

LIQUID BASED OR CONVENTIONAL? A CYTOLOGICAL INSIGHT INTO BREAST FNAC

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Abstract

Background: Breast lesions range from benign to malignant, requiring accurate diagnostic methods. FNAC is a safe and cost-effective initial diagnostic tool for evaluating breast masses. Traditionally, conventional smears have been regarded as the gold standard in cytological assessment of breast lesions. Multiple smears are typically prepared from the aspirated material. However, cytologists often find slide screening to be labor-intensive and exhausting. The challenge is further intensified by technical limitations such as poor smear preparation and sub-optimal fixation, which can compromise the preservation of cellular details. This study was conducted to evaluate the efficacy of LBC in breast FNAC by comparing with conventional smears. **Methodology:** A total of 100 cases were studied. Both conventional and Liquid based cytology smears were prepared. The conventional and LBC smears were evaluated by two separate Pathologists and scoring was done on the parameters like cellularity, background blood/debris, informative background, monolayer spread, nuclear details, and cytoplasmic details. **Results:** The results showed that there was no statistically significant difference between LBC and conventional smears in terms of providing informative background and preserving cytoarchitecture. However, LBC showed higher cellularity, reduced background blood and debris, improved formation of monolayers, and enhanced nuclear and cytoplasmic details over conventional cytology. **Conclusion:** Based on the findings of this study, we conclude that LBC can initially serve as a complementary technique alongside conventional breast FNA. As cytopathologists become more familiar with the cytomorphological features unique to LBC, it holds the potential to eventually replace conventional smears, much like its established role in cervical cytology.

Keywords: Breast Cytology, FNAC, Liquid-Based Cytology, Conventional Smears, LBC.

INTRODUCTION

Benign breast lesions are the most prevalent lesions, accounting for 90% of clinical presentations linked to breast¹. Fibroadenoma of the breast is the most prevalent cause of benign breast lumps². Breast cancer is the most common malignancy in women globally. With an anticipated 2.3 million new cases in 2020—11.7% of all cancer cases—it has already overtaken lung cancer as the primary driver

of cancer incidence worldwide³. As per the Globocan data 2020, in India, breast cancer accounted for 13.5% (178361) of all cancer cases and 10.6% (90408) of all death⁴. By the year 2030 global burden of breast cancer will be more than two million every year⁵. In India the incidence of carcinoma of breast is increasing and the mortality rate for breast cancer in India is 11.1 per 10,000⁶. When identifying breast masses, fine-needle aspiration cytology (FNAC) is a safe, economical first-line diagnostic method.

Conventional smears (CS) have long been considered the gold standard method in cytology for the diagnosis of breast lesions. The material that was aspirated is used to prepare variable number smears. The cytologists have found that screening the slides has been time-consuming and tiresome. Technical issues, such as inadequate smear preparation and fixation that results in inadequate preservation of cellular features, exacerbate the issue. Smear reporting is hampered by thick smears, cellular overlapping, and inflammatory infiltrates that obscure the smears.

A growing number of centers worldwide are using the liquid-based methodology for both gynecological and non-gynecological samples in order to address the aforementioned drawbacks of the old method. The US Food and Drug Administration (FDA) has approved two systems, called Thin-prep and Sure-path, which are widely utilized. They are both first-generation liquid-based cytology systems made up of vacuum devices, plastic containers, automated equipment, and filters.

Liquid based cytology (LBC) is widely used for gynecological cytology and has almost replaced CS except in peripheral centers where resources and cost per test is a limitation. But its utility in non-gynecologic cytology is still under research.

The current clinical study is carried out to compare the usefulness and efficacy LBC versus CS for the assessment of the breast lesion and to evaluate their use as an alternative to the conventional preparation. This is being done due to the rise in the incidence of breast lesions worldwide, particularly in India, and their potential for cure. The study also sought to see whether LBC might be used to evaluate breast lesions instead of traditional smears¹²

Finally, additional tests like immunocytochemistry and molecular research can also be performed on the remaining liquid-based sample. They can also be used to prepare cell blocks. Nevertheless, they are not included in this analysis.

