

Comparative Evaluation of Conventional Smear and Liquid Based Cytology in Diagnosis of Thyroid Lesions Using Bethesda System

Sumiti Gupta, Ajay Kumar, Renuka Verma*, Rajnish Kalra, Veena Gupta, Meenu Gill and Rajeev Sen

Pt. B.D.S, PGIMS, Pathology, Rohtak, Rohtak, Haryana, India

*Corresponding author: Verma R, Pt. B.D.S, PGIMS, Pathology, Rohtak, Rohtak, Haryana, India, Tel: 8295953200; E-mail: renuka138pathology@gmail.com

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Abstract

Introduction: The prevalence of palpable thyroid nodules in the worldwide population ranges from 4 to 7%. Approximately 95% of these lesions are benign and thyroid cancer is responsible for only 0.4% of all cancer deaths. The main cytological goal is to distinguish malignant lesions, which require appropriate surgical procedures, from benign nodules which, on the contrary, may be often treated conservatively. The Bethesda system for reporting thyroid cytology allows standardization in reporting with improved diagnostic terminology between cytopathologists and clinicians, and leads to more consistent management approaches.

Materials and Methods: In this prospective study, 60 cases with palpable thyroid lesions were considered. After detailed history, clinical examination and informed consent of patient, FNA of thyroid lesion was performed under sterile aseptic condition. Comparative study of Conventional smear (CS) and Liquid based cytology (LBC) was done using a standardized nomenclature by Bethesda system. The results were correlated with clinico-imaging diagnosis and histopathology wherever possible.

Results: LBC smears contained adequate diagnostic cells in most cases and were greatly superior to CS regarding the absence of background blood-debris, and recognition of nuclear and cytoplasmic details ($p < 0.001$). LBC showed cellularity, preserved cell architecture, and informative background as good as CS, expressed by no statistically significant differences ($p = 0.297, 0.083, \text{ and } 1.000$ respectively). In LBC amount of colloid was diminished and appear dense, fragmented, and in droplets.

Conclusion: LBC is also a relatively simple technique; reducing the number of slides and area per slide to be screened than the conventional preparation. However, caution must be applied to interpret the slides and secure a diagnosis, especially if the LBC is the first and only method applied as adequate experience is required to familiarize with various lesions on LBC. Other aspect to be considered is cost efficacy.

Keywords: Liquid based cytology; Conventional smear; Bethesda system; Thyroid lesions

Introduction

The prevalence of palpable thyroid nodules in the worldwide population ranges from 4 to 7%. Approximately 95% of these lesions are benign and thyroid cancer is responsible for only 0.4% of all cancer deaths. The sampling of tissue with needles for the purpose of investigating neoplastic processes has been reported over the past 160 years [1].

Fine Needle Aspiration Cytology (FNAC) represents an invaluable diagnostic tool for characterizing thyroid nodules with a worldwide consensus for its simplicity, safety and regarded as the most accurate and cost effective method for the selection of surgical patients. The liquid based cytology technique, originally developed for application to gynaecologic cervical smears, has progressively gained consensus for both non gynaecologic and fine needle aspiration cytology material [2].

In the year, 2007 the national cancer Institute, Bethesda, Maryland, US, organized the NCI Thyroid Fine Needle Aspiration State of the Science Conference, and an initiative was undertaken to public an atlas

and guidelines using a standardized nomenclature for the interpretation of thyroid FNAs, known as the Bethesda system for reporting thyroid cytopathology [3]. The atlas describes the six diagnostic categories of lesions: Non-diagnostic or unsatisfactory, benign, atypia of undetermined significance/atypical Follicular Lesion of undetermined significance (AFLUS), follicular Neoplasm/Suspicious for follicular neoplasm (SFN), suspicious for malignancy (SM), and malignant.

Controversy exists about the relative value of liquid based preparations (LBPs) versus conventional smears (CS) for the evaluation of thyroid fine needle aspirations. Proponents of CS note the simplicity and lack of expense, retention of important background clues that can be lost in LBPs. However, CS is tedious and time consuming to screen due to non-uniform slide preparation and fixation.

The advantages of LBC include improved sensitivity and specificity because fixation is better and nuclear details are well preserved. Abnormal cells are not obscured or diluted by other epithelial or inflammatory cells. The residual material in fixative solution allows ancillary studies, such as immuno-histochemistry, to be performed [4].

Hence, present study was aimed to classify thyroid lesions as per Bethesda system and to do comparative evaluation of utility of LBC versus CS in assessing thyroid lesions and whether it can be used as an alternative to CS preparation.

