



ORIGINAL RESEARCH PAPER

Pathology

LIQUID BASED CYTOLOGY VERSUS CONVENTIONAL CYTOLOGY IN FINE NEEDLE ASPIRATES OF BREAST MASSES

KEY WORDS:

cytomorphological, liquid based cytology, breast masses, conventional cytology

Garima Agarwal	Senior Resident, Lady Hardinge Medical College, New Delhi.
Gaurav Garg*	Senior Resident, Dr. Hedgewar Arogya Sansthan, New Delhi, *Corresponding Author.
Preeti Agarwal	Associate Professor, King George's Medical College, Lucknow
Madhu Mati Goel	Professor, King George's Medical College, Lucknow

ABSTRACT

This was the prospective observational comparative study conducted in a tertiary care hospital which include 211 breast masses. Both conventional aspiration cytology and LBC smears were prepared to compare cytomorphological features and to analyze their results in terms of cellularity, ease of interpretation, concordance, pitfalls and diagnostic efficiency. In terms of adequacy we found that both LBC and conventional smear (CS) were similar. Concordance between LBC and CS for making correct diagnosis was observed in 61.13% of cases. Diagnostic efficiency, interpretations were equal in both LBC and CC where as the ease of diagnosis was more with the conventional cytology.

INTRODUCTION:

Liquid Based Cytology (LBC) is a method of preparing smears from cytological material for assessment which was started in 1996^[1]. It has been developed as a competitive method to conventional cytopreparatory methods, with a wide spectrum of application (gynecologic and non-gynecologic cases). The aim of this prospective study was compare the efficacy, merits and demerits of Conventional FNA versus LBC (SurePath) for the cytodagnosis of breast mass lesions and to compare the cytomorphological features of in conventional FNA and LBC from breast masses.

MATERIAL AND METHODS:

This was a comparative observational prospective study which includes 211 cases conducted at the tertiary care hospital from a period of one year from August 2014 to August 2015. Patients with palpable breast lumps were included in the study. A thorough workup and follow-up were performed for all cases including detailed clinical history, general, local, and systemic examinations, routine and special investigations like mammography. Both conventional smear (CS) and LBC smear were prepared for each case. CS was prepared first and then the material from second pass was collected in a vial with BD CytoRich™ Red Preservative for LBC.

Initially, to establish the morphological alterations and train ourselves in LBC interpretation in breast lesions, both LBC and conventional smears were examined simultaneously with diagnosis and the morphological features were interpreted under the following headings i.e., loss of polarity, chromatin clumping, membrane irregularity, nuclear pleomorphism, prominent nucleoli and 3D clusters. After establishment of diagnostic features, the smears were again interpreted randomly and the differences were noted under following points - adequacy, cellularity, interpretation, ease of diagnosis, concordance, diagnostic efficiency and pitfalls. Statistical analysis was done using mean, standard deviation, chi square test and p value.

RESULTS:

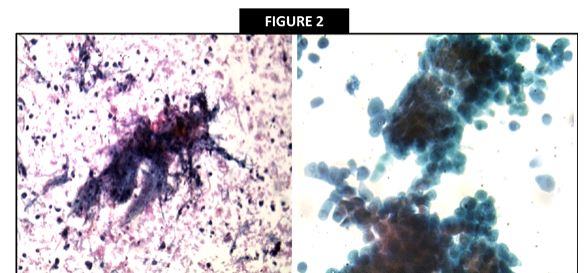
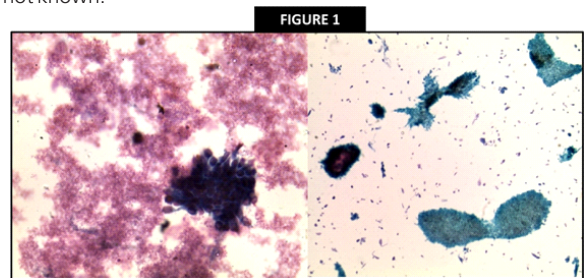
The present study was carried out to evaluate the usefulness of liquid based cytology as compared to conventional FNAC procedure. For this purpose a total of 211 suspected breast lesion cases were enrolled in the study. In our study ranged mean age of benign and malignant cases were 29.35±10.14 and 46.93±11.25 years respectively. Both sides of breast were almost equally involved i.e., right (47.9%) and left (48.3%) in both benign and malignant cases. Upper outer quadrant was the most common quadrant involved (61.6%). On analyzing the clinical features of malignancy we found that 58.8% presented with advanced clinical disease in our set-up with one fourth of patients having axillary lymphadenopathy. Among 211 cases included in the present study inadequacy rates were significantly lower for conventional smear as compared to LBC as shown in table 1. On