MATERIALS AND METHODS

This prospective observational study was conducted in the Department of Pathology, School of Medical Sciences and Research (SMS&R), Sharda University after taking approval from the Ethics Committee, SMS&R (Approval Letter number: 2023/81). A total of 100 cases were included in the study. After approval and informed consent from the patient, FNAs of all the palpable breast lumps coming to the cytopathology section of the Central Lab, Sharda Hospital (May 2023–January 2025) were performed under aseptic precautions using a 23G needle (Romsons) with a 10ml syringe by the principal investigator. From the first pass, two conventional smears were prepared—one air-dried and one alcohol-fixed (100% methanol). And the residual material left in the needle hub and syringe was utilised for LBC (Becton and Dickson SurePath). The air-dried smear was stained with Toluidine stain to assess adequacy. If inadequate, a repeat FNA was performed, and both conventional and LBC material were collected using an additional pass. CS's was stained with Papanicolaou (PAP) stain and May-Grunwald Giemsa (MGG) stain. For the preparation of the LBC smear, the sample was processed using the Becton and Dickson SurePath™ system. After processing, a single pap-stained smear with homogenized material in the center of the slide was obtained.

Both the conventional smears and LBC were examined by two separate pathologists. The parameters such as cellularity, blood in the background, informative background, cytoarchitectural details, monolayer, nuclear details, and cytoplasmic details were scored on both conventional and LBC smears separately (Table 1). The final diagnosis on both conventional and LBC was recorded separately along with The International Academy (IAC) Yokohama Grading (Table 2). The pathologist who evaluated LBC smears recorded the findings without knowing the diagnosis on conventional smears.

Demographic data analysis included sex ratio, age distribution, and laterality of benign and malignant cases. The distribution of benign and malignant cases was assessed, and the quality of LBC smears was compared with conventional smears based on cellularity, background blood debris, informative background, monolayer, cytoarchitectural details, nuclear details, and cytoplasmic details.

RESULTS

In this study, 100 samples were processed, and both conventional and LBC smears were prepared. Among the conventional smears, 87 were benign, and 13 were malignant. In LBC, 5 out of 100 smears were inadequate due to scant cellularity. Of the 95 adequate LBC smears (Table 3), 82 were benign, and 13 were malignant. The male-to-female ratio was 1:49. The age range for benign cases was 9–56 years, while malignant cases ranged from 18–87 years. Among the 100 cases with palpable breast lumps, 47% had lumps on the left side, while 53% presented with right-sided lumps.

Out of 100 cases, the most common diagnosis on conventional smears was fibroadenoma (including fibroadenoma with apocrine or cystic change), accounting for 54 cases, followed by acute on chronic breast abscess (10 cases) and fibrocystic change (7 cases). Usual ductal hyperplasia (4 cases), granulomatous mastitis (3 cases), tubercular abscess (3 cases), gynecomastia (2 cases), epidermal inclusion cyst (2 cases), fat necrosis (1 case), and galactoceles (1 case) were also observed. Among malignant cases, 11 were diagnosed as invasive ductal carcinoma, no special type (IDC NST), while malignant phyllodes 9 (Figure 1) and invasive papillary carcinoma were found in one case each. The IAC Yokohama Grading was 2 for benign cases and 5 for malignant cases.

The most common benign tumour in both LBC and conventional smears was fibroadenoma while the most common malignant tumour was Invasive Ductal Carcinoma of No Special Type (IDC NST).

The comparison of cytomorphological parameters between LBC and conventional smears revealed statistically significant differences in cellularity ($p = 0.003$), loss of background blood/debris ($p = 0.010$), presence of a monolayer ($p = 0.001$), nuclear details ($p = 0.002$), and cytoplasmic details ($p = 0.002$). However, parameters such as informative background ($p = 0.378$) and retention of cell architecture ($p = 0.458$) did not show significant differences between the two methods (Table 4).