Material and Methods

Present study was a prospective study conducted in Department of Pathology, Pt. B. D. Sharma University of Health Sciences, Rohtak. The study group constituted 60 cases with palpable thyroid lesions. After detailed history, clinical examination and informed consent of patient, FNA of thyroid lesion was performed under sterile aseptic condition using sterile disposable 23 gauge needles and 20 cc syringes (without giving local anaesthesia). The sample was distributed as follows:

- For conventional smear (CS) - one air dried smear subjected to adequacy evaluation under toluidine preparation on the site. One wet smear immediately fixed in 96% ethanol for minimum 30 minutes and later stained with Papanicolaou stain (PAP stain) and three air dried smears stained with May-Grunwald Giemsa in the laboratory [5].

- For liquid based cytology (LBC) - a portion of the aspirate added to a tube containing 5 to 7 ml of cytorich preservative fluid and used for LBC. LBC was done by sure path method on BD Prep Stain instrument [2].

Interpretation of results

Comparative study of CS and LBC was done using a standardized nomenclature by Bethesda system. The results were correlated with clinico-imaging diagnosis and histopathology wherever possible.

Adequacy criteria lay down by Bethesda system was followed for cellularity. It described that at least 6 well preserved follicular groups, containing at least 10 cells for solid nodules was considered as adequate [2].

The CSs and LBC smears compared by a semi-quantitative scoring system (Table 1) [6].

Cytologic features	0	1	2	3
Cellularity	Zero	Scanty	Adequate	Abundant
Background blood – debris	Zero	Occasional	Good amount	Abundant
Informative background	Absent	Present	-	-
Cytoarchitectural pattern	Non-recognised	Moderately recognized	Well recognised	-
Nuclear details	Poor	Fair	Good	Excellent
Cytoplasmic details	Poor	Fair	Good	Excellent

Table 1: Scoring system.

Statistical analysis

The whole data was entered in Microsoft Excel master sheet and analyzed using Statistical Package for Social Sciences (SPSS) version 20 software. The score then obtained by applying the semi quantitative scoring system was compared statistically by using Wilcoxon Signed Rank Test and P value was calculated. P value <0.05 was considered statistically significant and <0.01 as highly significant.

Biomedical waste disposal

Samples submitted for study processed for slide preparation. Slides will be preserved for five years in the department of pathology. All the biochemical waste generated during the study will be discarded as per

the Biomedical Waste Management and Handling Rules 2011 guidelines [7].

Results

The ages of study group ranged from 11-70 years, with the mean age of 39.18 (±13.65) years. Females were affected more than males with M: F ratio of 1:6.5.

Table 2 shows distribution of cases according to Bethesda System by both CS and LBC method. Maximum number of cases was reported in benign Category, among which colloid goiter accounted for maximum number of cases.

Cytological categories	CS- No. of cases (%)	LBC- No. of cases (%)
Non-diagnostic/unsatisfactory (Category 1)	05 (8.3%)	02 (3.3%)
Benign (Category 2)	44 (73.3%)	47 (78.3%)
Atypia of undetermined significance/follicular lesion of undetermined significance (Category 3)	02 (3.3%)	02 (3.3%)
Follicular neoplasm/suspicious for follicular neoplasm (Category 4)	01 (1.7%)	01 (1.7%)
Suspicious for malignancy (Category 5)	03 (5.0%)	02 (3.3%)

Malignant (Category 6)	05 (8.3%)	06 (10%)
Total	60 (100%)	60 (100%)

Table 2: Distribution of cases according to Bethesda System.

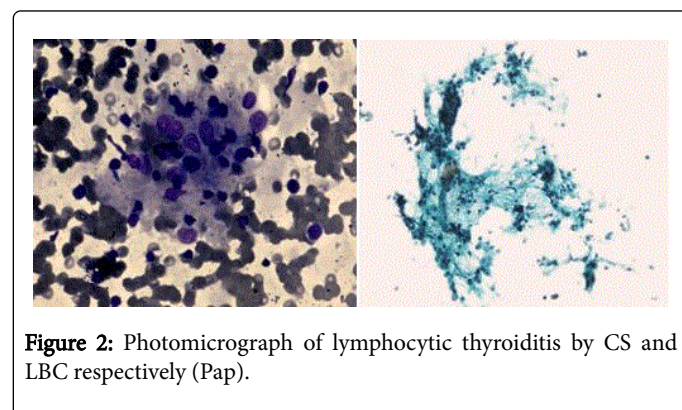
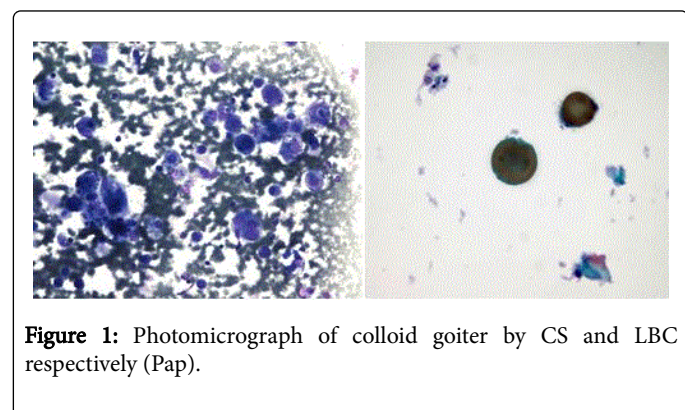
Ultrasonographically, colloid goiter was most common diagnosis (50.0%) and solitary nodule of thyroid was least common (1.7%). Out of 60 cases, 5 cases diagnosed as inadequate by CS method, but when same cases analyzed by LBC method, 3 diagnosed as lymphocytic thyroiditis and 2 as colloid goiter. One of the case diagnosed as suspicious for malignancy by CS method was found to be poorly differentiated carcinoma by LBC.

