/Non-diagnostic	32 (10.8)	15 (12.5)

Table 3. Diagnostic Classification of the Thyroid Cases as Per Bethesda Classification

A comparison of all the cases diagnosed by LBC was done with that by routine FNAC (CP) on one to one basis to find out the concordant and discordant rate. It was seen that the overall concordant rate for diagnosing all cases of thyroid swellings by LBC as compared to routine FNAC was 70.8% (table 4), and this was found to be highest for malignancy cases (as per Bethesda) accounting to 92.8% (table 4). Thus, the discordant rate by LBC was found to be minimum whenever these were cases of malignancy.

Sl. No.	Bethesda reporting	Diagnosis on LBC	No. (%)	Concordance with Routine FNAC (%)
	Benign		**68 (56.7)**	**45 (66.2)**
1		Colloid nodule	19 (15.8)	14 (73.6)
2		Colloid goitre	15 (12.5)	4 (26.7)
3		Adenomatoid/ Hyperplastic goitre	10 (8.3)	7 (70.0)
4		Benign follicular nodule	5 (4.2)	5 (100.0)
5		Granulomatous thyroiditis	2 (1.7)	2 (100.0)
6		Thyroiditis	4 (3.3)	2 (50.0)
7		Lymphocytic thyroiditis	12 (10.0)	11 (91.7)
8		NCG	1 (0.8)	0 (0.0)
	Atypia of Undetermined Significance		**6 (5.0)**	**3 (50.0)**
9		AUS	6 (5.0)	3 (50.0)
	Suspicious for Malignancy		**3 (2.5)**	**1 (33.3)**
10		Suspicious carcinoma	3 (2.5)	1 (33.3)
	Malignancy		**28 (23.3)**	**26 (92.8)**
11		Papillary carcinoma	15 (12.5)	15 (100.0)
12		Follicular carcinoma	10 (8.3)	9 (90.0)
13		Medullary carcinoma	2 (1.7)	1 (50.0)
14		Anaplastic carcinoma	1 (0.8)	1 (100.0)
	Non-diagnostic		**15 (12.5)**	**10 (66.7)**
15		Inadequate smear	15 (12.5)	10 (66.7)
	Total		**120 (100.0)**	**85 (70.8)**

Table 4. Comparison and Diagnostic rates of LBC with Bethesda and FNAC Reporting (n=120)

One case was Suspicious for follicular neoplasm (SFN) as per Bethesda. There were 43 cases under the benign cytologic category in CP. Both CP and the histologic diagnosis were consistent with a benign diagnosis in 40 cases that were true-negative for malignancy (Table 5). Overall diagnostic accuracy for diagnosing malignancy using CP among thyroid lesions showed a sensitivity of 82.4% (56.6 – 96.2) and specificity of 100.0% (93 – 100.0). The area under the curve (AUC) was 0.912 (0.818 – 1.00). The PPV and NPV were found to be 100.0% (76.8 – 100.0) and 94.4% (84.6 – 98.8) respectively. LR- was moderately sufficient to rule out disease (0.176) (Table 6).

There were 29 cases under the benign category in LBC. Both the LBC and histologic examination were consistent

with benign diagnosis in 25 cases that were true-negative for malignancy (Table 7). Overall diagnostic accuracy for diagnosing malignancy among thyroid lesions using LBC showed a significant AUC value of 0.86 (0.747 – 0.974). Sensitivity was found to be 75.0% (47.6 – 92.7) while specificity was 97.1% (74.6 – 97). PPV and NPV were 92.3% (64.0 – 99.8) and 89.2% (74.6 – 97.0) respectively. LR+ and LR- were found to be 25.5 and 0.258 respectively (Table 6). Thus, this test was very helpful for ruling in malignancy cases (One with positive result for malignancy on LBC had high chances for ruling in cases of malignancy).

Diagnosis in CP@	Nos. Diagnosed	True Positive	False Positive	False Negative	True Negative
Carcinoma	25	14	0	0	11
Papillary	9	9	0	0	0
Medullary	2	2	2	2	2
Anaplastic	1	1	0	0	0
Follicular	13	2	0	0	11
Benign	43	0	0	3	40
Total	**68**	**14**	**0**	**3**	**51**
(For malignancy)					
Table 5. Diagnostic Accuracy of Cytopathology as Compared to Histopathology (Gold Standard) (n=68)					

@All cases considered for diagnostic accuracy are considered for possibility of carcinoma (TP, FP, TN, FN are True positive, False Positive, True Negative and False Negative respectively as compared to gold standard i.e. Histopathology)