DISCUSSION

The liquid-based method has gained popularity in cytology over the past two decades due to its advantages, including a homogenous cellular layer, clean background, and better cell morphology preservation. However, it has not entirely replaced the conventional approach, and opinions on the optimal method remain divided. After thorough search of literature on Pub Med and Medline, we found very few studies done on the utility of LBC in breast cytology. The findings of the research work done on the same and their comparison with the current study are summarised in Table 5. **Cellularity:** - A sample is considered acceptable if it is cellular, high-quality, and representative of the lesion. In our study, cellularity was retained in 95% of cases ($p = 0.003$), with only 5% showing inadequate LBC smears. This may be due to using the second pass for LBC, as blood accumulation after the first pass can reduce cellularity. Had the first pass been used for LBC, those 5% might have been adequate, but prioritizing diagnostic material justified this approach. Studies by Dey et al⁷ (2000), Gerhard et al⁸,

Ryu et al⁹. (2013), Jose et al. (2015), and others suggest LBC and conventional smears have comparable cellularity, while some report slight variations. Our study also found that most cases showed moderate to high cellularity, with only a few exhibiting low cellularity, requiring an additional pass for better assessment. **Background material (blood debris and necrosis):** -In our study, background blood and debris were higher in conventional smears ($p = 0.010$), whereas LBC smears showed significant reduction. This is due to the SurePath LBC technique, which uses density gradient sedimentation to separate mucus and blood, enhancing cell visualization. In contrast, conventional smears often trap diagnostic material in blood clots, obscuring morphology. Our findings align with studies by Sharma et al¹⁰. (2019) and Jakheta et al¹¹. (2022), which also reported that LBC processing removes background material, providing a cleaner slide for easier screening. **Informative Background:** - Our study found that the loss of informative background in LBC is a major drawback, particularly in malignant lesions where tumor diathesis aids diagnosis. Three cases graded as Yokohama 5 on conventional smears were downgraded to Yokohama 4 on LBC, likely due to this loss. Similarly, six cases of fibroadenoma (Yokohama 2) on conventional smears were classified as atypia probably benign (Yokohama 3) on LBC (Figure 4), possibly due to the absence of bare bipolar nuclei, a key diagnostic feature. Our findings align with studies by Veneti et al¹². (2003), Michael et al¹³. (2000), Hoda et al¹⁴. (2007), and others, who also reported the loss of useful background in LBC. However, some authors, such as Jakheta et al¹¹. (2022) and Mygdakos et al¹⁵. (2009), found that LBC retained informative background better than conventional smears. **Cell architecture:** - Our study found that LBC alters cell architecture by fragmenting large clusters into smaller ones and causing cellular dissociation. This affected diagnosis, as six cases classified as Yokohama Grade 2 on conventional smears were graded as Yokohama Grade 3 on LBC, likely due to the loss of cohesion in fibroadenoma cell clusters, making them appear loosely cohesive and suspicious. These findings align with Michael et al¹³. (2000), who also observed cluster fragmentation in LBC, and Gerhard et al⁸. (2013), who noted that small clusters and loss of cohesion could lead to misdiagnosis of malignancy. However, Sharma et al¹⁰. (2019) found no significant difference in cytoarchitectural retention, while studies by R. Kaushalya¹⁶ (2023) and Komatsu et al¹⁷. (2008) reported better preservation of cell architecture in LBC due to reduced cellular overlap. **Presence of monolayer:** - Our study found that LBC smears had better monolayer cell arrangements with less overlap, allowing improved visualization of nuclear and cytoplasmic details compared to conventional smears. This may be due to greater cell cluster fragmentation and discohesion in LBC. These findings align with studies by Michael et al¹³. (2000), Komatsu et al¹⁷. (2008), and Pawar PS et al¹. (2014).