comparing the morphological features between benign and malignant lesions in 115 cases we found that loss of polarity was found in almost 100% of malignant cases. Other features are shown in table 2 and 3. When relative efficacy was compared, it was found that diagnostic efficiency and interpretation were equal for both CS and LBC whereas ease of diagnosis was more with CS as shown in table 4. Histopathology was taken as gold standard to test the definitive strength of LBC with CS. We found histopathological correlation in 172 cases out of 211. Diagnostic strength for correlation with histopathology was lower with LBC as compared to CS (table 5). Major pitfall of LBC found in benign cases was that we could not make out the diagnosis of granulomas. In malignancy we found that diagnosis of mucinous carcinoma breast could not be made with LBC due to lack of background.

DISCUSSION:

Fine needle aspiration cytology is an established diagnostic method for breast lesions. The morphological interpretation of liquid-based preparations remains a diagnostic challenge due to considerably altered cytomorphology.

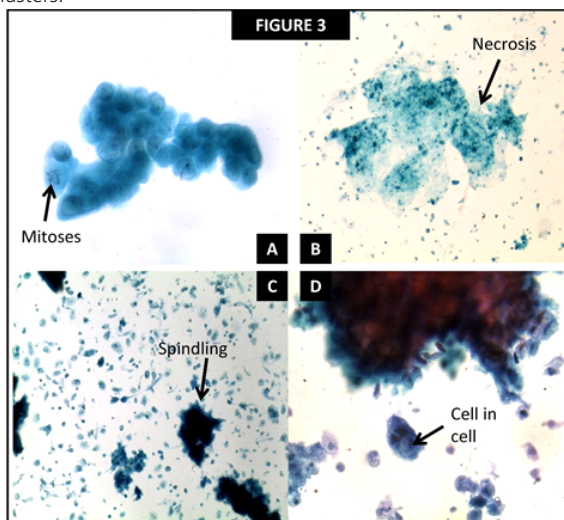
Initially, we processed our specimens in cytorich blue but, due to presence of cellular debris and poor cellularity, LBC smears processed in Cytorich blue were excluded from our study. Smears processed in cytorich red were taken for further interpretation. This confirms that Cytorich red preservative should be used in FNA aspirates of breast masses (Fig 1 and 2). The reason for this is yet not known.



As far as clinical details were concerned, we found that overall pattern of occurrence with benign lesions was seen in younger age group while malignant lesions were seen in older age group. Our findings of equal involvement of right and left breast by both benign and malignant lesions were in contrast with the findings of other workers Meena et al^[2] and Reddy and Reddy et al^[3] who reported the left side more common. Upper and outer quadrant were the most commonly involved quadrant (61.6%) in the present study corroborated by other studies [Rocha et al (45.20%)^[4], Zuk et al(42.20%)^[5], Reddy and Reddy(54.20%)^[3]]. The overall clinical presentation showed that majority of the patients (58.8%) presented with locally advanced disease at the time of first diagnosis, as reported by other workers (Pandya Amrish et al^[6]).

Inadequacy rates was much higher in LBC for benign than malignant lesions in our study. The reason can be attributed to the procedure of SurePath. In SurePath centrifugation is done two times, and we decant the material. In benign lesions fat is more, so this might be the reason that ductal epithelial cells are entrapped with in adipose tissue and are decanted accounting for the lower cellularity and acellularity of LBC smear.