Diagnostic method	Sensitivity	Specificity	PPV	NPV	LR+	LR-	Accuracy
CP	82.4%	100.0%	100.0%	94.4%	-	0.176	96%
LBC	75.0%	97.1%	92.3%	89.2%	25.5	0.258	90%
Table 6. Diagnostic Accuracy of FNAC and LBC as Compared to HISTOPATHOLOGY (Gold Standard) (n=68 for CP, n=50 for LBC)							

Diagnosis in LBC@	Nos. Diagnosed	True Positive	False Positive	False Negative	True Negative
Carcinoma	21	12	1	0	8
Papillary	7	7	0	0	0
Medullary	2	1	1	0	0
Anaplastic	1	1	0	0	0
Follicular	11	3	0	0	8
Benign	29	0	0	4	25
Total	**50**	**12**	**1**	**4**	**33**
(For malignancy)					
Table 7. Diagnostic Accuracy of LBC as Compared to Histopathology (Gold Standard) (n=50)					

@All cases considered for diagnostic accuracy are considered for possibility of carcinoma (TP, FP, TN, FN are True positive, False Positive, True Negative and False Negative respectively as compared to gold standard i.e. Histopathology).

DISCUSSION
Studies conducted on thyroid lesions have usually reported female to male cases of thyroid in the ratio of around 4-5:1, 11, 12 but in the current study the ratio was found to be more than 10:1 (91% females to 9% males). But the age group to which the thyroid cases belonged were almost the same as in studies conducted elsewhere.[11,12]

FNAC usually has its inherent limitations related to inadequate sampling, inability to distinguish between benign and malignant follicular lesions in the absence of nuclear features of papillary carcinoma, etc. For correctly characterizing the group of indeterminate lesion or follicular neoplasms, LBC was introduced. Since its introduction, the LBC processor has gained popularity as a collection and preparation technique for fine needle aspiration biopsy. LBC has its own advantages and disadvantages, with disadvantages limited if adequacy of sample is ensured.[13] It allows specimens to be collected in an alcohol-based preservative solution thereby eliminating air-drying artefacts, thus reducing unsatisfactory results as shown in some studies.[14] The solution aids in the disintegration of red blood cells and mucus that may interfere with cytologic preparation. Additionally, it has been reported that screening time is reduced, although processing time increased.

Other methods of diagnosis may be used in the diagnosis of thyroid lesions. Non-invasive methods like ultrasound have shown 90% sensitivity, 98.8% specificity with 90% positive predictive value in some studies (compared to histopathology as a gold standard).[12] Invasive techniques like CP have a sensitivity of 92.8%, specificity 94.2%, and positive and negative predictive values of 94.9% and 91.8% respectively with an overall total accuracy of 93.6%.[11]

Studies in India on Bethesda reporting systems conducted by Mehra et.al. On 225 cases of thyroid nodules have identified that only 7.2% were ND/UNS and the majority (80.0%) were benign. Malignant cases were limited to 2.2%, and suspicious for malignancy were 3.5%. 4.9% were AUS/FLUS, and 2.2% FN.[9]

The findings of the present study are quite similar to the findings of Hawkins et.al., where they had estimated a sensitivity of 85%, specificity of 95% and accuracy of 94%.[15] Another study conducted by Mamoon et al. (n=327) had inconclusive results in 18 (5.5%), benign in 230 (70.3%), suspicious in 64 (19.6%) and malignant in 15 (4.6%) cases. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of fine needle aspiration cytology (n=59) was calculated and correlated from patients undergoing thyroid surgery and was found to be 85.7%, 73.3%, 50%, 94.2% and 76.2% respectively, probably due to a small sample size.[16] Studies with a larger sample has mostly shown higher sensitivity and specificity.[4,17]

CONCLUSION

Benign group of lesions were the most common form of diagnosis as per Bethesda classification (both by CP and LBC). Concordance rate of liquid-based cytology was found to be around 71% overall and was highest in cases of malignancy (concordance 93%) as per Bethesda classification. LBC was found to have high specificity and with a strong positive likelihood ratio of 25.5 it increases the chances of ruling in disease, and had an overall accuracy of 90% as compared with the gold standard. But with higher accuracy along with 100% specificity and PPV, CP may always be regarded as the choice of diagnostic procedure as compared to LBC. In situations demanding less screening time, avoiding artefacts and errors in specimen preparation, and confirming cases of thyroid malignancy LBC may be considered as the method of choice.

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