Nuclear and cytoplasmic details: - In our study, cytoplasmic details were rated excellent in 81% of LBC smears compared to 50% in conventional smears, while nuclear details were excellent in 79% of LBC cases versus 50% in conventional smears. This improvement in LBC may be due to reduced cellular overlap, better monolayer formation, loss of background blood/debris, and fewer fixation artifacts. Our findings align with studies by Dey et al⁷. (2000), Michael et al¹³. (2000), Gerhard et al⁸. (2014), and Tripathy et al¹⁸. (2015), who also observed superior cytoplasmic details in LBC. However, some authors, like Sharma et al¹⁰. (2019) and Perez et al¹⁹. (1994), reported poorer cellular details in LBC compared to conventional smears. LBC provides better monolayer cell spread and enhanced nuclear and cytoplasmic details, the loss of informative background, such as tumor diathesis and bare bipolar nuclei, may lead to diagnostic challenges. So, from this research work we conclude that LBC can be initially used as an adjunct to conventional breast FNA and eyes can be trained to the cytomorphological patterns of this new technique. And once our eyes get accustomed to LBC smears of breast FNA, it can actually replace conventional as in cervical cytology. This would not only save time in screening the slides of FNA but the residual material can also be used for ancillary tests like immunocytochemistry and molecular testing though it was not done in the current study. However, there were some drawbacks in this study, not all the cases had histopathology done and inadequate

cellularity must have resulted from using residual material after preparing conventional smears. Had it been done from the first pass material; we would have no inadequate cases on LBC.

Table 1: Scoring of various parameters on LBC and conventional smears

Cytologic features	Score 0	Score 1	Score 2	Score 3
Cellularity	Nil	Scanty	Adequate	Abundant
Background blood, cell debris	Nil	Occasional	Good amount	Abundant
Informative background	Absent	Present		
Monolayer	Absent	Occasional monolayer cells	Many monolayer cells	
Cell architecture	Not recognized	Moderately recognized	Well recognized	
Nuclear detail	Poor	Fair	Good	Excellent
Cytoplasmic detail	Poor	Fair	Good	Excellent

Table 2: International Academy of Cytologists Yokohama Grading system of Breast cytopathology

C1	Insufficient
C2	Benign
C3	Atypical
C4	Suspicious of malignancy
C5	Malignant

Table 3: Cytological Diagnosis and IAC Yokohama grading on LBC smears

S. No	Diagnosis on LBC smear	No. of Cases(N=100)	IAC Yokohama
1	Inadequate **	5	1
2	Fibroadenoma (FA)/ FA with apocrine change /FA with cystic change	47	2
3	Usual ductal hyperplasia (UDH)	2	2
4	Fibrocystic change	6	2
5	Granulomatous Mastitis	3	2
6	Tubercular Abscess (Figure 3)	3	2
7	Acute on Chronic breast Abscess	9	2
8	Fat Necrosis	1	2
9	Gynecomastia	2	2
10	Galactocele	1	2
11	Epidermal Inclusion cyst	2	2
12	Atypia probably Benign** (Figure 4)	6	3
13	Suspicious for malignancy**	3	4
14	Invasive ductal carcinoma, no special type (IDC NST)	9	5
15	Malignant Phyllodes (Figure 2)	1	5
16	Papillary Carcinoma	1	5

** Discrepancy in LBC results.

Table 4: Comparison between liquid -based and conventional cytology for different cytological features

Characteristic / Technique	Score 0 (CS/LBC)	Score 1 (CS/LBC)	Score 2 (CS/LBC)	Score 3 (CS/LBC)	p-value
Cellularity	0 / 5.0	9.0 / 2.0	39.0 / 28.0	52.0 / 65.0	0.003
Background Blood/Debris	1.0 / 96.0	14.0 / 2.0	32.0 / 2.0	53.0 / 0.0	0.010

Informative Background	30.0 / 33.0	70.0 / 67.0	–	–	0.378
Monolayer	1.0 / 6.0	48.0 / 29.0	51.0 / 65.0	–	0.001
Cell Architecture	1.0 / 7.0	61.0 / 13.0	38.0 / 80.0	–	0.458
Nuclear Details	0.0 / 6.0	5.0 / 2.0	45.0 / 13.0	50.0 / 79.0	0.002
Cytoplasmic Details	0.0 / 5.0	6.0 / 2.0	44.0 / 12.0	50.0 / 81.0	0.002