Interpretation of LBC smear was done by looking at loss of polarity, chromatin clumping, membrane irregularity, nuclear pleomorphism, prominent nucleoli and 3D clusters (figure 3). Loss of polarity was observed to be a consistent feature of all malignant cases in LBC in our study as reported by Feoli et al^[7]. Nuclear membrane irregularity was observed in both benign and malignant lesions in our study and it was difficult to discriminate between the two on the basis of membrane irregularity alone. Possibly it requires more experience in interpretation of LBC smears. Our study showed definite chromatin clumping in malignant lesions, and bland chromatin as a feature of benign breast lesions which confirms the findings done by Pranab Dey et al^[8] who observed excellent nuclear chromatin and outlines in LBC preparations in malignant lesions. Our result are in agreement with Benoit JL et al^[9] who observed fibroadenoma is the most common cause of a false-positive diagnosis of malignancy in breast FNAC as in few benign cases, we also observed prominent nucleoli leading to false positive diagnosis of malignancy; which were diagnosed as fibroadenoma on histology. Our study and study done by Kollur SM et al^[10] reported the similar observations that nuclear pleomorphism was not present in every case of malignancy in our study, suggesting that it may not be a specific features of malignancy in LBC preparations. Prominent three dimensional configuration of cell clusters was the most prominent characteristic of SP compared with CS, the findings was in close conformity with those of Ryu et al^[11] However, 3D clusters were observed in both benign as well malignant lesions, therefore it was difficult to interpret the malignant lesions only on the basis of 3D clusters.



A comparative assessment of conventional versus LBC was made based on following cytomorphological features viz: adequacy,

cellularity, interpretation, concordance, diagnostic efficiency, ease of diagnosis and pitfalls.

Both LBC and conventional smears were adequate in 97.6% (111/211) of cases, 39.3% (83/211) of cases were adequate in conventional smear only and 8.1% (17/211) of cases in LBC only. Acellular and paucicellular smears were observed mainly in benign lesions in LBC findings in agreement with other studies [Michael CW et al^[12], Leung CS et al^[13] and Perez Reyex N et al^[14]]. In our study 40.3% (85/211) of cases were diagnosed with conventional smear, 55.5% (117/211) cases with both conventional and LBC smear and only 4.3% (09) cases with LBC smear. This was due to better cellularity in conventional smears than in LBC smears. In our study, the two techniques were concordant in 61.13% (129/211) of cases and were discordant in 38.87% (82/211) of cases. 100% concordant rate was seen in CS with histopathology, where as it was 69.2% with LBC showing difference of statistical significance between the two techniques. In the present study, equal diagnostic performances of LBC and CS were observed in 55.2% (117/211) of cases. In 41.0% (86/211) of cases diagnosis could not be made on LBC and it was possible in conventional smear. The low diagnostic rate of LBC in 41.0% cases, were mainly in cases of fibroadenoma as reported by other workers also [Perez Reyex N et al^[14]]. They observed that the diagnosis of fibroadenoma seems to be most problematic on LBC preparations. Along with fibroadenoma, in our study LBC was also found to be poor in detecting granulomas which were easily detected in CS (Figure 4). LBC in our study was in agreement with that reported by Ryu et al^[11]. 5 cases which were inadequate in conventional smear were diagnosed only by LBC, of these 3 were diagnosed as benign breast disease in LBC specimen and 2 cases were diagnosed as filariasis breast (Figure 5). Ease of diagnosis was observed to be more with conventional smears i.e. in 49% of cases (103/211). It was equal with both LBC and CS in 46.7% (98/211) and only 4.3% (9/211) in LBC smears. Therefore, ease of diagnosis was more with conventional smears in our study. However, it can be subjective at our stage.