Conventional and LBC method comparison

Table 3 and Figures 1-6, shows comparison of diagnosis according to Bethesda System made by CS and LBC method. The kappa measure of agreement between diagnosis made by CS and LBC method came out to be 0.734, which was statistically significant ($p < 0.01$).

		Diagnosis by LBC method*											Total (%)#
		IA	CC	CG	HT/LT	AUS	FN	SFM	AC	NHL	PDC	PTC	
Diagnosis by Conventional method*	IA	-	-	2	3	-	-	-	-	-	-	-	5 (8.3)
	CC	1	4	3	-	-	-	-	-	-	-	-	8 (13.3)
	CG	1	1	22	-	-	-	-	-	-	-	-	24 (40.0)
	HT	-	-	-	12	-	-	-	-	-	-	-	12 (20.0)
	AUS	-	-	-	-	2	-	-	-	-	-	-	2 (3.3)
	FN	-	-	-	-	-	1	-	-	-	-	-	1 (1.7)
	SFM	-	-	-	-	-	-	2	-	-	1	-	3 (5.0)
	AC	-	-	-	-	-	-	-	1	-	-	-	1 (1.7)
	NHL	-	-	-	-	-	-	-	-	1	-	-	1 (1.7)
	PDC	-	-	-	-	-	-	-	-	-	1	-	1 (1.7)
	PTC	-	-	-	-	-	-	-	-	-	-	2	2 (3.3)
Total (%)#		2 (3.3)	8 (13.3)	24 (40)	15 (25)	2 (3.3)	1 (1.7)	2 (3.3)	1 (1.7)	1 (1.7)	2 (3.3)	2 (3.3)	60 (100)
Kappa measure of agreement=0.734 $p < 0.01$													
#Percentages in parenthesis.													
*1A: Inadequate, CC: Colloid cyst, CG: Colloid Goitre, HT/LT: Hashimoto/Lymphocytic Thyroiditis, AUS: Atypia of indeterminate significance, FN: Follicular Neoplasm, SFM: Suspicious for Malignancy, AC: Anaplastic Carcinoma, NHL: Non-Hodgkin Lymphoma, PDC: Poorly Differentiated Carcinoma, PTC: Papillary Thyroid Carcinoma													

Table 3: Comparison of diagnosis made by CS and LBC method (n=60).



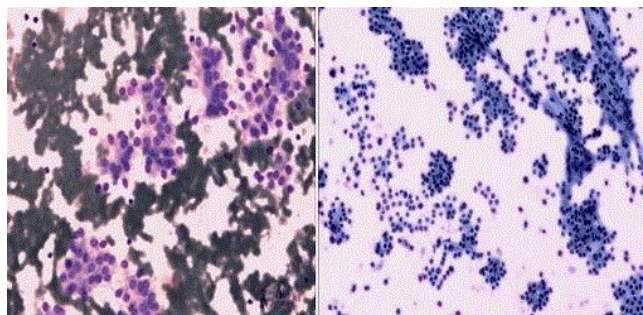


Figure 3: Photomicrograph of follicular neoplasm by CS and LBC respectively (Pap).

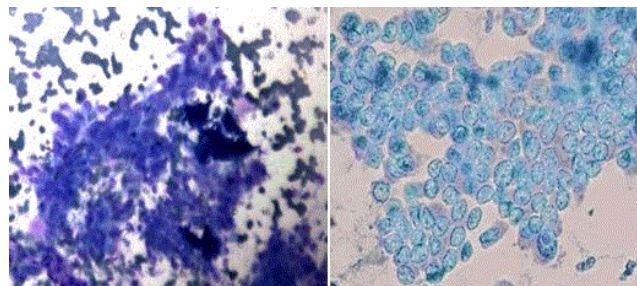


Figure 4: Photomicrograph of papillary thyroid carcinoma by CS and LBC respectively (Pap).