Table 5: Comparative Summary of Study Results

Study	Cellularity	Nuclear/ Cytoplasmic Details	Background Clarity	Informative Background	Cell architecture
Dey et al. (2000)	Comparable	Improved	Cleaner background	Loss of informative background	Well recognized
Michael et al. (2000)	Comparable	Improved	Reduced artifacts	Altered informative background	Altered cell architecture
Gerhard et al. (2013)	Comparable	Improved	Better clarity	Comparable	Altered cell architecture
Ryu et al. (2013)	Comparable	Superior	Cleaner	Loss of architecture	Loss of architecture
Sharma et al. (2019)	Comparable	Inferior	Cleaner	Loss of informative background	Comparable
Jakhetia et al. (2022)	Comparable	Comparable	Cleaner	Retained informative background	Comparable
Kousalya et al. (2023)	Comparable	Improved	Cleaner	Loss of informative background	Well recognized
Current study (2025)	Comparable	Improved	Cleaner	Loss of informative background	Altered cell architecture

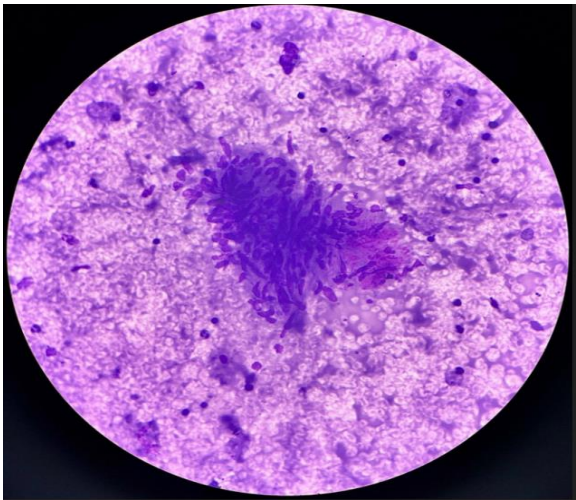


Figure 1: Malignant phyllodes Tumour400x (CS)stromal fragment with increased cellularity and pleomorphic cells on May Grunwald Giemsa stain

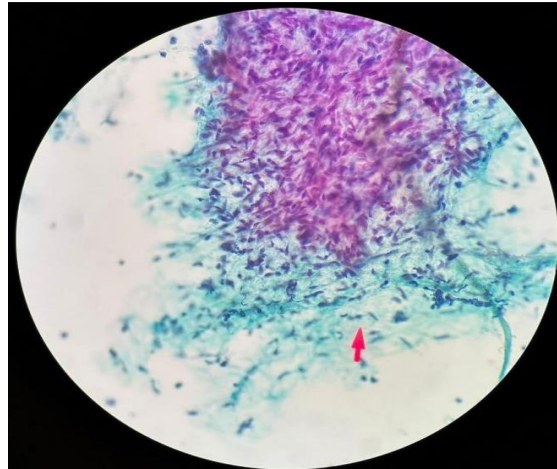


Figure 2: Malignant Phyllodes 400x (LBC) stromal fragment with increased cellularity and pleomorphic cells on Papanicolaou stain.

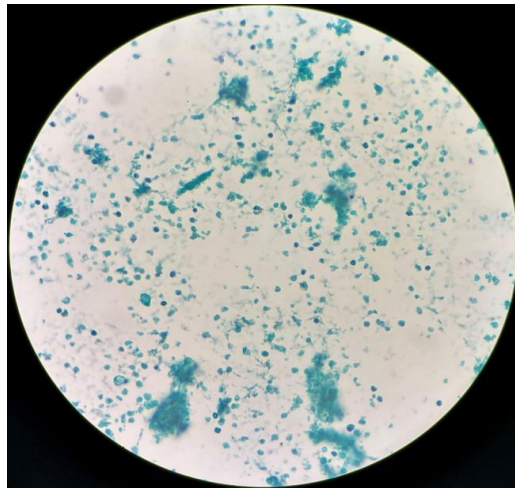


Figure 3: Tubercular Abscess 400x (LBC). Necrotic background with few degenerated lymphocytes and epithelioid cells on Papanicolaou stain



Figure 4: Atypia probably benign 100x (LBC). A monolayered cluster of bland ductal cells but absence of myoepithelial cells and bare bipolar nuclei in the background on Papanicolaou stain**

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