FIGURE 4

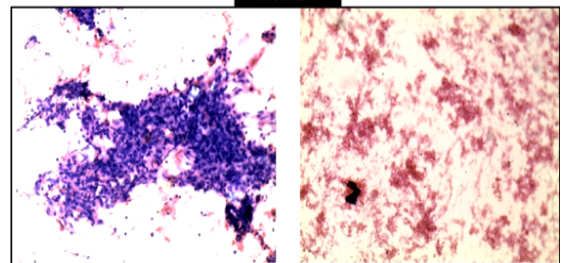
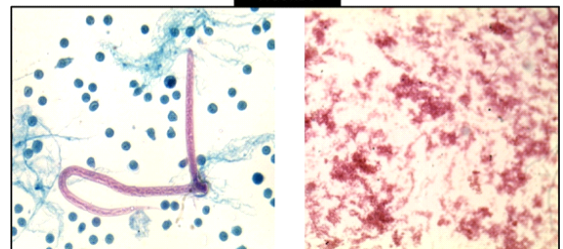


FIGURE 5



Despite the similar findings in both CS and LBC, the conventional methods are fast and inexpensive more suitable for a tertiary care center where cost affordability is a major constraint. The majority of patients report from rural background. The conventional FNA can be done free or at nominal charges of less than Rs 100/- whereas the basic cost of LBC is approximately Rs.350-400 which is not affordable to these patients.

Further the breast LBC requires specific training for the practicing pathologist. However, some advantages of LBC in respect to conventional cytology, it is less time-taking technique, well-preserved cytomorphology of cells with clear background.

Table 1: Specimen adequacy for the purpose of diagnosis between Liquid Based Cytology and Conventional Cytology

	Adequate	Inadequate	Total
Benign			
Conventional	121	5	126
LBC	83	43	126
$\chi^2=37.162$; $p<0.001$			
Malignant			
Conventional	85	0	85
LBC	66	19	85
$\chi^2=21.391$; $p<0.001$			

Table 2: Detailed morphological features in LBC in 115 cases

SN	Feature	Benign (n=49)		Malignant (n=66)		Total (n=115)		Significance of difference	
		No.	%	No.	%	No.	%	Z	P
1.	Loss of polarity	0	0.0	66	100.0	66	57.4	115	<0.001
2.	Membrane irregularity								
	Absent	18	36.7	3	4.5	21	18.3	20.43	<0.001
	Mild	31	63.3	61	92.4	92	80.0		
	Definite	0	0.0	2	3.0	2	1.7		
3.	Chromatin Clumping								
	Absent	0	1	2.0	1	1.5	2	89.502	<0.001
	Mild to moderate	9	18.4	8	12.1	17			
	Definite	0	0.0	55	83.3	55			
	Fine chromatin	6	12.2	2	3.0	8			
	Bland	33	67.3	0	0.0	33			
4.	Nucleoli								
	Absent	18	36.7	0	0.0	18	15.7	89.00	<0.001
	Pinpoint nucleoli	31	63.3	8	12.1	39	33.9		
	Prominent	0	0.0	58	87.9	58	50.4		
5.	Nuclear pleomorphism								
	Absent	48	98.0	1	1.5	49	42.6	107.02	<0.001
	Mild to moderate	1	2.0	36	54.5	37	32.2		
	Severe	0	0.0	29	43.9	29	25.2		
6.	3-D Clustering	27	55.1	65	98.5	92	80.0	33.08	<0.001
7.	Background								
	Others	1	2.0	14	21.2	15	13.0	107.71	<0.001
	Bare bipolar	48	98.0	1	1.5	49	42.6		
	Well preserved cells	0	0.0	51	77.3	51	44.3		
8.	Spindling	0	0.0	54	81.8	54	47.0	75.58	<0.001
9.	Cell in cell	0	0.0	5	7.6	5	4.3	3.881	0.049
10.	Necrosis	0	0	7		7		5.534	0.019

Table 3: Prominent features observed in malignancy in descending order

S. NO.	MORPHOLOGICAL FEATURES	PERCENTAGE
1.	Loss of polarity	100%
2.	Prominent nucleoli	87.9%
3.	Definite chromatin clumping	83.3%
4.	Spindling	81.8%
5.	Well preserved cells	77.3%
6.	Severe nuclear pleomorphism	43.9%
7.	Necrosis	10.6%
8.	Cell in cell	7.6%
9.	Definite membrane irregularity	3.0%