Table 4 shows comparison of cellularity: the overall cellularity observed by LBC method was slightly higher than CS method.

Scoring of Cellularity	Conventional (CS) method		LBC method	
	No. of subjects	Percentage (%)	No. of subjects	Percentage (%)
0	02	3.3%	01	1.7%
1	09	15%	08	13.3%
2	44	73.3%	44	73.3%
3	05	8.3%	07	11.7%
Total	60	100%	60	100%
Mean score (±S.D.)	1.87 (±0.60)		1.95 (±0.56)	
Significance*	Z=-1.043; p=0.297			

Table 4: Comparison of cellularity observed by CS and LBC method (n=60).

Table 5 shows that LBC technique was more valuable in producing clear background by reducing the background obscuring materials such as blood and inflammatory cells, so that the interpretation of smear becomes very easy.

Scoring of background blood-debris	Conventional (CS) method		LBC method	
	No. of subjects	Percentage (%)	No. of subjects	Percentage (%)
0	-	-	16	26.7%
1	01	1.7%	43	71.7%
2	50	83.3%	01	1.7%
3	09	15.0%	-	-
Total	60	100%	60	100%
Mean score (±S.D.)	2.13 (±0.39)		0.75 (±0.47)	
Significance*				
Z=-6.925				
p<0.001				

Table 5: Comparison of background blood-debris observed by CS and LBC method (n=60).

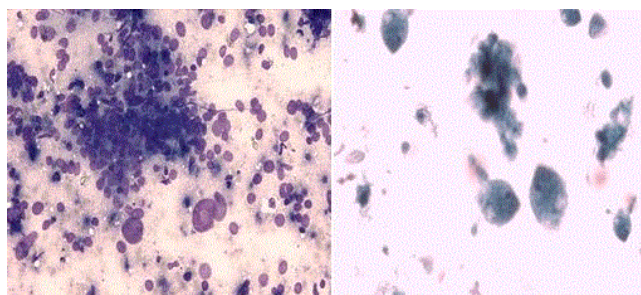


Figure 5: Photomicrograph of anaplastic carcinoma by CS and LBC respectively (Pap).

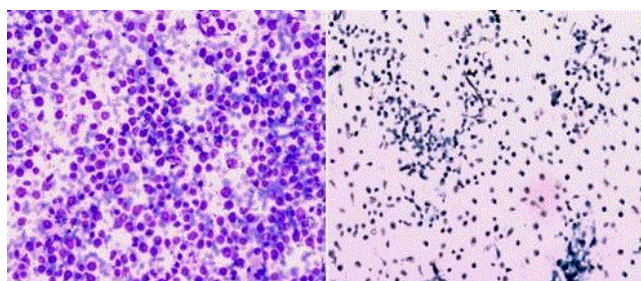


Figure 6: Photomicrograph of non-hodgkin lymphoma by CS and LBC respectively (Pap).

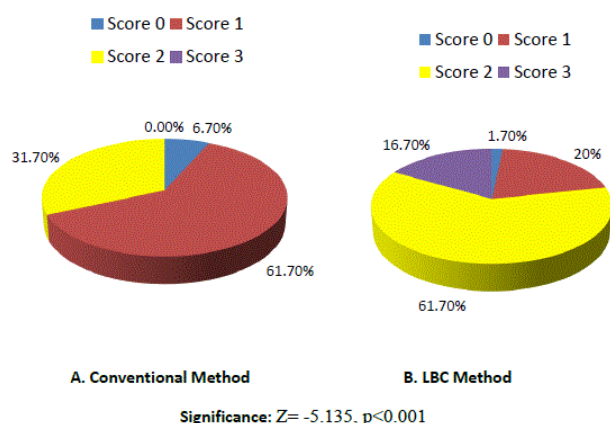


Figure 7: Comparison of cytoplasmic details observed by CS and LBC method (n=60).

According to present study both the methods were almost equally good regarding the informative background ($p=1.000$) ($Z=0.000$) and cytoarchitectural pattern ($p=0.083$) ($Z=-1.732$). By each method, 45 (75%) cases showed presence of informative background and 15 (25%) cases showed absence of informative background and by each method maximum number of cases (55 for LBC and 53 for CS method) showed moderately recognized cytoarchitectural pattern.