Table 4: Comparison of relative efficacy of Liquid Based Cytology as compared to Conventional Cytology

Feature	Conventional better		Conventional is equal to LBC		LBC better	
	No.	%	No.	%	No.	%
Cellularity	83	39.3	111	97.6	17	8.1
Interpretation	85	40.3	117	55.5	9	4.3
Diagnostic efficacy	86	41.0	117	55.2	8	3.8
Ease of diagnosis	103	49.0	99	46.7	9	4.3

Table 5: Concordance of CS and LBC with HPE

CS=HPE	LBC=HPE
172 (100%)	119 (69.2 %)

$\chi^2=62.653$; $p<0.001$

REFERENCES:

- Randall K Gibb, and Mark G Martens. The Impact of Liquid-Based Cytology in Decreasing the Incidence of Cervical Cancer . Rev Obstet Gynecol 2011
- Meena SP, Hemrajani DK, Joshi N. A comparative and evaluative study of cytological and histological grading system profile in malignant neoplasm of breast - An important prognostic factor. Indian J Pathol Microbiol 2006;49:199-202
- Reddy DG, Reddy CRR. Carcinoma of the breast, its incidence and histological variants among South Indians. Indian J Med Sci 1958;12:228-34.
- Rocha PD, Nadkarni NS, Menezes S. Fine needle aspiration biopsy of breast lesions and histopathologic correlation. Acta Cytol 1997;41:705-12.
- Zuk JA, Maudsley G, Zakhour HD. Rapid reporting on fine needle aspiration of breast lumps in outpatients. J Clin Pathol 1989;42:906-11.
- Amrith N. Pandya, , Neelam P. Shah, Breast Fine Needle Aspiration Cytology Reporting : A Study of Application of Probabilistic Approach 2013
- Francesco Feoli , Lieveke Ameye ,Pascal Van Eeckhout Marianne Paesmans Vincenzo Marra Riccardo Arisio Liquid-Based Cytology of the Breast: Pitfalls Unrecognized before Specific Liquid-Based Cytology Training – Proposal for a Modification of the Diagnostic Criteria Acta Cytologica 2013;57:369–376.
- Pranab Dey, Usha K. Luthra, Josely George, Fatma Zuhairy, Sara Shirley George, and Bahiya Ibrahim Haji. Comparison of ThinPrep and Conventional Preparations on Fine Needle Aspiration Cytology Material. Acta Cytol 2000;44:46–50.
- Benoit JL, Kara R, McGregor SE, Duggan MA. Fibroadenoma of the breast: diagnostic pitfalls of fine-needle aspiration. Diagn Cytopathol, 1992;8: 643-8.
- Kollur SM, El Hag IA: FNA of breast fibroadenoma: observer variability and review of cytomorphology with cytohistological correlation. Cytopathology 2006; 17: 239-244.
- Ryu H S, Park IA, Park SY, Jung YY, Park SH, Shin HC. A pilot study evaluating liquid-based fine needle aspiration cytology of breast lesions: a cytomorphological comparison of SurePath® liquid-based preparations and conventional smears. Acta Cytol. 2013;57:391-9.
- Michael CW, Hunter B: Interpretation of fine-needle aspirates processed by the Thin Prep® technique: cytologic artifacts and diagnostic pitfalls. Diagn Cytopathol 2000; 23: 6– 13.
- Chung Shan Leung, Brian Chiu and Valerie Bell, Comparison of ThinPrep and Conventional Preparations: Nongynecologic Cytology Evaluation , Diagnostic Cytopathology, 1997, 16:368–371.
- Perez-Reyes N, Mulford DK, Rutkowski MA, Logan-Young W, Dawson AE Breast fine-needle aspiration. A comparison of thin-layer and conventional preparation. American Journal of Clinical Pathology . 1994; 102:349-353.
- Bradley Gornstein, Timothy Jacobs, Yvan Bédard, Charles Biscotti, Barbara Ducatman et al. Interobserver agreement of a probabilistic approach to reporting breast fine-needle aspirations on ThinPrep. Diagnostic Cytopathology 2004;30: 389–395.