In the present study, LBC was superior to CS regarding the recognition of nuclear and cytoplasmic details as shown in Figures 7

and 8. In LBC the epithelial cells were crowded and tightly clustered with nuclear overlapping while in CS cells were present in flat sheets or in a honeycomb arrangement. In LBC loss of cellular preservation was apparent in large aggregates; additionally the peripheral edge of the preparation was commonly blurred and poorly stained as an artifact.

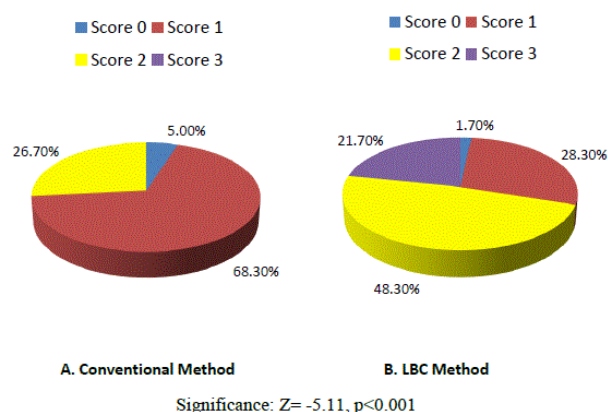


Figure 8: Comparison of nuclear details observed by CS and LBC method (n=60).

Discussion

It was seen that the distribution of cases as per the six-tier Bethesda system in our study differed from studies done by Mamatha et al. and Hershman et al. with the percentages of cases in the benign category being higher (73.3% by CS and 78.3% by LBC) and Suspicious for neoplasm category being lower [8,9].

The reason for the number of cases in the benign category being higher can be attributed to the fact that, our institute, despite being a tertiary care centre, not only caters to the needs of patients on a referral basis, but also patient come here directly without referral. So a large population, representative of general population, is encountered in our institute. Therefore, the proportion of benign cases that is a lot higher in general population is reflected proportionately in our study.

The reason for the lower percentage in the non-diagnostic category with LBC method can be attributed to the fact that LBC method is superior to conventional method (CS) regarding the absence of background blood and debris and better recognition of nuclear and cytoplasmic details.

Cellularity in the LBC smears was slightly higher than CS method. It was also found that where LBC was prepared from needle/syringe rinse after initial CS preparation, significant loss of cells, background material blood-debris, and cell detail was seen. However, when a special pass was done for LBC, most of the time there were adequate diagnostic cells, preserved architecture and extracellular material.

Geers et al. showed in their study that LBC is a reliable technique for assessment of thyroid nodules and offers the advantages of easy identification of colloid (informative background). Present study also showed the presence of colloid in the smears prepared by LBC method, but the colloid was slightly diminished, dense and was present in fragmented or droplets forms [10].

Koo et al conducted a study which showed that in LBC method malignant tumor cells evidenced the large and more vesicular nuclei, prominent nucleoli, and distinct nuclear membrane [11]. In present study LBC appeared to have more disruption of the cytoplasm, an increased number of naked nuclei with larger nuclei and prominent nucleoli. Nuclear grooves and pseudo inclusions were less apparent in papillary carcinoma.

Level of agreement means how many times diagnosis made by both the methods was same. Cohen's k statistic test was used to measure the level of agreement. In present study the sample size was 60 and the kappa agreement came out to be 0.734 (N=60, k=0.734), which was statistically significant. Similar study was done by Jung et al., the sample size of their study was 193. In their study overall agreement of CS and LBC was quite high (N=193, k=0.687) [12]. While in a study by Cochand-Priollet et al., the diagnostic accuracy of CS was better than that of LBC. Oncocytic tumors and lymphocytic thyroiditis presented diagnostic problems in their study, the lack of background colloid with LBC was also a confounding factor [13].

Conclusion

LBC smears contained adequate diagnostic cells in most cases and were greatly superior to CS regarding the absence of background blood-debris, and recognition of nuclear and cytoplasmic details ($p<0.001$). LBC showed cellularity, preserved cell architecture, and informative background as good as CS, expressed by no statistically significant differences ($p=0.297$, 0.083 , and 1.000 respectively). In LBC amount of colloid was diminished and appear dense, fragmented, and in droplets.

LBC is also a relatively simple technique; reducing the number of slides and area per slide to be screened than the conventional preparation. A potential major advantage of LBC is the ability to perform Immunocytochemical studies, which may be required in some cases to reach a definitive diagnosis. However, caution must be applied to interpret the slides and secure a diagnosis, especially if the LBC is the first and only method applied as adequate experience is required to familiarize with various lesions on LBC. Other aspect to be considered is cost efficacy.

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